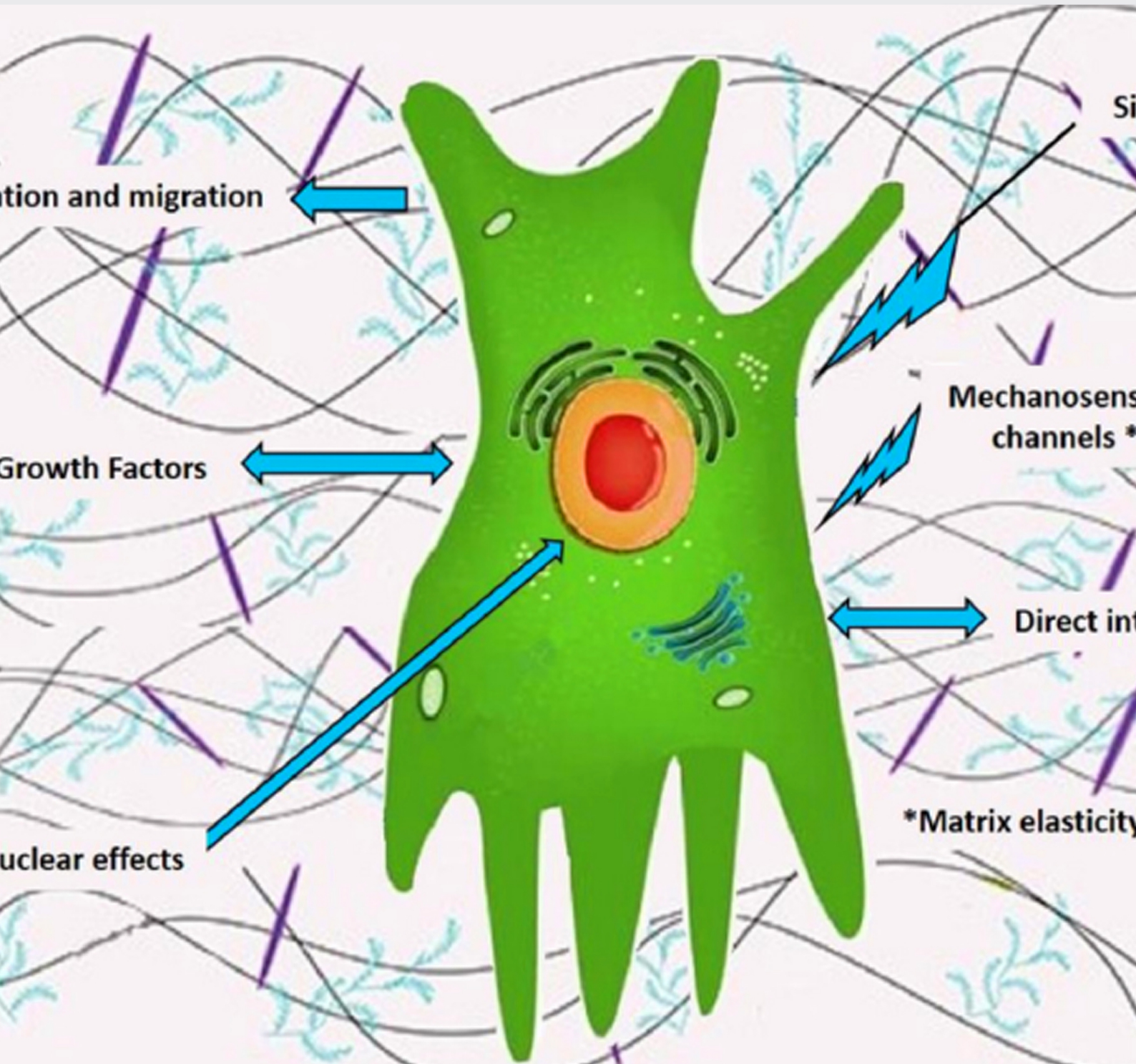


# Plastic and Aesthetic Research

PAR



## EDITORIAL BOARD

---

### Editor-in-Chief

Raúl González-García (Spain)

### Associate Editors

Charles E. Butler (USA)  
Yi-Lin Cao (China)  
Wen-Guo Cui (China)  
Rui Fernandes (USA)  
Raymund E. Horch (Germany)  
Zhi-Qi Hu (China)  
Pedro Infante-Cossio (Spain)  
Hua Jiang (China)  
Francesca Toia (Italy)

### Editorial Board Members

Fabio Caviggioli (Italy)  
Chang-Cheng Chang (Taipei, China)  
Hctxg{ Chim (USA)  
Ravi Kumar Chittoria (India)  
Jonathan R. Clark (Australia)

Peter D. Costantino (USA)  
Salvatore D'Arpa (Belgium)  
Adrian Dragu (Germany)  
Francesco M. Egro (USA)  
Damien G. Grinsell (Australia)  
Francesca Romana Grippaudo (Italy)  
Matthew L. Iorio (USA)  
Richard Irving (UK)  
Yong Ju Jang (South Korea)  
Ruben Yap Kannan (UK)  
Kenji Kawamura (Japan)  
Vaughan Keeley (UK)  
Umar Daraz Khan (UK)  
Isao Koshima (Japan)  
Charles Yuen Yung Loh (UK)  
Raman C. Mahabir (USA)  
Hiroshi Mizuno (Japan)  
Stan Monstrey (Belgium)  
Tqpcrf "Ngqpctf "Moy (USA)  
Elias Polykandriotis (Germany)  
Lee L. Q. Pu (USA)

Oscar M. Ramirez (USA)  
Raffaele Rauso (Italy)  
Salah Rubayi (USA)  
Gennaro Selvaggi (Sweden)  
Andrea Sisti (Italy)  
Jia-Ming Sun (China)  
Pierluigi Tos (Italy)  
Carroll Ann Trotman (USA)  
Jon P. Ver Halen (USA)  
You-Bin Wang (China)  
Stephen Anthony Wolfe (USA)  
Alex K. Wong (USA)  
Bin Yang (China)  
Da-Ping Yang (China)  
Cheng-Gang Yi (China)  
James E Zins (USA)

### Editorial Staffs

Min-Jie Zhang (China)  
Huan-Liang Wu (China)  
Cai-Hong Wang (China)



# GENERAL INFORMATION

---

## About the Journal

*Plastic and Aesthetic Research (PAR)*, ISSN 2349-6150 (Online), ISSN 2347-9264 (Print), is a peer-reviewed online journal with print on demand compilation of articles published. The journal's full text is available online at [www.parjournal.net](http://www.parjournal.net). The journal allows free access (Open Access) to its contents and permits authors to self-archive final accepted version of the articles on any OAI-compliant institutional/subject-based repository. The journal focuses on plastic and aesthetic surgery, and the coverage extends to other basic and clinical studies related to plastic surgery, including microsurgery, laser treatment, orthopaedic surgery, psychology, social ethics, *etc.* The journal is indexed by Google Scholar, EBSCO, Hinari, Eurasian Scientific Journal Index (ESJI), Root Indexing, JournalTOCs, JournalGuide, CNKI, Wanfang Data, J-Gate, DRJI, SHERPA/RoMEO, ResearchBib, Chaoxing "Domain" Publishing Platform, Cite Factor, and Worldcat.

## Information for Authors

Manuscripts should be prepared in accordance with Author Instructions.

Please check [www.parjournal.net/pages/view/author\\_instructions](http://www.parjournal.net/pages/view/author_instructions) for details.

All manuscripts should be submitted online at [www.editorialmanager.com/par](http://www.editorialmanager.com/par).

## Copyright

The entire contents of the *PAR* are protected under international copyrights. The journal, however, grants to all users a free, irrevocable, worldwide, perpetual right of access to, and a license to copy, use, distribute, perform and display the work publicly and to make and distribute derivative works in any digital medium for any reasonable purpose, subject to proper attribution of authorship and ownership of the rights. The journal also grants the right to make small numbers of printed copies for their personal use under the Creative Commons Attribution 4.0 License.

Copyright is reserved by © The Author(s) 2020.

## Permissions

For information on how to request permissions to reproduce articles/information from this journal, please visit [www.parjournal.net](http://www.parjournal.net).

## Disclaimer

The information and opinions presented in the journal reflect the views of the authors and not of the journal or its Editorial Board or the Publisher. Publication does not constitute endorsement by the journal. Neither the *PAR* nor its publishers nor anyone else involved in creating, producing or delivering the *PAR* or the materials contained therein, assumes any liability or responsibility for the accuracy, completeness, or usefulness of any information provided in the *PAR*, nor shall they be liable for any direct, indirect, incidental, special, consequential or punitive damages arising out of the use of the *PAR*. *PAR*, nor its publishers, nor any other party involved in the preparation of material contained in the *PAR* represents or warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such material. Readers are encouraged to confirm the information contained herein with other sources.

## Partner Affiliation/Society

Italian Federation of Aesthetic Medicine (FIME)

## Contacts

E-mail: [gf.kqtknqHegB rctlqwtpcrfpgv](mailto:gf.kqtknqHegB rctlqwtpcrfpgv)

Website: [www.parjournal.net](http://www.parjournal.net)

## Published by

OAE Publishing Inc.

245 E Main Street st122, Alhambra, CA 91801, USA

Website: [www.oaepublish.com](http://www.oaepublish.com)

# CONTENTS

---

- 1 Targeting facial aging with nano and regenerative technologies and procedures**  
Marek Dobke, Adam Hauch  
*Plast Aesthet Res* 2020;7:1 <http://dx.doi.org/10.20517/2347-9264.2019.65>
- 2 Face transplantation for massive mandibular defects: considerations for allograft design and surgical planning**  
William Jackson Palmer, Laurel Nelms  
*Plast Aesthet Res* 2020;7:2 <http://dx.doi.org/10.20517/2347-9264.2019.34>
- 3 A case of congenital aneurysm of the ulnar artery of the palm**  
Hin-Lun Liu, Melody Man-Kuen Wong, Joseph Hon-Ping Chung  
*Plast Aesthet Res* 2020;7:3 <http://dx.doi.org/10.20517/2347-9264.2019.50>
- 4 Expanding the top rungs of the extremity reconstructive ladder: targeted muscle reinnervation, osseointegration, and vascularized composite allotransplantation**  
Saïd C. Azoury, Andrew Bauder, Jason M. Souza, John T. Stranix, Sammy Othman, Christine McAndrew, Scott M. Tintle, Stephen J. Kovach, Lawrence Scott Levin  
*Plast Aesthet Res* 2020;7:4 <http://dx.doi.org/10.20517/2347-9264.2019.44>
- 5 Mesh and plane selection: a summary of options and outcomes**  
Yewande Alimi, Chamilka Merle, Michael Sosin, Marielle Mahan, Parag Bhanot  
*Plast Aesthet Res* 2020;7:5 <http://dx.doi.org/10.20517/2347-9264.2019.39>
- 6 Indocyanine green lymphangiography-guided liposuction in breast cancer-related lymphedema treatment - patient selection and technique**  
Hin-Lun Liu , Melody Man-Kuen Wong , Joseph Hon-Ping Chung  
*Plast Aesthet Res* 2020;7:6 <http://dx.doi.org/10.20517/2347-9264.2019.62>
- 7 Abdominal wall procedures: the benefits of prehabilitation**  
Nathan Knapp, Breanna Jedrzejewski, Robert Martindale  
*Plast Aesthet Res* 2020;7:7 <http://dx.doi.org/10.20517/2347-9264.2019.69>
- 8 Comparison of adipose particle size on autologous fat graft retention in a rodent model**  
Xiaonan Yang, Francesco M. Egro, Taraneh Jones, W. Vincent Nerone1, Michael Yousefpour, Jeffrey A. Gusenoff, J. Peter Rubin, Lauren E. Kokai  
*Plast Aesthet Res* 2020;7:8 <http://dx.doi.org/10.20517/2347-9264.2019.63>



- 9        The benefit of combined radiofrequency and ultrasound to enhance surgical and nonsurgical outcomes for the face and neck**  
Christine A. Catinis, Suneel Chilukuri  
*Plast Aesthet Res* 2020;7:9 <http://dx.doi.org/10.20517/2347-9264.2019.68>
- 10      Differential diagnoses and treatment of lipedema**  
Maria Wiedner, Donia Aghajanzadeh , Dirk F. Richter  
*Plast Aesthet Res* 2020;7:10 <http://dx.doi.org/10.20517/2347-9264.2019.51>
- 11      A minimally invasive midface suspension**  
Jorge I. de la Torre, John Lindsey Jr., Dean Cerio, Luis O. Vasconez  
*Plast Aesthet Res* 2020;7:11 <http://dx.doi.org/10.20517/2347-9264.2019.54>
- 12      The “central six” of ptosis repair: eliminating contour as a variable in external levator surgery**  
Benjamin C. Campbell, Susuana T. Adjei, William R. Nunery, H. B. Harold Lee  
*Plast Aesthet Res* 2020;7:12 <http://dx.doi.org/10.20517/2347-9264.2019.59>
- 13      Review of soft tissue coverage options in distraction osteogenesis of the extremity**  
Jacqueline Stoneburner, Beina Azadgoli, Anna C. Howell, Douglass Tucker, Geoffrey Marecek, Joseph Carey  
*Plast Aesthet Res* 2020;7:13 <http://dx.doi.org/10.20517/2347-9264.2019.028>
- 14      Role of the extracellular matrix in skin aging and dedicated treatment - State of the art**  
Adele Sparavigna  
*Plast Aesthet Res* 2020;7:14 <http://dx.doi.org/10.20517/2347-9264.2019.73>
- 15      Breast cancer-related lymphedema: focus on surgical treatment**  
Maria Luisa Nardulli  
*Plast Aesthet Res* 2020;7:15 <http://dx.doi.org/10.20517/2347-9264.2019.56>
- 16      The use of botulinum toxin A in chemical component separation: a review of techniques and outcomes**  
Sharbel A. Elhage, Eva B. Deerenberg, Jenny M. Shao, Vedra A. Augenstein, B. Todd Heniford  
*Plast Aesthet Res* 2020;7:16 <http://dx.doi.org/10.20517/2347-9264.2020.03>
- 17      Safety and efficacy of surgical treatments for axillary osmidrosis: a retrospective cohort study comparing conventional open excision with cartilage-shaver closed curettage**  
Ryutaro Tanaka, Daichi Morioka, Syuryo Akamine, Takafumi Shimizu, Koichi Kadomatsu  
*Plast Aesthet Res* 2020;7:17 <http://dx.doi.org/10.20517/2347-9264.2020.12>

- 18 Flap reconstruction of the abdominal wall**  
Sneha Patel, Alexander F. Mericli, Sahil K. Kapur, Margaret S. Roubaud, Charles E. Butler  
*Plast Aesthet Res* 2020;7:18 <http://dx.doi.org/10.20517/2347-9264.2019.15>
- 19 Surgical debulking, lymphaticovenous anastomosis, vascularised lymph node transfer in lower limb lymphoedema**  
Hari Venkatramani, Rajasabapathy Raja Shanmugakrishnan, Murugesan Senthil Kumaran, Shanmuganathan Raja Sabapathy  
*Plast Aesthet Res* 2020;7:19 <http://dx.doi.org/10.20517/2347-9264.2019.70>
- 20 Evaluation and management of acquired ptosis**  
Stephanie E. Farber, Mark A. Codner  
*Plast Aesthet Res* 2020;7:20 <http://dx.doi.org/10.20517/2347-9264.2020.05>
- 21 Establishing a center of excellence in abdominal wall reconstruction**  
Jenny Shao, Sharbel Elhage, Eva Deerenberg, Vedra Augenstein, B. Todd Heniford  
*Plast Aesthet Res* 2020;7:21 <http://dx.doi.org/10.20517/2347-9264.2020.04>
- 22 Introduction of special issue “Advances in Microsurgery for Upper and Lower Extremity Reconstruction and Limb Preservation”**  
Matthew L. Iorio  
*Plast Aesthet Res* 2020;7:22 <http://dx.doi.org/10.20517/2347-9264.2020.86>
- 23 Exploring adherence to daytime compression in women with breast cancer related lymphedema: a multi-methods study**  
Mona Al Onazi, Naomi Dolgoy, Joanna Parkinson, Margaret L. McNeely  
*Plast Aesthet Res* 2020;7:23 <http://dx.doi.org/10.20517/2347-9264.2019.74>
- 24 Dorsal hand reconstruction with radial artery perforator-based adipofascial flap**  
Sho Yamakawa, Kenji Hayashida  
*Plast Aesthet Res* 2020;7:24 <http://dx.doi.org/10.20517/2347-9264.2020.20>
- 25 Endoscopic assisted facial rejuvenation: a 35 year personal journey**  
Oscar M. Ramirez  
*Plast Aesthet Res* 2020;7:25 <http://dx.doi.org/10.20517/2347-9264.2019.78>
- 26 Cryopreserved fat: our clinical experience and applications**  
Masanori Ohashi  
*Plast Aesthet Res* 2020;7:26 <http://dx.doi.org/10.20517/2347-9264.2020.15>



- 27     **Role of limited access dressing in achieving improved aesthetic results during resurfacing of wounds**  
Pramod Kumar  
*Plast Aesthet Res* 2020;7:27 <http://dx.doi.org/10.20517/2347-9264.2020.07>
- 28     **Stem cells and tissue engineering in plastic surgery: an update**  
Gregory R. D. Evans, Alan D. Widgerow  
*Plast Aesthet Res* 2020;7:28 <http://dx.doi.org/10.20517/2347-9264.2019.53>
- 29     **Secondary damage in trauma and limited access dressing: a review**  
Pramod Kumar, Akriti Gupta, Apoorva Gupta  
*Plast Aesthet Res* 2020;7:29 <http://dx.doi.org/10.20517/2347-9264.2019.71>
- 30     **Lympho-SPECT/CT as a tool to evaluate postoperative outcomes after LVA for lymphedema repair**  
Jose M. Lasso  
*Plast Aesthet Res* 2020;7:30 <http://dx.doi.org/10.20517/2347-9264.2019.75>
- 31     **Stem cells and tissue engineering: an alternative treatment for craniofacial congenital malformations and articular degenerative diseases**  
Cristina Velasquillo, Antonio Madrazo-Ibarra, Claudia Gutiérrez- Gómez, Marcia Rosario Pérez-Dosal, Yaaziel Melgarejo-Ramírez, Clemente Ibarra  
*Plast Aesthet Res* 2020;7:31 <http://dx.doi.org/10.20517/2347-9264.2020.30>
- 32     **Nerve transfers in distal forearm and in the hand**  
Alfio Luca Costa, Paolo Titolo, Bruno Battiston, Michele Rosario Colonna  
*Plast Aesthet Res* 2020;7:32 <http://dx.doi.org/10.20517/2347-9264.2020.43>
- 33     **Established and experimental techniques to improve phalloplasty outcomes/optimization of a hypercomplex surgery**  
Erin E. Carter, Curtis N. Crane, Richard A. Santucci  
*Plast Aesthet Res* 2020;7:33 <http://dx.doi.org/10.20517/2347-9264.2020.81>
- 34     **Psychological stress enhances keloid development via stress hormone-induced abnormal cytokine profiles and inflammatory responses**  
Ya-Ting Yang, Xiao-Li Wu, Wei Liu  
*Plast Aesthet Res* 2020;7:34 <http://dx.doi.org/10.20517/2347-9264.2020.24>
- 35     **Equality in cleft and craniofacial care**  
Nicholas D. Sharratt, Jean Calleja Agius, Gareth Davies, Felicity V. Mehendale, Peter Hagell, Martin Persson  
*Plast Aesthet Res* 2020;7:35 <http://dx.doi.org/10.20517/2347-9264.2020.99>

- 36     **The interaction between hyaluronidase and hyaluronic acid gel fillers - a review of the literature and comparative analysis**  
Michael K. Paap, Rona Z. Silkiss  
*Plast Aesthet Res* 2020;7:36 <http://dx.doi.org/10.20517/2347-9264.2020.121>
- 37     **Impact of different surgical protocols on dental development in oro-facial cleft children**  
Rosa Guagnano, Federica Romano, Ernesto Pepe, Patrizia Defabianis  
*Plast Aesthet Res* 2020;7:37 <http://dx.doi.org/10.20517/2347-9264.2020.21>
- 38     **Olecranon bone grafting for the treatment of nonunion after distal finger replantation**  
Burak Sercan Ercin, Fatih Kabakas, Musa Kemal Keles , Ismail Bulent Ozcelik, Berkan Mersa  
*Plast Aesthet Res* 2020;7:38 <http://dx.doi.org/10.20517/2347-9264.2020.56>
- 39     **Strategies for operative management of abdominal wall hernia after solid organ transplant**  
Devinder Singh, Luther Holton, Lauren Antognoli, Salman Choudhry  
*Plast Aesthet Res* 2020;7:39 <http://dx.doi.org/10.20517/2347-9264.2019.76>
- 40     **Prevention of hyper- and hypotrophic scars through surgical incisions in the direction of the “main folding lines” of the skin**  
Gottfried Lemperle  
*Plast Aesthet Res* 2020;7:40 <http://dx.doi.org/10.20517/2347-9264.2020.14>
- 41     **Stem cells and periodontal regeneration: present and future**  
Filippo Citterio, Giacomo Gualini, Ludovica Fierravanti, Mario Aimetti  
*Plast Aesthet Res* 2020;7:41 <http://dx.doi.org/10.20517/2347-9264.2020.29>
- 42     **Major upper limb replantation: a review of clinical pearls**  
Margaret Luthringer, Margaret Dalena, Haripriya S. Ayyala  
*Plast Aesthet Res* 2020;7:42 <http://dx.doi.org/10.20517/2347-9264.2020.35>
- 43     **Metoidioplasty as a one-stage phallic reconstruction in transmen**  
Marta Bizic, Borko Stojanovic, Marko Bencic, Noemi Bordas, Miroslav Djordjevic  
*Plast Aesthet Res* 2020;7:43 <http://dx.doi.org/10.20517/2347-9264.2020.80>
- 44     **Complications after cosmetic periocular filler: prevention and management**  
Mike Zein, Ryan Tie-Shue, Nathan Pirakitikulr, Wendy W. Lee  
*Plast Aesthet Res* 2020;7:44 <http://dx.doi.org/10.20517/2347-9264.2020.133>
- 45     **Midline raphe scroti artery flap for penile shaft reconstruction**  
Ursula Mirastschijski, Carla Schwenke, Igor Schwab, Andreas Buchhorn, Andreas Schmiedl  
*Plast Aesthet Res* 2020;7:45 <http://dx.doi.org/10.20517/2347-9264.2020.44>



- 46 Effects of various surgical protocols on maxillofacial growth in patients with unilateral cleft lip and palate: a systematic review**  
Pasquier Corthouts, Fien Boels, Elke Van de Castele, Nasser Nadjmi  
*Plast Aesthet Res* 2020;7:46 <http://dx.doi.org/10.20517/2347-9264.2020.97>
- 47 Conventional surgical techniques and emerging transplantation in complex penile reconstruction**  
Nima Khavanin, Richard J. Redett  
*Plast Aesthet Res* 2020;7:47 <http://dx.doi.org/10.20517/2347-9264.2020.63>
- 48 Facial morphospace: a clinical quantitative analysis of the three-dimensional face in patients with cleft lip and palate**  
Chihiro Tanikawa  
*Plast Aesthet Res* 2020;7:48 <http://dx.doi.org/10.20517/2347-9264.2020.136>
- 49 Clinical application of mesenchymal stem cells for cartilage regeneration**  
Yu-Chun Chen, Chih-Hung Chang  
*Plast Aesthet Res* 2020;7:49 <http://dx.doi.org/10.20517/2347-9264.2020.28>
- 50 50+ years of replantation surgery experience: are we progressing or regressing?**  
Karen Noh, Jacques H. Hacquebord  
*Plast Aesthet Res* 2020;7:50 <http://dx.doi.org/10.20517/2347-9264.2020.49>
- 51 Phalloplasty: understanding the chaos**  
Megan Lane, Emily C. Sluiter, Shane D. Morrison, Devin Coon, Katherine M. Gast, Jens U. Berli, William M. Kuzon  
*Plast Aesthet Res* 2020;7:51 <http://dx.doi.org/10.20517/2347-9264.2020.106>
- 52 Skin grafting for penile skin loss**  
Alysen Demzik, Charles Peterson, Bradley D. Figler  
*Plast Aesthet Res* 2020;7:52 <http://dx.doi.org/10.20517/2347-9264.2020.93>
- 53 Periocular rejuvenation using hyaluronic acid fillers**  
Kasra Ziai, Jessyka G. Lighthall  
*Plast Aesthet Res* 2020;7:53 <http://dx.doi.org/10.20517/2347-9264.2020.151>
- 54 Inflammation as an orchestrator of cutaneous scar formation: a review of the literature**  
Traci A. Wilgus  
*Plast Aesthet Res* 2020;7:54 <http://dx.doi.org/10.20517/2347-9264.2020.150>
- 55 Wolfring dacryops: a case of acquired ptosis in a child**  
Sammie E. Fung, Clara J. Men, Bobby S. Korn, Don O. Kikkawa, Catherine Y. Liu  
*Plast Aesthet Res* 2020;7:55 <http://dx.doi.org/10.20517/2347-9264.2020.60>

- 56     **Current techniques in adult-acquired buried penis repair: where are we now**  
Katherine M. Theisen, Ashley V. Alford, Nicholas Kim, Joseph J. Pariser  
*Plast Aesthet Res* 2020;7:56 <http://dx.doi.org/10.20517/2347-9264.2020.83>
- 57     **Botanicals for photoprotection**  
Angeli E. Torres, Kevin M. Luk, Henry W. Lim  
*Plast Aesthet Res* 2020;7:57 <http://dx.doi.org/10.20517/2347-9264.2020.87>
- 58     **Partial flap loss in transgender phalloplasty using the anterolateral thigh or forearm - a systematic literature review**  
Isabel Cylinder, Aaron Heston, Breanna Jedrzejewski, Zbigniew Sikora, Blair Peters, Jens Urs Berli  
*Plast Aesthet Res* 2020;7:58 <http://dx.doi.org/10.20517/2347-9264.2020.85>
- 59     **Environmental aging of the skin: new insights**  
Karen E. Burke  
*Plast Aesthet Res* 2020;7:59 <http://dx.doi.org/10.20517/2347-9264.2020.154>
- 60     **Ptosis repair: external levator advancement vs. Müller's muscle-conjunctiva resection - techniques and modifications**  
Jacquelyn F. Laplant, Julia Y. Kang, Kimberly P. Cockerham  
*Plast Aesthet Res* 2020;7:60 <http://dx.doi.org/10.20517/2347-9264.2020.69>
- 61     **Metoidioplasty using labial advancement flaps for urethroplasty**  
Toby R. Meltzer, Nick O. Esmonde  
*Plast Aesthet Res* 2020;7:61 <http://dx.doi.org/10.20517/2347-9264.2020.122>
- 62     **Hyaluronic acid for lower eyelid and tear trough rejuvenation: review of the literature**  
Alberto Diaspro, Giuseppe Sito  
*Plast Aesthet Res* 2020;7:62 <http://dx.doi.org/10.20517/2347-9264.2020.143>
- 63     **Aging skin and non-surgical procedures: a basic science overview**  
Amy R. Vandiver, Sara R. Hogan  
*Plast Aesthet Res* 2020;7:63 <http://dx.doi.org/10.20517/2347-9264.2020.159>
- 64     **Microsurgical salvage of complex dorsal shearing injuries of the hand and wrist**  
Praveen G. Murthy, Adam B. Strohl  
*Plast Aesthet Res* 2020;7:64 <http://dx.doi.org/10.20517/2347-9264.2020.72>
- 65     **Strategies for innervation of the neophallus**  
Rayisa Hontscharuk, Charalampos Siotos, Loren S. Schechter  
*Plast Aesthet Res* 2020;7:65 <http://dx.doi.org/10.20517/2347-9264.2020.124>



- 66     **Review on the treatment of scars**  
Daniel J. Callaghan  
*Plast Aesthet Res* 2020;7:66 <http://dx.doi.org/10.20517/2347-9264.2020.166>
- 67     **Laser Resurfacing for the Management of Periorbital Scarring**  
Nathan Pirakitikulr, John J. Martin, Sara T. Wester  
*Plast Aesthet Res* 2020;7:67 <http://dx.doi.org/10.20517/2347-9264.2020.77>
- 68     **Surgical management of jaw-winking synkinesis and ptosis in Marcus Gunn syndrome: a systematic outcomes analysis**  
Henry Bair, Giancarlo A. Garcia, Benjamin P. Erickson  
*Plast Aesthet Res* 2020;7:68 <http://dx.doi.org/10.20517/2347-9264.2020.74>
- 69     ***Deschampsia antarctica* extract (Edafence®) as a powerful skin protection tool against the aging exposome**  
Manuel Mataix, Azahara Rodríguez-Luna, María Gutiérrez-Pérez, Massimo Milani, Alberto Gandarillas, Jesús Espada, Azahara Pérez-Davó  
*Plast Aesthet Res* 2020;7:69 <http://dx.doi.org/10.20517/2347-9264.2020.138>
- 70     **Wound repair and scarring of genital skin**  
Ursula Mirastschijski, Dongsheng Jiang, Yuval Rinkevich, Refaat Karim, Heiko Sorg  
*Plast Aesthet Res* 2020;7:70 <http://dx.doi.org/10.20517/2347-9264.2020.147>
- 71     **Botulinum toxin in facial plastic surgery**  
Nicole Favre, David Sherris  
*Plast Aesthet Res* 2020;7:71 <http://dx.doi.org/10.20517/2347-9264.2020.149>
- 72     **A review of nonsurgical facial rejuvenation**  
Stephanie E. Farber, Mathew T. Epps, Emily Brown, Julie Krochonis, Rena McConville, Mark A. Codner  
*Plast Aesthet Res* 2020;7:72 <http://dx.doi.org/10.20517/2347-9264.2020.152>
- 73     **Nonsurgical rhinoplasty using soft tissue fillers**  
Yuyang Chu, Jonathan Bacos, Sasha Becker  
*Plast Aesthet Res* 2020;7:73 <http://dx.doi.org/10.20517/2347-9264.2020.169>

Review

Open Access



# Targeting facial aging with nano and regenerative technologies and procedures

Marek Dobke, Adam Hauch

Division of Plastic and Reconstructive Surgery, Department of Surgery, University of California San Diego, San Diego, CA 92103, USA.

**Correspondence to:** Prof. Marek Dobke, Division of Plastic and Reconstructive Surgery, Department of Surgery, UC San Diego, 200 West Arbor Drive, San Diego, CA 92103-8890, USA. E-mail: mdobke@health.ucsd.edu

**How to cite this article:** Dobke M, Hauch A. Targeting facial aging with nano and regenerative technologies and procedures. *Plast Aesthet Res* 2020;7:1. <http://dx.doi.org/10.20517/2347-9264.2019.65>

**Received:** 30 Nov 2019 **First Decision:** 2 Jan 2020 **Revised:** 2 Jan 2020 **Accepted:** 7 Jan 2020 **Published:** 16 Jan 2020

**Science Editors:** John Yousif, Kai O. Kaye **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

This study reviews information about the most novel ideas and modalities being incorporated into facial and neck cosmetic care. We seek to identify trends and developments within these areas as well as perceptions of plastic surgeons regarding their probable significance, and discussion on how these modalities may impact future practice patterns. It is hypothesized that nano and regenerative technologies are considered the most “hopeful”. Emerging invasive and non-invasive modalities utilizing nano and regenerative concepts were reviewed. We intentionally sought to investigate approaches to beautification, including maintenance and reversal of signs of aging, utilizing methods lacking an established level of evidence. This included promising modalities which are currently at the investigational stage. Twelve board-certified plastic surgeons were surveyed regarding the clinical importance of twelve concepts and their expected impact on facial and neck rejuvenation. Scientific and technological creativity in aesthetics is rapidly changing, and the efficacy of innovations and safety margins are improving. Nano and regenerative medicine-based technologies and procedures were ranked most promising for the future of cosmetics. Their potential applications and research were reviewed in the context of surgical and non-surgical modalities in clinical practice. There has been an advent of new approaches to facial and neck aesthetic surgery and tissue care, which is well-beyond just skin care. With this new level of knowledge regarding variability in care responses and indications for procedures on an almost “molecular level”, personalized and precise aesthetic surgery and medicine are quickly becoming a reality.

**Keywords:** Face, neck rejuvenation, nano, regenerative technologies



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



## INTRODUCTION

Many technologies are currently available for developing effective cosmeceuticals with the goals of facial rejuvenation through procedures with varying levels of “invasiveness”. These include non-invasive and invasive (requiring anesthesia) modalities; multi-platform devices for tissue contouring, comprising skin tightening modalities, fat reduction, and/or volumization procedures; genomic/molecular-level intervention to slow down the aging process; and nano and regenerative technologies<sup>[1,2]</sup>. Molecular concepts in the field of cosmetology indicate that even seemingly distant human body components and parameters such as gut microbiome and skin quality are related<sup>[3]</sup>. Furthermore, nanorobots and/or specific nanocosmeceuticals may form micellar nanostructures capable of carrying both hydrophilic and hydrophobic agents with rapid uptake into cells. These agents can be, for example, antibiotics to treat acne or agents targeting interventions on the molecular level such as prevention of telomere attrition, thus protecting skin DNA and structural proteins from aging and loss of native growth factors<sup>[1,4]</sup>.

The goal of this review is to present and discuss current and potential future trends in facial and neck rejuvenation. In doing so, this review links current technologies with what may be considered somewhat futuristic approaches and perspectives. The authors felt that it was important to survey practicing board-certified plastic surgeons to determine how they felt about the significance of emerging trends (not only nano and regenerative), their expected prevalence and applicability to the areas of facial and neck aesthetic surgery practices in the coming decade, and to determine whether the perspective of scientific researchers is congruent with non-academic practitioners. Furthermore, the authors sought to potentially determine educational priorities among plastic surgeons.

From this analysis, the question that arises is whether the familiarity with nano and regenerative concepts is useful to a practitioner. The answer should be “yes”, as trends clearly indicate that many procedures may be combined and include approaches which may appear to be distant and esoteric today, but are clearly going to be a large part of the daily practice of facial rejuvenation in the near future. Lastly, the question arises as to why to review nanoscience and regenerative technologies in one article. The simple reason is that it appears that both are tightly intermingled and one of the primary goals of this review is to point out and comment on these links<sup>[2,5]</sup>.

## DEFINITIONS

For the purpose of this review, the following definition of nanotechnology was applied: nanoscience is the area of science, including healthcare, that deals with developing and producing extremely small devices and molecules. Nanoscale materials are classified in the range of  $10^{-9}$  m (there are 25,400,000 nanometers in an inch)<sup>[6]</sup>.

Regeneration is defined as the process of renewal, restoration, and regrowth of damaged tissue or tissue replaced in the process of natural fluctuation or events (e.g., hair). Regeneration can be either complete, where the new tissue is the same as the tissue replaced, or incomplete, where different qualities of tissue replace the original (e.g., scar formation). Aspects of the regenerative processes for nerves, bones, and skin were traditionally emphasized and studied in plastic surgery. Advances in molecular biology and empiric clinical observations provided a basis for the development of interventions utilizing the potential regenerative properties of stem cells obtained from adipose tissue and perivascular tissue components<sup>[2,7,8]</sup>.

## SAMPLING PLASTIC SURGEONS' VISIONS REGARDING TRENDS IN FACIAL PLASTIC SURGERY

A review of the literature was conducted using the following keywords as search criteria: nanotechnology, nanocosmeceuticals, and regenerative procedures for facial and neck rejuvenation. The results of this

**Table 1. Survey of plastic surgeons regarding modalities impacting the practice of facial and neck rejuvenation**

Modality rank	Score
1. Non-invasive stem cell and regenerative approaches	135
2. Molecular level interventions, e.g., prevention of telomeres attrition protecting skin DNA and structural proteins from aging, from loss of native growth factors	119
3. Improvements in percutaneous delivery of cosmeceuticals, personalized cosmetics	115
4. Genomic interventions	109
5. Tissue contouring/repair by energy delivering modalities	100
6. Refinements in body shaping by surgical techniques including tissue fillers and scaffolds	96
7. Skin resurfacing, tightening techniques (light, lasers)	69
8. Nutrition, specific, personalized diets, skin nutrients, control of obesity and malnutrition	48
9. Nanorobotic tissue rebuilding techniques	44
10. Alterations/adjustments in skin and gut microbiota	41
11. Immune system manipulations	33
12. Auto- and allo- transplantations for cosmetic purposes (other than fat transfers)	33

search were evaluated by the author and two independent reviewers (non-academic practitioners) in an attempt to identify for this review and summarize the literature on the topic in the context of relevancy to current research and practitioners' interests as affirmed by a survey of practicing board-certified plastic surgeons. These board-certified plastic surgeons participated in the survey ranking the clinical importance (current and future) of twelve issues and their predicted or expected impact on the practice of face and neck rejuvenation. Each respondent ranked the twelve categories by assigning a score "12" to the modality expected to exert the most prominent impact in descending fashion, ending on "1" given to the treatment with the least expectations. Scores were then totaled and ranked for each of the issues.

Twelve board-certified plastic surgeons were surveyed, ranking areas of current and future research and clinical developments targeting facial and neck aging which will likely have the highest impact on the practice of rejuvenation [Table 1]. The results of the survey indicate that nano and regenerative medical and surgical developments and new technologies are expected to have the largest influence moving forward and reaffirm the hypothesis reflected originally.

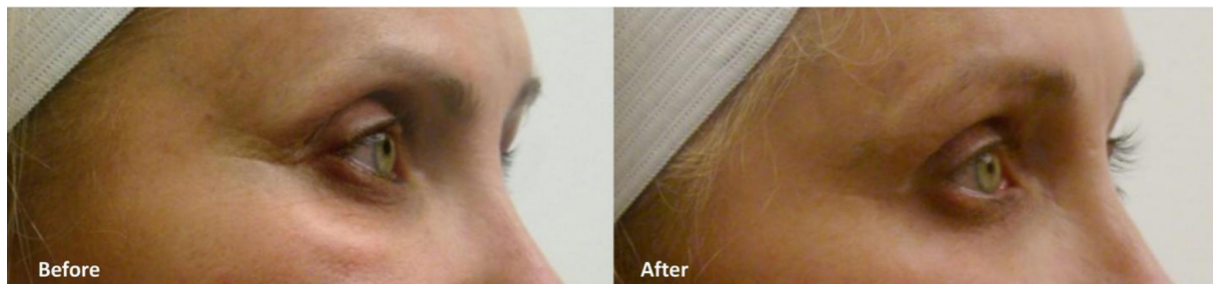
## COMBINING NANO AND REGENERATIVE TISSUE RESEARCH AND TECHNOLOGIES

Primary technologies enabling interventions at the molecular level, e.g., prevention of telomere attrition and subsequent protection of DNA and structural proteins of the skin from loss of growth factors and ultimately aging or the use of platelet-rich plasma for skin rejuvenation, are examples of applications of such emerging nanotechnology and regenerative techniques<sup>[1,4,9,10]</sup>.

Nano materials and formulations currently tested in cosmetic medicine include scaffolds for cells and fillers, agent (e.g., growth factors) delivery, applications for cellular modification, isolation and tracking, and nanodevices/robots (e.g., biosensors). Their small size and ability to enter even cell organelles as well as technologies enabling the release of active agents are key<sup>[9]</sup>. Nano systems, nano-assisted cosmetic interventions, and regenerative medicine and surgery have the potential to change facial rejuvenation. Leveraging and enhancement of endogenous stem cells and self-repair mechanisms will likely lead to the development of precision cosmetology (similar to precision medicine) by identification of signaling and effective tissue intercellular substrates and intracellular targets; and current trends, which are generally based on physician intuition and experience, will begin to disappear as palliation of signs of facial and neck aging progress<sup>[11]</sup>. Futuristically, one can imagine a synergy of nano and regenerative systems by cosmeceuticals or stem cell delivery by nanocarriers steering towards a specific target organ, e.g., face or neck, by external magnetic fields and light-triggered release of active agents<sup>[12]</sup>.



**Figure 1.** Nano technology for skin care: From “Capture Totale” antiaging cream - skin cosmeceutical utilizing nano-liposomal technology for improved transepidermal and percutaneous delivery introduced by Dior® and representing technology with moisture-loaded liposomes which reduce skin water deprivation - to “Atelokolagen” by Colway which contains sericin - capable of carrying both hydrophilic and hydrophobic agents with intracellular uptake



**Figure 2.** Crows feet skin wrinkles before and after daily application of nano-collagen for 3 weeks (Marine Collagen Biologicals, Inc., San Diego, CA). Results were less spectacular in other cases, however, perhaps due to different individual nuclear gene expression profiles and skin biology<sup>[16]</sup>

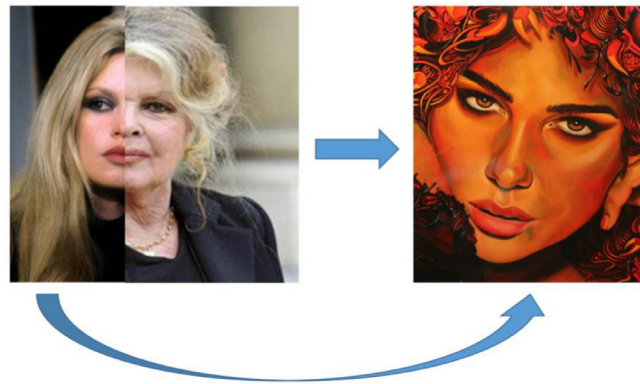
Topical and cutaneous delivery of cosmeceuticals evolved from formulations utilizing nano systems to enhance percutaneous delivery of tissue-rejuvenating agents (e.g., the first nano cream Capture Totale developed by Dior®, France) to agents with nanocarriers ensuring specific targeted extra- or intracellular cosmeceutical delivery (e.g., sericin containing Atelokolagen developed by Colway, Poland) [Figure 1]<sup>[4,13]</sup>. Certainly, variability in tissue rejuvenating techniques and agents depends not only on the modality of delivery, including surgery, but also on the pheno- and genotypic characteristics of the subject. Currently, personalized cosmetic selection to effectively target specific features is likely mostly through empiric processes which lead to the establishment of an individualized beauty and rejuvenation “regimen”<sup>[14,15]</sup> [Figure 2].

It is probably only a matter of time until personalized skin care and facial rejuvenation techniques will include interventions on a genotypic level (e.g., by shutting down unfavorable genes) or by triggering regenerative processes<sup>[16]</sup>. These beliefs and modalities were consistently demonstrated and highly ranked within the plastic surgery community<sup>[5,16]</sup>. Transcription factors activate gene supporting processes which lead to cell repair and such factors as forkhead-box protein O3a have a high capacity to preserve skin DNA in younger individuals. As with aging agents, such as sericin, which may be useful to counteract the reduction of cells’ capacity for self-repair and dermal regeneration, enhancing the targeted delivery of cosmeceuticals to tissues and even cellular organelles may ultimately reduce and/or reverse skin “decay”<sup>[1,4,5]</sup> [Figure 3].

Surveyed physicians recognized that there is a growing level of evidence that mesenchymal stem cells from adipose tissue and perivascular cellular components exert a strong potentiating effect on angiogenesis,



## Can we slow down or even reverse aging?

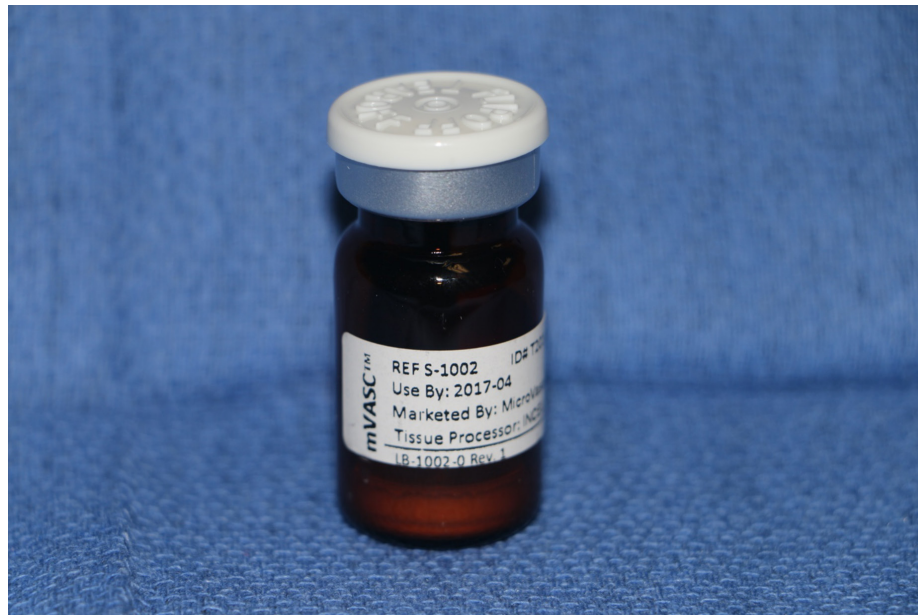


**Figure 3.** How far are we willing to go to alter, edit, or prevent decay of the skin and/or body genome? Are we able to slow down aging (lower arrow) or reverse skin decay that has already taken place (upper arrow)?

and that they stimulate the regenerative ability of skin by maintaining stem cells that are present in the skin or by assuming the role of native cells upon injection/deposition. Signaling pathways for cellular interactions within the skin and their “controls” appear to be at the core for the maintenance of youthful facial and neck tissues or for the reversal of the loss of a smooth skin surface, dermal cohesion, and/or firmness<sup>[2,8]</sup>. One of the already existing and commercially available systems (SkinMedica TNS Recovery Complex®, SkinMedica®, An Allergan Company, Anaheim, CA), capable of upregulation of extracellular matrix regeneration controlling genes, exemplifies the feasibility and effectiveness of skin rejuvenation interventions at a molecular level<sup>[17]</sup>.

Technical refinements of tissue replacement by fat grafting are based on at least level III evidence, and they include such factors as the physical form of transferred fat, ratios between the vascularized recipient site and surface/volume of the adipose tissue graft, and the proportion between the stroma and adipocytes<sup>[18]</sup>. Adipose-based tissue graft volumization in combination with customized laser-based skin resurfacing may lead to marked improvements in facial rejuvenation outcomes, as it appears that both modalities may augment specific individual effects<sup>[7]</sup>. Potentiation of the viability, and ultimately the retention rate of the adipose cell grafts by the stromal vascular fraction (including enhancements by the addition of stem cells obtained by culturing), may require more than one operative step. For example, obtaining extra stem cell tissue cultures for 2-3 weeks may be required prior to the addition of stem cells to newly procured adipose tissue. Increased ratio between stem cells (or stroma) and the adipose cells may enhance composite autograft potential, but the entire procedure requires two steps (harvest of adipose tissue to obtain stroma and produce stem cells and procurement of fat to be enriched with cultured stem cells prior to usage)<sup>[19]</sup>.

Therefore, from the standpoint of practicality in cosmetic surgery, tissue regeneration and/or enhancing products which can be stored and “taken off the shelf” - just as many synthetic tissue fillers are - is of particular interest<sup>[8,20,21]</sup>. For example, the mVASC (Microvascular Tissues, Inc., San Diego, CA) product, which consists of aseptically-processed and freeze-dried allogeneic microvascular tissue derived from donated tissue, requires less than one minute to prepare for use. This is in contrast to procedures involving commercially available closed systems for isolating platelet rich plasma, which typically require approximately 30 min to obtain<sup>[10]</sup>. The proprietary mVASC manufacturing process eliminates the proliferative ability of cells within the graft as well as its immunogenicity. The resultant injectable tissue graft (which can also be applicable as a topical agent, e.g., to senescent wounds) is stable at room temperature for four years<sup>[20]</sup> [Figure 4]. Ongoing trials indicate enormous angiogenic potential of mVASC through upregulation of signaling cascades resulting in tissue remodeling, regeneration, rejuvenation of



**Figure 4.** A vial of mVASC with the lyophilized and sterilized pellet containing microvascular tissue fragments. The standardized formulation of mVASC contains an amount of the graft dispersed into one vial such that is ready for dilution, and forms isotonic solution upon the addition of 1 mL of sterile water

its architecture, and thickness, which - no doubt - will find future aesthetic applications because results of ongoing trials are so encouraging<sup>[8,20]</sup>.

## CONCLUSION

Notably, the survey demonstrated that such modalities as tissue contouring by energy delivering devices, refinements in surgical techniques, alterations in skin and gut microbiota, and transplantation-based (other than adipose tissue) methods were perceived as methods that exert a lesser effect on the future management of facial and neck aging than those “top modalities” including nano and regenerative techniques<sup>[16,19,20]</sup> [Table 1].

Considering the relatively fast-paced changes and progress in cosmetic surgery and medicine, as well as the novelties that are frequently thrown on the market - oftentimes without prior rigorous clinical studies or trials - suggests that words of caution are appropriate. Recipients of cosmetic care are particularly vulnerable due to the fact that, in many countries, cosmeceuticals are not regulated as rigorously as pharmaceutical drugs, and there is also the risk of addiction to aesthetic procedures. Patients may develop a psychological obsession with repeated or multiple cosmetic procedures and may obsessively seek invasive and non-invasive aesthetic novelties. These patients lose objectivity and criticism, and oftentimes become victims of unscrupulous providers. Even those who observe safety issues may not appreciate the importance of the fact that there is a separate categorization of the quality of evidence and strength of recommendations available<sup>[22,23]</sup>. Failure to recognize the quality of evidence can lead to misguided recommendations. The assessment of trends and developments in facial and neck rejuvenation requires particular attention to the quality of research available prior to its clinical application, as emerging evidence includes an element of uncertainty about tradeoffs - the balance among safety and desirable and undesirable effects is not well-defined. With the inevitability of scientific progress, this new level of knowledge regarding the variability in care responses and indications for procedures on almost a “molecular level” will likely lead to personalized and precise aesthetic surgery and medicine becoming a nearby reality.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Dobke M, Hauch A

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Lohani A, Verma A, Joshi H, Yadav N, Karki N. Nanotechnology-based cosmeceuticals. *ISRN Dermatol* 2014;2014:843687.
2. Gaur M, Dobke M, Lunyak VV. Mesenchymal stem cells from adipose tissue in clinical applications for dermatological applications and skin aging. *Int J Mol Sci* 2017;18:1-29.
3. Dobke M, Sikora NC, Vargas F. Skin aging and beauty exploring the gut microbiota connection. *J Aesth Rec Surg* 2019;5:1-3.
4. Mandal BB, Kundu SC. Self-assembled silk sericin/poloxamer nanoparticles are nanocarriers of hydrophobic and hydrophilic drugs for targeted delivery. *Nanotechnology* 2009;20:355101.
5. Goldfaden R, Goldfaden G. Target wrinkle formation with novel peptides. *Life Extension* 2014. Available from: <https://www.lifeextension.com/magazine/2014/4/target-wrinkle-formation-with-novel-peptides> [Last accessed on 10 Jan 2020]
6. Limongi T, Canta M, Racca L, Ancona A, Tritta S, et al. Improving dispersal of therapeutic nanoparticles in the human body. *Nanomedicine (Lond)* 2019;14:797-801.
7. Cohen SR, Womack H. Injectable tissue replacement and regeneration: anatomic fat grafting to restore decayed facial tissues. *Plast Reconstr Surg Glob Open* 2019;7:1-11.
8. Terlizzi V, Kolibabka M, Burgess JK, Hammes HP, Hamsen MC. The pericytic phenotype of adipose tissue-derived stromal cells is promoted by NOTCH2. *Stem Cells* 2018;36:240-51.
9. Prow TW, Grice JW, Lin LL, Faye R, Butler M, et al. Nanoparticles and microparticles for skin drug delivery. *Adv Drug Deliv Rev* 2011;63:470-91.
10. Samadi P, Sheykhasan M, Khoshinani HM. The use of platelet-rich plasma in aesthetic and regenerative medicine: a comprehensive review. *Aesthetic Plast Surg* 2019;43:803-14.
11. Verma S, Domb A, Kumar N. Nanomaterials for regenerative medicine. *Nanomedicine (Lond)* 2011;6:157-81.
12. Jin Z, Ngyuen KT, Go G, Kang B, Min HK, et al. Multifunctional nanorobot systems for active therapeutic delivery and synergistic chemo-photothermal therapy. *Nano Lett* 2019;19:8550-64.
13. Dominguez S, Mackert GA, Dobke M. Nanotechnology to enhance transdermal delivery of hydrophilic humectants for improved skin care: a model for therapeutic applications. In: Andronescu E, Grumezescu AM, editors. *Nanostructures for drug delivery*. Amsterdam: Elsevier; 2017. pp. 919-39.
14. Sikora NC, Dobke M. Postmenopausal skin aging - exploring the significance of estrogen-gut microbiota axis. *JJ Expt Derm* 2019;3:1-4.
15. Dobke M, Sikora NC, Vargas F. Skin aging and beauty exploring the gut microbiota connection. *J Aesthet Reconstr Surg* 2019;5:1-3.
16. Velmeshev D. Single-cell genomics in disease research and diagnostics. *Clin Lab Manager* 2019;4:18-21.
17. Kadoya K, Makino ET, Jiang LI, Bachelor M, Chung R, et al. Upregulation of extracellular matrix genes corroborates clinical efficacy of human fibroblast-derived growth factors in skin rejuvenation. *J Drugs Dermatol* 2017;16:1190-6.
18. Cohen SR, Womack H. Injectable tissue replacement and regeneration: anatomic fat grafting to restore decayed facial tissues. *Plast Reconstr Surg Glob Open* 2019;7:e2293.

19. Park JY. Stem cell fat graft. In: Liposuction. Singapore; Springer; 2018. pp. 253-74.
20. Dobke M, Berger JS. Allogeneic microvascular tissue graft restores healing capacity in recalcitrant cleft palate fistula: a case report. *J Den Max Surg* 2019;2:164-70.
21. Raper V. Gene therapies are being brought to a wider audience. *Genet Eng Biotechnol News* 2019;39:7-9.
22. Guyatt GH, Oxman AD, Vist GE, Kunz R, Falck-Ytter Y, et al. GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. *BMJ* 2008;336:924-6.
23. Leask F. Spotting 'unproven' stem cell therapies in the wild. *Biotechniques* 2019;67:253-5.

Review

Open Access



# Face transplantation for massive mandibular defects: considerations for allograft design and surgical planning

William Jackson Palmer<sup>1</sup>, Laurel Nelms<sup>2</sup>

<sup>1</sup>Boston University School of Medicine, Boston, MA 02118, USA.

<sup>2</sup>University of California Riverside School of Medicine, Riverside, CA 92521, USA.

**Correspondence to:** Mr. William Jackson Palmer, Boston University School of Medicine, 72 E. Concord St., Boston, MA 02118, USA.  
E-mail: wjpalmer@bu.edu

**How to cite this article:** Palmer WJ, Nelms L. Face transplantation for massive mandibular defects: considerations for allograft design and surgical planning. *Plast Aesthet Res* 2020;7:2. <http://dx.doi.org/10.20517/2347-9264.2019.34>

**Received:** 2 Oct 2019 **First Decision:** 30 Dec 2019 **Revised:** 7 Jan 2020 **Accepted:** 13 Jan 2020 **Published:** 20 Jan 2020

**Science Editor:** Ali-Farid Safi **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

Modern face transplant techniques have advanced to allow for the transfer of vascularized skeletal components in addition to overlying soft tissue. This represents significant opportunity for individuals with mandibular defects that are not amenable to traditional reconstruction. Care must be taken when planning and executing transplants with these complex grafts, as satisfactory functional and aesthetic outcomes rely on achieving proper spatial relationships between the mandible, skull base, and midface. Which donor skeletal elements are included in the allograft and how they are harvested are important considerations in this planning and are associated with controversy. To optimize outcomes in the reconstruction of single-jaw defects, some advocate for transplantation of only the affected jaw while others support bimaxillary transplantation. Clinical evidence in this debate is not conclusive at this time. In current practice, including donor dentoalveolar anatomy by utilizing a bilateral sagittal split osteotomy of the mandible is favored to optimize outcomes such as dental occlusion. It has been suggested that harvesting the mandible at the level of the condyle or even the temporal bone may also be possible and may improve temporomandibular joint-related outcomes. Despite encouraging preclinical evidence, these strategies remain controversial. After allograft design, successful mandibular reconstruction with face transplantation relies on surgical precision in the donor and recipient procedures. Computerized surgical planning, computer-aided design and manufacturing, and intraoperative navigation are technologies currently in use to mitigate operative complexity. Results in both cadaveric and clinical face transplantations suggest these technologies are reliable and beneficial, although some room for improvement remains.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Keywords:** Face transplantation, mandibular reconstruction, allograft design, bimaxillary transplantation, temporomandibular joint reconstruction, computerized surgical planning, computer-aided design and manufacturing, intraoperative navigation

## INTRODUCTION

Face transplantation offers significant aesthetic and functional improvements for patients with devastating injuries that cannot be managed with conventional reconstruction<sup>[1-3]</sup>. Including the first procedure performed in 2005, 44 face transplantations have been reported in the scientific literature to date<sup>[2,4]</sup>. Successful transplantations have demonstrated the feasibility of this procedure and paved the way for technique refinement and increased operative complexity<sup>[3,4]</sup>. Surgeons are now able to include vascularized bone in addition to soft tissue in facial allografts<sup>[4,5]</sup>. The ability to incorporate varying amounts of donor facial skeleton allows the development of grafts that are customized to a patient's individual defect and reconstructive goals<sup>[2,5]</sup>. While many autologous reconstructive options for the mandible exist, they may fail to correct severe defects in some patients<sup>[6]</sup>. Modern face transplantation therefore represents a powerful tool for surgeons seeking to reconstruct massive facial defects involving the mandible.

Use of osteomyocutaneous allografts in this setting requires thorough planning and meticulous execution. To improve facial aesthetics and mandibular function, transplantation must not only replace missing or defective mandible; it is also necessary to restore proper spatial relationships between the mandible and other skeletal elements including the midface and skull base<sup>[4,7]</sup>. Establishing these relationships starts with thoughtful allograft planning based on an understanding of how the skeletal components included in the graft and the locations of osteotomies and osteosyntheses will influence functional outcomes. Precision in the subsequent steps of allograft harvest and inset is challenging and imperative. In many cases, recipients have endured injuries and reconstructive procedures that can disfigure anatomy and make graft alignment difficult<sup>[7,8]</sup>. Furthermore, when the recipient's defect warrants an allograft including both the midface and mandible, attachment of the graft is complicated by the paucity of recipient landmarks other than the skull base and the potential for misalignment in three dimensions<sup>[4,9]</sup>. Observed outcomes have reflected the procedural challenges, as transplants involving the maxilla and mandible have been associated with trismus, malocclusion, and impaired airway function, for example<sup>[4,7]</sup>. To avoid these functional complications and best restore this critical anatomy, transplants involving the mandible require detailed planning and precise execution<sup>[4]</sup>.

This review seeks to define some of the important considerations in the surgical planning of facial allografts involving the mandible as well as to highlight some of the emerging technologies available to optimize patient outcomes in these cases.

## DESIGNING THE MANDIBLE-CONTAINING ALLOGRAFT

One of the earliest considerations in planning for facial transplantation to correct a mandibular defect is how much donor skeleton to include in the allograft. Given the goal of restoring function and appearance, it follows that, in the case of patients with defects involving both the maxilla and mandible, reconstruction with an allograft including both of these skeletal components is most appropriate<sup>[2,10]</sup>. Determining the extent of donor bone inclusion is more controversial in the case of patients with defects limited to only the mandible. In such circumstances, some advocate for transplantation of only the affected jaw while others support bimaxillary transplantation<sup>[4,11,12]</sup>.

In 2011, Gordon *et al.*<sup>[13,14]</sup> introduced through cadaveric studies the concept of “hybrid occlusion”, or the occlusal relationship achieved after allotransplantation between the donor maxilla and recipient's



native mandible. The concept of hybrid occlusion has since been extended to include cases where the native dentoalveolar structure is the maxilla, and the mandible is being transplanted<sup>[4,10]</sup>. Single-jaw transplantation resulting in hybrid occlusion has been utilized extensively in clinical cases of face transplantation<sup>[11]</sup>. Proponents of this technique argue that poor occlusive outcomes may arise with single-jaw as well as with bimaxillary transplantation, but only the more conservative approach accounts for the risk of graft failure by preserving functional recipient anatomy<sup>[12,15]</sup>. Furthermore, these individuals suggest that occlusion can be improved with subsequent orthognathic and orthodontic interventions<sup>[12]</sup>. Those advocating for bimaxillary transplantation in cases of single-jaw defects cite the importance of adequate postoperative occlusion in restoring vital patient functions such as mastication and speech and suggest that preserving the donor occlusal relationship may optimize these outcomes<sup>[4,11]</sup>. Additionally, by obviating the need to find a donor jaw that properly fits the recipient's native jaw anatomy, bimaxillary transplantation may expand the possible donor pool<sup>[11]</sup>. No studies have directly compared single-jaw with bimaxillary transplantation, and the number of patients receiving these grafts is still relatively small; at this point, arguments for one strategy over the other based on functional outcomes remain experiential and speculative<sup>[4,11]</sup>.

In both mandible-only and bimaxillary transplantation, the next consideration is where to dissect the mandible. The earliest facial allotransplantation to utilize donor mandible included only the chin<sup>[16]</sup>. Subsequent procedures included donor mandible from angle to angle, which is now a common practice<sup>[16,17]</sup>. The bilateral sagittal split osteotomy of the mandible, a common procedure in traditional orthognathic surgery that involves splitting the mandible posterior to the alveolar process, has been widely incorporated into facial transplantation because it offers maximal donor-to-recipient bony contact<sup>[8,18]</sup>. Furthermore, this technique is favored over including only a portion of the mandibular tooth bed because incorporation of the entire donor alveolar structure is thought to better allow establishment of occlusion<sup>[10,18]</sup>. The exact path of bilateral sagittal split osteotomy can be customized for an individual recipient by using computer surgical planning techniques that are discussed below.

An exciting concept in the field currently is the prospect of transplanting the temporomandibular joint (TMJ) along with the mandible. Individuals with injuries warranting consideration of facial transplantation often have significant impairment of the TMJ<sup>[19]</sup>. Traumatic injuries themselves can cause scarring and other articular pathologies, and reconstructive efforts may similarly contribute to or exacerbate these conditions through scarring, reduction of jaw mobility, and subsequent muscle shortening<sup>[19]</sup>. Together, jaw injury and surgery may significantly impact a patient's quality of life by imposing difficulties opening the mouth, chewing, and speaking<sup>[19]</sup>. Donor mandibles void of articular anatomy, such as those prepared with the bilateral sagittal split osteotomy, do not alone address the potential for TMJ dysfunction in this population<sup>[16]</sup>. While it is possible to identify and treat TMJ pathology before facial transplantation, clinical evidence in the face transplant population suggests this strategy does not offer a definitive solution<sup>[4,19]</sup>. This fact is further complicated by the observation in at least one facial transplant candidate that TMJ impairments may not be apparent on preoperative imaging<sup>[19]</sup>. In cases where jaw dysfunction persists postoperatively, secondary revisions such as coronoidectomy and condylectomy are viable options that have been described in the literature with acceptable results<sup>[4,16,20]</sup>. In any case, transplantation with an allograft including the TMJ represents an alternative treatment option for these patients that may eventually offer comparable or superior functional outcomes and the possibility of fewer revision surgeries<sup>[16]</sup>.

The feasibility of TMJ transplantation and associated outcomes are still being elucidated. To date, there has been one case reported in the scientific literature of unilateral mandibular condyle inclusion in a facial allograft<sup>[6,16]</sup>. The patient in this case received a graft including donor mandible from right angle to left condyle to treat a large mandibular defect and known left TMJ ankylosis secondary to radiation therapy<sup>[6,16]</sup>. The transplantation was successful, and, although the patient demonstrated only 10 mm of mandibular

excursion postoperatively, this was attributed to prior right TMJ injury<sup>[6,16]</sup>. No facial transplantation reported to date has included both mandibular condyles, and, accordingly, the feasibility and practicality of this procedure remain unclear<sup>[4,16]</sup>. For adequate postoperative jaw function in the case of allografts including both donor mandibular condyles, the transplanted segment must align with the recipient's glenoid fossae in a way that enables functional articulation<sup>[16]</sup>. It follows that this may make donor selection burdensome, as mandible morphology including intercondylar distance is known to vary significantly between individuals<sup>[4,16]</sup>. However, preclinical evidence has been encouraging. Khavanin *et al.*<sup>[16]</sup> utilized the computed tomography (CT) scans of 100 adults to evaluate the viability of bilateral condylar transplant and concluded that the procedure would be anatomically feasible and clinically practical in most cases given adequate average interglenoid widths and the fact that the glenoid fossa itself is wider than the mandibular condyle<sup>[4]</sup>. It should be noted that this study excluded individuals with mandibular trauma or defects<sup>[16]</sup>. Candidates for mandibular condyle transplantation may have anatomical changes in the glenoid region that impact the feasibility of accepting a donor condyle. More targeted research is warranted. Bilateral condyle transplantation has also been challenged on the grounds that violating the TMJ's supportive connective tissue may result in complications such as ankylosis or joint instability<sup>[4]</sup>. As a potential solution to these concerns, it has been suggested that the entire TMJ including donor temporal bone could be transplanted, although this would increase procedural complexity and operative time<sup>[16]</sup>. Additionally, the vascular anastomoses necessary to support this anatomy have not been demonstrated, and until outcomes of these procedures are reported, suggesting the superiority of one method over the other remains speculation<sup>[4,16]</sup>. With further characterization, transplanting the TMJ along with the mandible may become a valuable reconstructive modality for individuals with severe mandibular defects and impairments<sup>[16]</sup>.

## OPTIMIZING SURGICAL PRECISION

Once the skeletal components of the allograft and the general osteotomy locations have been determined, two operative procedures must be completed: the donor harvest and the recipient inset<sup>[8]</sup>. Both of these procedures are technically challenging and time-intensive<sup>[8,21]</sup>. The donor harvest demands a thorough understanding of the recipient's defect and an appropriate surgical plan; otherwise, the surgical team would be faced with the time-consuming and error-prone challenge of adjusting the allograft to fit the recipient during transplantation<sup>[7,21]</sup>. The recipient procedure, including preparation and allograft attachment, requires similar precision to yield proper spatial relationships between regional anatomy, a crucial factor in the restoration of aesthetics and functional parameters such as dental occlusion<sup>[5,7,8,22-24]</sup>. Achieving proper alignment between the allograft and the recipient's native anatomy may be particularly challenging in face transplantation, where devastating injuries and previous reconstructive efforts can mar regional anatomy<sup>[7,8]</sup>. To address these challenges, surgical teams have begun incorporating computer-based technology, including computerized surgical planning (CSP), computer-aided design and manufacturing (CAD/CAM), and intraoperative navigation<sup>[4,8,21]</sup>.

Prior to their use in facial transplantation, these computer-aided techniques demonstrated promise in other craniomaxillofacial applications<sup>[8]</sup>. With this technology, preoperative CT images can be rendered in three dimensions in a virtual workspace, allowing the user to develop a surgical plan based on virtual manipulation and measurement of the patient's anatomy<sup>[22]</sup>. This technology obviates the need to design a three-dimensional plan off of two-dimensional images<sup>[22]</sup>. Tangible stereolithographic models may also be produced from these virtual representations to further aid in operative planning<sup>[22]</sup>. In the case of fibular free flaps for midface or mandibular reconstruction, for instance, the osteotomies can be designed virtually, corresponding measurements can be used to produce stereolithographic models of these skeletal elements (a technique termed CAD/CAM), and reconstructive hardware can be pre-formed to these patient-specific models prior to surgery<sup>[24]</sup>. The virtual measurements can also be used to design patient-specific cutting guides that facilitate accurate osteotomies in the operating room<sup>[22]</sup>. Advancing virtual reality technology and the incorporation of haptic feedback devices into virtual workstations promise to

make the surgical planning process even more immersive, realistic, and accurate<sup>[25]</sup>. Virtual rendering of patient images has also offered intraoperative and postoperative utility. Intraoperative navigation, which uses a portable localizer and patient CT scans to show the surgeon the real-time position of an instrument, has demonstrated the ability to accurately locate anatomy and surgical tools within a small margin of error<sup>[22]</sup>. This strategy may be particularly useful in cases of structures that are challenging to visualize intraoperatively such as parts of the mandible and the base of the skull<sup>[22]</sup>. Augmented reality, which allows for the projection of computer-generated images onto the surgical field in real time, is another promising computer-based technology that has been applied in various areas of maxillofacial surgery to improve intraoperative navigation in challenging anatomical scenarios<sup>[25,26]</sup>. Postoperatively, three-dimensional rendering of CT scans can be compared via virtual superimposition on surgical plans to confirm results and evaluate fidelity to the preoperative plan<sup>[21,22]</sup>. These computer-aided tools have reduced surgical time and improved precision in multiple craniomaxillofacial applications, making them an attractive technology for face transplantation<sup>[8,24]</sup>.

Jacobs *et al.*<sup>[21]</sup> proposed and validated through cadaveric transplantation a planning protocol using CSP and CAD/CAM technologies that reflects their use in the face transplantation field at large and depicts their utility. While this team focused on allografts without mandible, their principles have implications for mandibular reconstruction as accurate handling of the maxilla is necessary for the establishment of a functional maxillomandibular relationship<sup>[21]</sup>. Furthermore, several teams have used similar protocols with allografts including varying amounts of donor mandible<sup>[8,9,27]</sup>. First, using preoperative CT images rendered in three dimensions, the recipient's defect is defined<sup>[21]</sup>. In the virtual workspace, the donor rendering can then be superimposed on the recipient in order to plan appropriate osteotomies<sup>[21]</sup>. Jacobs *et al.*<sup>[21]</sup> advocate for first virtually establishing optimal donor-to-recipient bony relationships including occlusion and then using the resulting model to design osteotomy paths that will yield these exact relationships. Other teams have since suggested that the osteotomies be defined in a way that preserves functional soft tissue<sup>[27]</sup>. In either case, once the osteotomy is depicted virtually, custom cutting guides based on these models can be manufactured to enable efficient and precise osteotomies designed specifically to establish these predetermined anatomical relationships<sup>[21,27]</sup>. In the case of single-jaw transplantation, a custom dental splint can also be prepared based on the virtual model to further aid in establishment of proper occlusion intraoperatively<sup>[21]</sup>. In cases of bimaxillary transplantation where the landmark of a native recipient jaw is absent, the donor jaws can be placed in intermaxillary fixation preoperatively for a similar effect<sup>[4,8,9]</sup>. Finally, the skeletal aspects of the transplantation procedure can be conducted in the virtual workspace to assess outcomes and refine the plan as necessary<sup>[21]</sup>.

Using the postoperative overlay analysis described previously, Jacobs *et al.*<sup>[21]</sup> found that the surgical result of their midface transplantation differed from the virtual plan by less than 5 mm. In a series of seven cadaveric transplantations including a portion of the mandible, Sosin *et al.*<sup>[27]</sup> utilized CSP and CAD/CAM and also demonstrated close adherence of the postoperative results to preoperative plans. This team also found that grafts prepared and attached based on these virtual plans did not require time-consuming *ad hoc* intraoperative adjustments<sup>[27]</sup>. This corroborates the idea that virtual surgical plans can be reproduced reliably in the operating room with a time-saving benefit<sup>[27]</sup>.

Intraoperative applications of computer-based technologies have also been validated through cadaveric face transplant models. As mentioned above, computer-based strategies that enable precise alignment of skeletal components may be particularly useful in face transplants that include both the midface and mandible, as recipients in these cases may lack obvious landmarks to guide graft attachment<sup>[9]</sup>. Brown *et al.*<sup>[8]</sup> preformed 10 bimaxillary face transplantations based on CSP principles. In addition to using CSP to plan the osteotomies and manufacture cutting guides, the team utilized a stereolithographic model of the recipient as well as intraoperative navigation during osteotomies and graft inset<sup>[8]</sup>. The stereolithographic model

was constructed from a representation of the recipient after virtual osteotomy; in other words, the model represented the recipient's facial skeleton prior to graft inset<sup>[8]</sup>. During the donor harvest, the proposed allograft was aligned to this stereolithographic model before severing the graft's supporting vasculature<sup>[8]</sup>. This technique allowed the surgical team to optimize the fit and adapt surgical hardware in advance of ischemia time<sup>[8]</sup>. The team demonstrated no significant difference in cephalometric variables including occlusal plane angles between the virtual surgical plans and the postoperative results<sup>[8]</sup>. Postoperative occlusion was also successfully achieved<sup>[8]</sup>. Dorafshar *et al.*<sup>[9]</sup> demonstrated similarly encouraging results in their study of five bimaxillary cadaver transplants conducted with donor osteotomies under intraoperative navigation and recipient osteotomies facilitated by custom-manufactured cutting guides. This team found that the postoperative results closely mirrored the computer-designed surgical plan in five of the six axes of movement with significant differences only appearing in lateral translation<sup>[9]</sup>.

Fidelity to the virtual surgical plan has also been demonstrated in several clinical face transplantations. Sosin *et al.*<sup>[28]</sup> successfully performed a facial transplantation including the genial segment of the mandible with CSP and CAD/CAM and noted postoperative results in close agreement with their virtual plan. Dorafshar *et al.*<sup>[18]</sup> utilized cutting guides, intraoperative navigation, and donor alignment to a stereolithographic model of the recipient in their double-jaw transplantation with high fidelity to the virtual plan and postoperative maintenance of occlusion. Dorafshar *et al.*<sup>[9]</sup> also followed their five cadaveric transplants with a clinical transplant employing the same protocol and noted similar postoperative results between the two groups. More recently, Ramly *et al.*<sup>[4]</sup> reported two bimaxillary transplants facilitated by CSP, CAD/CAM, and intraoperative navigation that both resulted in adherence to the virtual surgical plan and class I occlusion following transplantation. Together, these results support the feasibility of conducting face transplantation with computer-aided strategies and achieving reliable postoperative results. As these surgical plans are tailored to the reconstructive goals of each recipient-donor pair, it follows that technology enabling the accurate reproduction of these plans should optimize patient outcomes<sup>[7]</sup>.

While evidence to date supports the role of CSP and other computer-based strategies in maximizing operative efficiency and optimizing postoperative face transplantation results, there remains room for improvement<sup>[5]</sup>. The evidence provided by Dorafshar *et al.*<sup>[9]</sup> serves as a reminder that a degree of infidelity between the virtual plan and the postoperative results exists. These authors suggest that fidelity to the virtual plan may improve as teams become more comfortable with CSP strategies but that the surgeon will likely still be required to make decisions based on intraoperative observations, particularly in cases of challenging anatomy<sup>[9]</sup>. It is also important to understand that while encouraging, fidelity to a virtual plan does not necessarily result in enduring spatial relationships. Current CSP technology is not equipped to accurately account for the influence of recipient musculature on postoperative allograft positioning<sup>[4,5,11]</sup>. In the two clinical bimaxillary transplants reported by Ramly *et al.*<sup>[4]</sup>, both patients developed malocclusion during their recoveries despite the class I occlusion observed after transplantation. One of these patients also subsequently required mandibular coronoidectomy to improve mandibular mobility<sup>[4]</sup>. Improved CSP software that can predict dynamic bone-to-bone relationships in the context of postoperative muscle recovery may help to improve long-term occlusive and TMJ outcomes<sup>[4]</sup>.

## CONCLUSION

Face transplantation techniques have evolved to allow for the inclusion of vascularized bone in the donor allograft. For individuals with large, complex facial defects involving the mandible, these grafts represent a valid and important option for reconstruction when other strategies fail. Thoughtful allograft design, thorough surgical planning, and precise execution of these procedures are necessary to ensure proper postoperative relationships between the mandible and the maxilla as well as the mandible and the skull base. By establishing proper relationships between these skeletal elements, surgeons can restore the aesthetics and functionalities lost with massive mandibular injury. Transplantation of the TMJ and

computer-aided planning, manufacturing, and navigation are emerging technologies that, with deeper understanding and continued refinement, appear to promise these patients improved outcomes.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design, analysis, interpretation, and preparation of the review and manuscript: Palmer WJ

Assisted with manuscript preparation, as well as provided administrative, technical, and material support: Nelms L

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Fischer S, Lee TC, Krezdorn N, Alhefzi M, Kueckelhaus M, et al. First lower two-thirds osteomyocutaneous facial allograft perfused by a unilateral facial artery: outcomes and vascularization at 1 year after transplantation. *Plast Reconstr Surg* 2017;139:1175e-83e.
2. Pomahac B, Nowinski D, Diaz-Siso JR, Bueno EM, Talbot SG, et al. Face transplantation. *Curr Probl Surg* 2011;48:293-357.
3. Infante-Cossio P, Barrera-Pulido F, Gomez-Cia T, Sicilia-Castro D, Garcia-Perla-Garcia A, et al. Current role in facial allograft transplantation: what have we learned? *Plast Aesthet Res* 2016;3:211-8.
4. Ramly EP, Kantar RS, Diaz-Siso JR, Alfonso AR, Shetye PR, et al. Outcomes after tooth-bearing maxillomandibular facial transplantation: insights and lessons learned. *J Oral Maxillofac Surg* 2019;77:2085-103.
5. Rifkin WJ, David JA, Plana NM, Kantar RS, Diaz-Siso JR, et al. Achievements and challenges in facial transplantation. *Ann Surg* 2018;268:260-70.
6. Cavadas PC, Ibáñez J, Thione A. Surgical aspects of a lower face, mandible, and tongue allotransplantation. *J Reconstr Microsurg* 2012;28:43-7.
7. Plana NM, Malta Barbosa J, Diaz-Siso JR, Brecht LE, Rodriguez ED. Dental considerations and the role of prosthodontics and maxillofacial prosthetics in facial transplantation. *J Am Dent Assoc* 2018;149:90-9.
8. Brown EN, Dorafshar AH, Bojovic B, Christy MR, Borsuk DE, et al. Total face, double jaw, and tongue transplant simulation: a cadaveric study using computer-assisted techniques. *Plast Reconstr Surg* 2012;130:815-23.
9. Dorafshar AH, Brazio PS, Munding GS, Mohan R, Brown EN, et al. Found in space: computer-assisted orthognathic alignment of a total face allograft in six degrees of freedom. *J Oral Maxillofac Surg* 2014;72:1788-800.
10. Mohan R, Borsuk DE, Dorafshar AH, Wang HD, Bojovic B, et al. Aesthetic and functional facial transplantation: a classification system and treatment algorithm. *Plast Reconstr Surg* 2014;133:386-97.
11. Khalifian S, Brazio PS, Mohan R, Shaffer C, Brandacher G, et al. Facial transplantation: the first 9 years. *Lancet* 2014;384:2153-63.
12. Wall A, Bueno E, Pomahac B, Treister N. Intraoral features and considerations in face transplantation. *Oral Dis* 2016;22:93-103.
13. Gordon CR, Susarla SM, Peacock ZS, Kaban LB, Yaremchuk MJ. Le Fort-based maxillofacial transplantation: current state of the art and a refined technique using orthognathic applications. *J Craniofac Surg* 2012;23:81-7.
14. Gordon CR, Susarla SM, Peacock ZS, Cetrulo CL, Zins JE, et al. Osteocutaneous maxillofacial allotransplantation: lessons learned from a novel cadaver study applying orthognathic principles and practice. *Plast Reconstr Surg* 2011;128:465e-79e.

15. Pomahac B, Bueno EM, Sisk GC, Pribaz JJ. Current principles of facial allotransplantation: the Brigham and Women's Hospital Experience. *Plast Reconstr Surg* 2013;131:1069-76.
16. Khavanin N, Davidson EH, Lee DY, Byrne P, Dorafshar AH. Anatomic considerations for temporomandibular joint vascularized composite allotransplantation. *J Craniofac Surg* 2018;29:871-7.
17. Lantieri L, Hivelin M, Audard V, Benjoar MD, Meningaud JP, et al. Feasibility, reproducibility, risks and benefits of face transplantation: a prospective study of outcomes. *Am J Transplant* 2011;11:367-78.
18. Dorafshar A, Bojovic B, Christy M, Borsuk D, Iliff N, et al. Total face, double jaw, and tongue transplantation: an evolutionary concept. *Plast Reconstr Surg* 2013;131:241-51.
19. Krezdorn N, Alhefzi M, Perry B, Aycart MA, Tasigiorgos S, et al. Trismus in face transplantation following ballistic trauma. *J Craniofac Surg* 2018;29:843-7.
20. Aycart MA, Alhefzi M, Kueckelhaus M, Krezdorn N, Bueno EM, et al. A retrospective analysis of secondary revisions after face transplantation: assessment of outcomes, safety, and feasibility. *Plast Reconstr Surg* 2016;138:690e-701e.
21. Jacobs JMS, Dec W, Levine JP, McCarthy JG, Weimer K, et al. Best face forward: virtual modeling and custom device fabrication to optimize craniofacial vascularized composite allotransplantation. *Plast Reconstr Surg* 2013;131:64-70.
22. Bell RB. Computer planning and intraoperative navigation in cranio-maxillofacial surgery. *Oral Maxillofac Surg Clin North Am* 2010;22:135-56.
23. Caterson EJ, Diaz-Siso JR, Shetye P, Junker JPE, Bueno EM, et al. Craniofacial principles in face transplantation. *J Craniofac Surg* 2012;23:1234-8.
24. Rudman K, Hoekzema C, Rhee J. Computer-assisted innovations in craniofacial surgery. *Facial Plast Surg* 2011;27:358-65.
25. Kim Y, Kim H, Kim YO. Virtual reality and augmented reality in plastic surgery: a review. *Arch Plast Surg* 2017;44:179-87.
26. Wong K, Yee HM, Xavier BA, Grillone GA. Applications of augmented reality in otolaryngology: a systematic review. *Otolaryngol Head Neck Surg* 2018;159:956-67.
27. Sosin M, Ceradini DJ, Hazen A, Levine JP, Staffenberg DA, et al. Total face, eyelids, ears, scalp, and skeletal subunit transplant cadaver simulation: the culmination of aesthetic, craniofacial, and microsurgery principles. *Plast Reconstr Surg* 2016;137:1569-81.
28. Sosin M, Ceradini DJ, Levine JP, Hazen A, Staffenberg DA, et al. Total face, eyelids, ears, scalp, and skeletal subunit transplant: a reconstructive solution for the full face and total scalp burn. *Plast Reconstr Surg* 2016;138:205-19.



Case Report

Open Access



# A case of congenital aneurysm of the ulnar artery of the palm

Yoko Kishi

Department of Pediatric Surgery, Dokkyo Medical University Saitama Medical Center, Saitama 343-8555, Japan.

**Correspondence to:** Dr. Yoko Kishi, Department of Pediatric Surgery, Dokkyo Medical University Saitama Medical Center, 2-1-50 Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan. E-mail: kishi-y@dokkyomed.ac.jp

**How to cite this article:** Kishi Y. A case of congenital aneurysm of the ulnar artery of the palm. *Plast Aesthet Res* 2020;7:3.  
<http://dx.doi.org/10.20517/2347-9264.2019.50>

**Received:** 3 Nov 2019 **First Decision:** 23 Dec 2019 **Revised:** 8 Jan 2020 **Accepted:** 14 Jan 2020 **Published:** 21 Jan 2020

**Science Editor:** A Thione **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

Congenital aneurysms of the palm are uncommon in the pediatric population compared to aneurysms in adults. A seven-month-old boy presented with a true aneurysm of the ulnar artery with reconstruction with surgical excision and end-to-end microvascular anastomosis using a superficial vein of the dorsal foot. To our knowledge, there have been only 15 reports of congenital aneurysms of the palm.

**Keywords:** Congenital aneurysm of the palm, ulnar artery, microvascular reconstruction

## INTRODUCTION

Hypothenar hammer syndrome is characterized by aneurysm in the palm; it is most commonly caused by trauma. Congenital aneurysm in the palm is relatively rare.

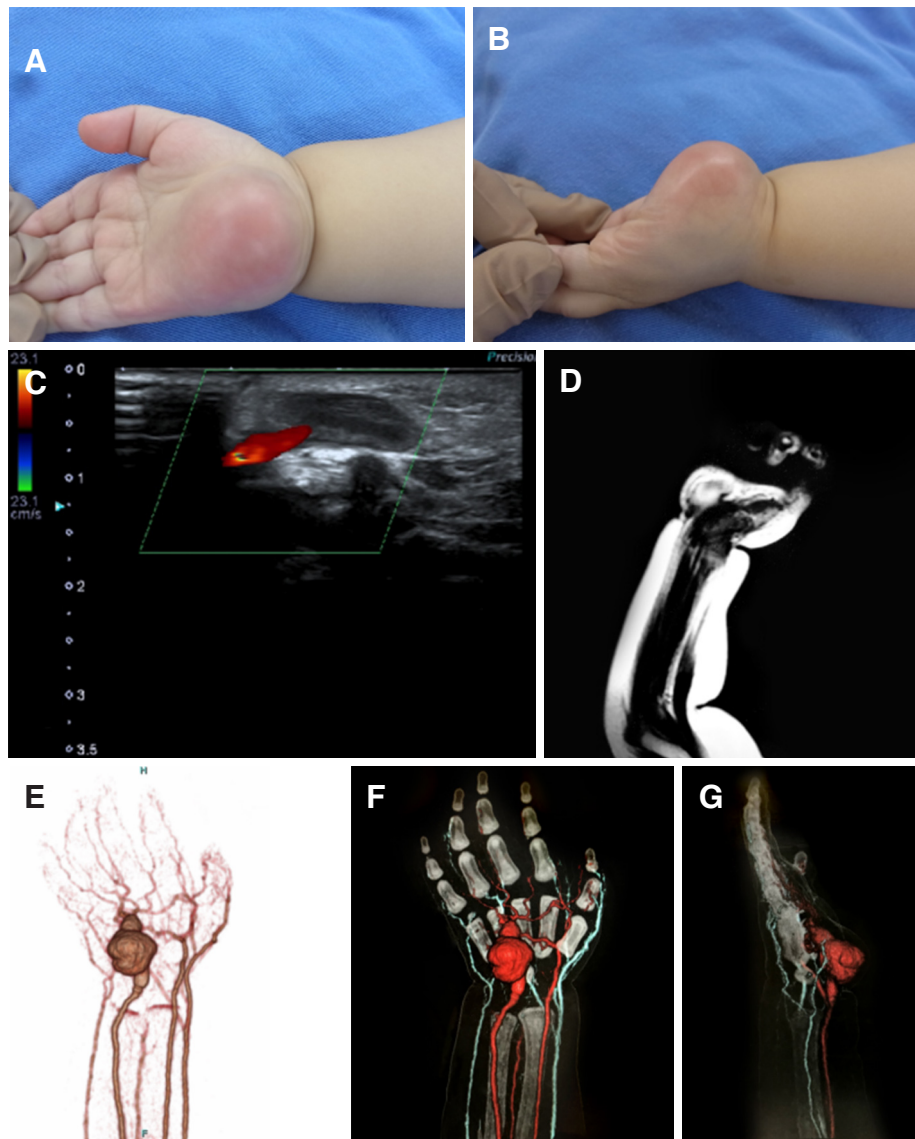
To our knowledge, there are only two reports<sup>[1,2]</sup> on congenital aneurysm in the palm in the Japanese literature and 13 reports<sup>[3-15]</sup> in the English literature.

We describe a case of congenital aneurysm of the ulnar artery in the palm of a nine-month-old baby boy. We reviewed the literature on congenital aneurysm to identify the site of occurrence, affected side, treatment procedure, and outcomes.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** Patient presented with a large pulsatile palmar mass. A: Clinical findings; front view; B: lateral view; C: color doppler ultrasound imaging; D: MRI findings; E: MRA findings; F: 3D view (frontal); G: 3D view (lateral)

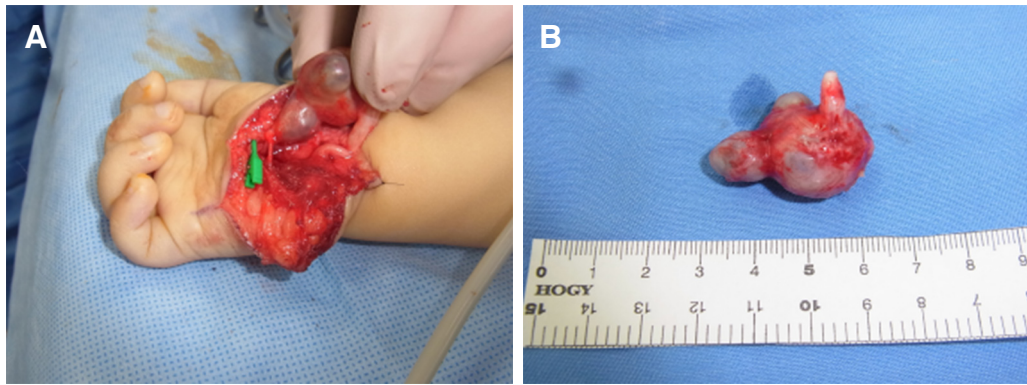
## CASE REPORT

The family of a seven-month-old boy noticed a protuberance on the palm of his right hand. His parents took him to a dermatology clinic, from where he was referred to our hospital for treatment. On the first visit, the protuberance was diagnosed as a pulsatile subcutaneous tumor, measuring 25 mm × 25 mm [Figure 1A and B].

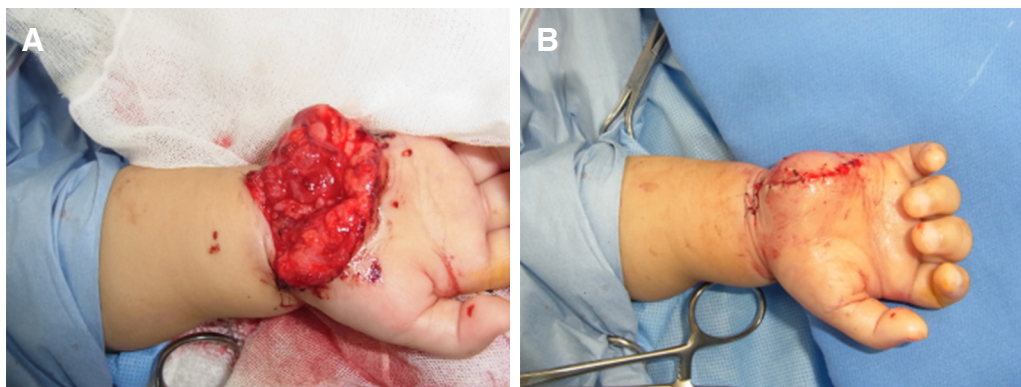
A Doppler ultrasound revealed marked expansion of the lumen of the ulnar artery distal to the wrist [Figure 1C].

T2-weighted magnetic resonance imaging (MRI) revealed uneven high signal intensity and T1-weighted MRI showed low signal intensity that was not fat-suppressed [Figure 1D].

Magnetic resonance angiography (MRA) revealed expansion of the artery extended between the palmar carpal branch of the ulnar artery and the superficial palmar arch [Figure 1E-G]. Surgery was scheduled for resection of the aneurysm and reconstruction of the artery using the superficial saphenous vein taken from



**Figure 2.** Intraoperative view A: the aneurysm was elevated; B: total view of the mass

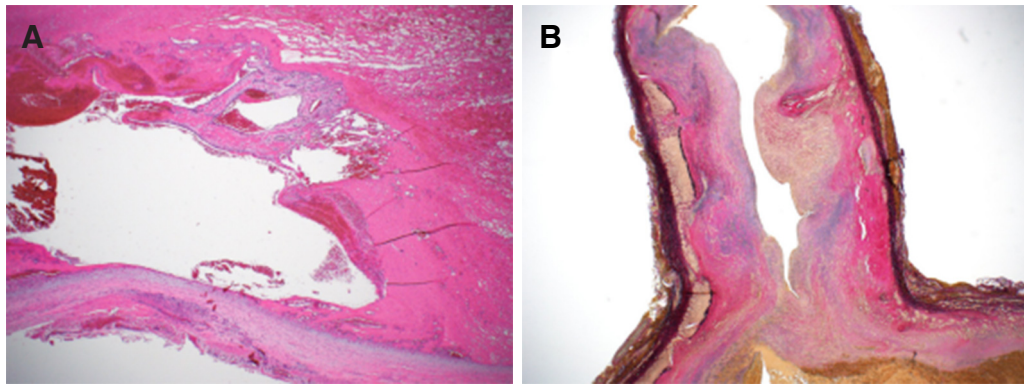


**Figure 3.** The recipient vein was anastomosis between the ulnar artery and superficial palmar arch

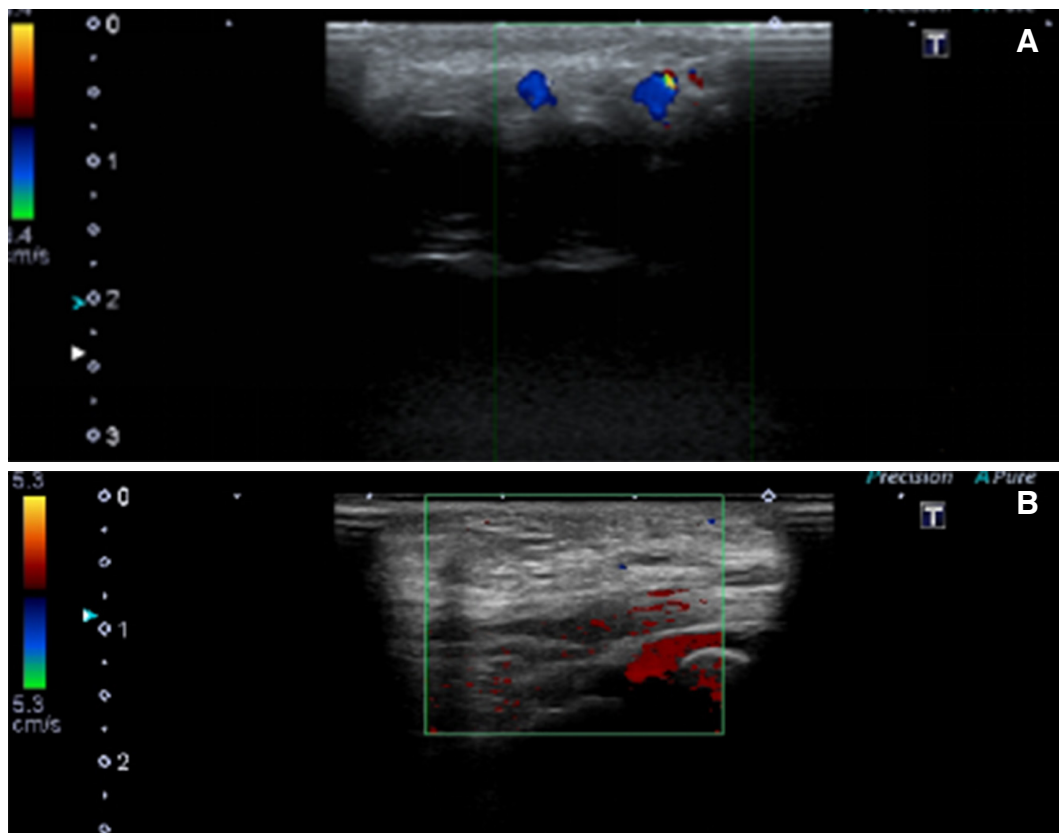
the dorsal foot at nine months of age. With the patient under anesthesia, a tourniquet was placed on the left upper arm and inflated to 200 mmHg. A lazy-S skin incision was drawn from the metacarpophalangeal joint of the little finger on the palmar side to the midpalm carpal line. The incision line was extended to transverse the wrist and elongated toward the forearm longitudinally. Next, the fat tissue layer was dissected to reach the lesion. The aneurysm was dissected from the ulnar artery at the wrist to the bifurcation of the proper palmar digital artery at the superficial palmar arch. Following the release of the cubital tunnel, the proximal part of the aneurysm was identified and a 30-g vessel clip was applied to both ends of the section that was judged to be the aneurysm expansion. The total size of the aneurysm was approximately 32 mm × 25 mm × 22 mm [Figure 2A and B].

The vein on the dorsal foot that was confirmed prior to the operation using AccuVein<sup>TX</sup> (Accu Vein LLC New York). About 2 cm of the vein was harvested as an interposition graft because the great saphenous vein is difficult to remove, although its diameter is good for anastomosis. The lumen of the vein was washed using a 1:20,000 solution of heparin in physiological saline. Under microscopic guidance, the aneurysm was resected, and the ulnar artery was anastomosed to the donor vein using 10-0 nylon suture. The clip was removed, and the pulsation was identified. The transverse palmar ligament, fat tissue, dermis, and epidermis were loosely sutured layer by layer [Figure 3A and B].

Bulky dressing was applied. To relieve shock on the palmar side, a cast with a Reston<sup>TM</sup> (3M. St Paul. Minnesota) self-adhering foam padding was fixed on the palmar side. Pathological examination showed



**Figure 4.** Pathological findings by Hematoxylin-Eosin stain A: the hemorrhage was shown and the normal structure of the artery was broken  $\times 20$ ; B: the neck of the aneurysm  $\times 4$



**Figure 5.** The left side is the ulnar artery and The longitudinal view of the the right side is the radial artery anastomotic graft vein (red). at the middle part of the palm (blue)

that the arterial wall was expanded with increased irregular fibrous tissue deposition. The normal architecture was distorted, and a thrombus had become a substrate [Figure 4A and B].

After surgery, heparin sodium and prostaglandin infusion were administered for one week to prevent thrombosis and spasm. At two weeks after surgery, prognosis was excellent, and the patient was discharged home safely. Every two weeks, he was checked for the condition of the anastomosed vessels by ultrasonography [Figure 5A and B].



**Table 1. Patient characteristics of all reports of congenital true palmar aneurysms**

Ref.	Published year	Age	Sex	Side	Dominant	Artery	Collagen vascular disease	Operation
Okuda <i>et al.</i> <sup>[1]</sup>	1987	5 years	F	R		Palmar arch 4		Resection
Rikukawa <i>et al.</i> <sup>[2]</sup>	1992	12 years	M	L		Ulnar		Resection: end to end anastomosis
Martin <i>et al.</i> <sup>[3]</sup>	1982	18 years	M	R	Left	Ulnar		Resection + vein graft, superficial v
Itoh <i>et al.</i> <sup>[4]</sup>	1992	8 months	M	L		Common digital		Resection
Offer <i>et al.</i> <sup>[5]</sup>	1999	1 year	M	L		Ulnar		Resection
Witt <i>et al.</i> <sup>[6]</sup>	2003	8 years	M	L		Ulnar		Resection + vein graft, reverse saphenous vein
Deune <i>et al.</i> <sup>[7]</sup>	2005	4 years	M	L		Ulnar	+	Resection + vein graft
Al-Omran <i>et al.</i> <sup>[8]</sup>	2007	18 months	M	L		Ulnar	+ Kawasaki	Resection
Parsa <i>et al.</i> <sup>[9]</sup>	2008	12 years	M	R	Right	Ulnar		Resection + vein graft
Amjad <i>et al.</i> <sup>[10]</sup>	2010	2 years	M	L		Ulnar		Resection
Iyer <i>et al.</i> <sup>[11]</sup>	2012	5 months	F	L		Ulnar		Resection ligated
Stalder <i>et al.</i> <sup>[12]</sup>	2016	15 years	M	L	Right	Ulnar		Resection + vein graft, cephalic vein
Shutze <i>et al.</i> <sup>[13]</sup>	2017	16 years	F	L		Common digital		Resection
Meals <i>et al.</i> <sup>[14]</sup>	2017	6 months	M	L		Common digital		Resection
Dean <i>et al.</i> <sup>[15]</sup>	2019	13 months	M	R		Common digital		Resection
Our case	2019	7 months	M	R		Ulnar		Resection + vein graft

A palmar gypsum cast with Reston<sup>TM</sup> was kept in place for one year and his condition was satisfactory as of this writing. There were no complications or abnormalities of blood flow or the median or ulnar nerve.

## DISCUSSION

In Japan, 12 reports have been published on aneurysms in the palm, of which 10 were caused by trauma, one had unknown etiology, and only one was diagnosed by Okuda *et al.*<sup>[1]</sup> as a congenital aneurysm. In the English literature, we found only 14 reports on aneurysms in the palm<sup>[2,3,15]</sup>, five of which were diagnosed as congenital aneurysm but with underlying arteriosclerosis. Most aneurysms were caused by blunt trauma, especially those located in the palm, and were thought to have arisen because the ulnar artery was sandwiched between the hamate bone and skin. Such cases are considered to represent hypothernar hammer syndrome. In 1772, Guattani<sup>[16]</sup> was the first to describe a palmar aneurysm with most of the 52 reported cases being caused by trauma and 30 involving the ulnar artery.

Doppler ultrasound can be used to detect the characteristic yin-yang sign of congenital aneurysm. MRI is also effective for examining the luminal of the artery. MRA is however the most effective method for examining vascular flow. According to the pathological findings, congenital aneurysms are characterized by a poor inflammation and no arteriosclerosis. It is easy to distinguish them from congenital arteriovenous fistula using arteriographic and MRA imaging findings. Furthermore, it is difficult to detect congenital aneurysms in one-year-old children because identification of its symptoms is challenging in this population, and the only physical finding is often a bump in the palm.

Among the 15 cases previously reported<sup>[1-15]</sup>, the site of occurrence was the ulnar artery in 10 cases, the common digital artery in four cases, and the palmar arch in one case; the ulnar artery was involved in 67% of cases [Table 1].

Eleven of the fifteen cases described were on the left side.

One of the cases in which the aneurysm occurred in the dominant hand was a right-sided aneurysm in a 12-year-old boy; one patient was very young, and investigators could not judge the dominant hand. Our case was not identified as occurring in the dominant side on the first visit; however, one year later, it appeared that the dominant side was the right side.

The age of onset ranged from 5 months to 16 years with a mean of six years. Treatment was carried out in all 15 cases. Simple excision was performed for eight cases and reconstruction for seven cases; one needed end-to-end anastomosis, five needed vein graft, and one needed a combination with sclerotherapy. Embolization was not selected because it may impair blood flow in the common digital arteries. We chose to use a vein graft from the saphenous vein because there was a 2-cm diameter gap between the affected arteries; the aneurysm was located at the edge of the deep palmar arch; and the mobility of the ulnar artery was poor. If the distance between the defect of the artery had been short, we could have directly sutured the artery. The donor vein was difficult to anastomose because its diameter was one-third that of the ulnar artery. Furthermore, it was difficult to obtain a good vein to fit the diameter of the recipient artery from a nine-month-old boy with minimal injury because the fat layer at that region around the saphenous vein was thick and therefore the resulting wound was considerably longer. Two cases in the literature had a history of collagen disease, namely Kawasaki disease, Marfan's syndrome, Loeys-Dietz syndrome, osteogenesis imperfect, and Ehlers-Danlos syndrome. Our patient had no evidence of these diseases. We diagnosed this as a congenital aneurysm in the palm because it was a true aneurysm, with poor infiltration, no arterial sclerosis on pathological findings, and no evidence of collagen disease or trauma.

In conclusion, arterial aneurysms of the palm in the pediatric population are rare. Our case was a right-handed seven-month-old boy. The patient underwent operative exploration with arterial reconstruction to interpose between the defect using a dorsal pedal vein. The patient recovered well without any complication. Including the present case, there have been only 16 cases of congenital aneurysms in the palm.

## **DECLARATIONS**

### **Authors' contributions**

The author contributed solely to the article.

### **Availability of data and materials**

Not applicable.

### **Financial support and sponsorship**

None.

### **Conflicts of interest**

The author declared that there are no conflicts of interest.

### **Ethical approval and consent to participate**

This study was conducted in accordance with the declaration of Helsinki.

### **Consent for publication**

Not applicable.



## Copyright

© The Author(s) 2020.

## REFERENCES

1. Okuda H, Koshikawa A, Kawada Y, Kurata A. Congenital aneurysm in the palm: a case report. *Orthop Surg* 1987.
2. Rikukawa H, Kudo T, Takahashi K, et al. A case report of true palmar aneurysm. *J. Japan Surgical Society* 1992;93:445-7.
3. Martin RD, Manktelow RT. Management of ulnar artery aneurysm in the hand: a case report. *Can J Surg* 1982;25:97-9.
4. Itoh M, Takato T, Yokota M. Upper extremity aneurysm in infants. *Ann Plast Surg* 1992;29:157-60.
5. Offer GJ, Sully L. Congenital aneurysm of the ulnar artery in the palm. *J Hand Surg Br* 1999;24:735-7.
6. Witt PD, Bowen KA, Johansen K. True ulnar artery aneurysm of the hand in an 8-year-old boy. *Plast Reconstr Surg* 2003;111:2475-6.
7. Deune EG, McCarthy EF. Reconstruction of a true ulnar artery aneurysm in a 4-year-old patient with radial artery agenesis. *Orthopedics* 2005;28:1459-61.
8. Al-Omran M. True ulnar artery aneurysm of the hand in an 18-month-old boy: a case report. *J Vasc Surg* 2007;45:841-3.
9. Parsa AA, Higashigawa K, Parsa FD. Arterial aneurysm of the hand. *Hawaii Med J* 2008;67:37-40.
10. Amjad I, Murphy T, Zahn E. Diagnosis and excision of an ulnar artery aneurysm in a two-year-old boy. *Can J Plast Surg* 2010;18:e15-6.
11. Iyer RS, Hanel DP, Enriquez BK, Weinberger E. Ulnar artery aneurysm causing palmar mass in 5-month-old girl. *Pediatr Radiol* 2012;42:1401-4.
12. Stalder MW, Sanders C, Lago M, Hilaire HS. Multilocation true ulnar artery aneurysm in a pediatric patient. *Plast Reconstr Surg Glob Open* 2016;4:e595.
13. Shutze RA, Leichty J, Shutze WP. Palmar artery aneurysm. *Proc (Bayl Univ Med Cent)* 2017;30:50-1.
14. Meals CG, Carey GB, Higgins JP, Chang B. Ulnar artery aneurysm in a 6-month-old: a case report. *Hand (N Y)* 2017;12:118-20.
15. Dean RA, Fleming SI, Zvavanjanja RC, Marques ES, Greives MR. Congenital aneurysm of the palmar digital artery: a case report and literature review. *Radiol Case Rep* 2019;14:83-7.
16. Guattani C. *De externis aneurysmatibus manu chirurgica methodice pertractandis*. Rome; 1772.

Review

Open Access



# Expanding the top rungs of the extremity reconstructive ladder: targeted muscle reinnervation, osseointegration, and vascularized composite allotransplantation

Saïd C. Azoury<sup>1</sup>, Andrew Bauder<sup>1</sup>, Jason M. Souza<sup>2</sup>, John T. Stranix<sup>3</sup>, Sammy Othman<sup>1</sup>, Christine McAndrew<sup>4</sup>, Scott M. Tintle<sup>2,4</sup>, Stephen J. Kovach<sup>1,4</sup>, Lawrence Scott Levin<sup>1,4</sup>

<sup>1</sup>Division of Plastic Surgery, Department of Surgery, University of Pennsylvania, Pennsylvania, PA 19104, USA.

<sup>2</sup>Department of Orthopaedic Surgery, Walter Reed National Military Medical Center, Bethesda, MD 20889, USA.

<sup>3</sup>Department of Plastic Surgery, University of Virginia, Charlottesville, VA 22908, USA.

<sup>4</sup>Department of Orthopaedic Surgery, University of Pennsylvania, Pennsylvania, PA 19104, USA.

**Correspondence to:** Dr. Lawrence Scott Levin, FACS; Paul B. Magnuson Professor of Bone and Joint Surgery; Chairman, Department of Orthopaedic Surgery; Professor of Surgery (Plastic Surgery); Penn Medicine University City; 3737 Market Street, 6th Floor, Philadelphia, PA 19104, USA. E-mail: scott.levin@pennmedicine.upenn.edu

**How to cite this article:** Azoury SC, Bauder A, Souza JM, Stranix JT, Othman S, McAndrew C, Tintle SM, Kovach SJ, Levin LS. Expanding the top rungs of the extremity reconstructive ladder: targeted muscle reinnervation, osseointegration, and vascularized composite allotransplantation. *Plast Aesthet Res* 2020;7:4. <http://dx.doi.org/10.20517/2347-9264.2019.44>

**Received:** 26 Oct 2019 **First Decision:** 16 Jan 2020 **Revised:** 17 Jan 2020 **Accepted:** 20 Jan 2020 **Published:** 12 Feb 2020

**Science Editor:** Matthew L. Iorio **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

## Abstract

Osseointegration (OI), targeted muscle reinnervation (TMR), and vascularized composite allotransplantation (VCA) are just a few ways by which our reconstructive ladder is evolving. It is important to recognize that amputation does not necessarily denote failure, but surgeons should strive to find ways to provide these patients with means for obtaining better satisfaction and quality of life postoperatively. TMR and OI have added options for mutilating lower extremity injuries that necessitate amputation. More recently, the senior author (Levin LS) described the “penthouse” floor of the reconstructive ladder being VCA. Despite the advances in VCA over the last 20 years, there are many challenges that face this discipline including indications for patient selection, minimizing immunosuppressive regimens, standardizing outcome measures, establishing reliable protocols for monitoring, and diagnosing and managing rejection. Herein, the authors review TMR, OI, and VCA as additional higher rungs of the reconstructive ladder.

**Keywords:** Targeted muscle reinnervation, osseointegration, vascularized composite allotransplantation, salvage, reconstruction



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



## INTRODUCTION

Harold Gillies introduced the reconstructive ladder for traumatic extremity wounds based on his experience during World War I. Decades later, the senior author (Levin LS) revisited this concept when he coined the collaborative orthoplastic approach between orthopedic and plastic surgeons in extremity reconstruction<sup>[1,2]</sup>. He elaborated on the necessity of a surgeon to be well versed with the various rungs of the reconstructive ladder. Although variations to the original ladder have been described as the reconstructive armamentarium expands with time, the basic tenants remain largely unchanged<sup>[3-7]</sup>. Most descriptions begin with healing by secondary intention on the lowest rung<sup>[3]</sup>. The next lower-level rungs of the ladder include simpler reconstructive options such as the use of split-thickness skin grafts and local tissue rearrangements and the higher rungs represent complex techniques such as free tissue transfer. In general, the simplest option that is able to cover the defect adequately and replace the missing tissue components should be the reconstruction of choice. However, it is understood that a more complex option such as free tissue transfer may be more appropriate even when simpler means achieve closure, such as the cases of severe traumatic wounds with associated fracture repair, hardware, or in the case of oncologic reconstruction when future radiation is anticipated.

Targeted muscle reinnervation (TMR) and osseointegration (OI) have added additional options for mutilating lower extremity injuries that necessitate amputation<sup>[8-13]</sup>. More recently, the senior author Levin<sup>[14]</sup> described the “penthouse” floor of the reconstructive ladder being vascularized composite allotransplantation (VCA). Despite the successes of prosthetic technology and targeted muscle reinnervation, transplantation offers the ability to restore sensation, fine motor control, and tactile aesthetics while improving overall quality of life<sup>[15,16]</sup>. Herein, the authors review TMR, OI, and VCA as additional higher rungs of the reconstructive ladder [Figure 1].

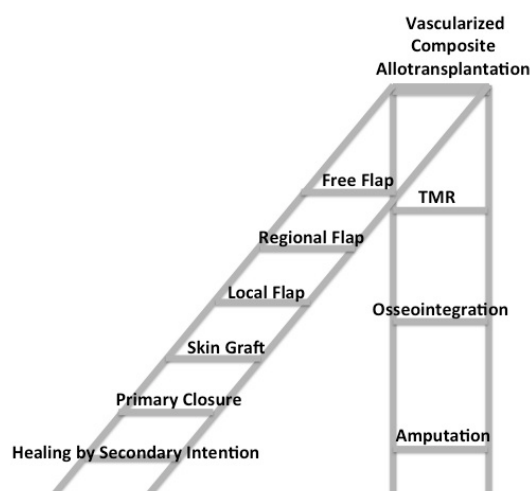
## TARGETED MUSCLE REINNERVATION

### Background

When a limb is lost, there are residual muscles rendered incompetent without a joint to act across. Similarly, there are nerves that not only are purposeless without distal targets to reinnervate, but can also become a functional hindrance should they form a chronically painful neuroma. TMR was originally developed to make use of these redundant muscles and nerves in amputees for improved upper extremity prosthetics. In TMR, nerves transected during amputation are coapted to nearby motor nerves of redundant muscles, providing a conduit to grow along and a muscle to reinnervate. The muscle acts as a biologic amplifier, creating a myoelectric signal that can be picked up through surface electromyography (EMG). This signal detection can be further amplified surgically by superficializing and separating the myoelectric unit from other nearby signals. Signal mapping and feedback then allows for complex movement of a myoelectric prosthesis.

Evidence for the functional capabilities of TMR in providing advanced prosthetic control came in the form of myoelectrical signal decoding involving physical and practical movements with a prosthetic arm. As research evolved, it was shown that pattern recognition technology could allow for intuitive movement-learning, potentially enhancing both control and timing of movements through the prosthetic limb as an alternative to conventional prosthetics that were plagued with slower learning due to a rather unintuitive motion process<sup>[17]</sup>. The research demonstrated that not only is TMR effective in allowing for complex physical movements, but was also efficient in signal transmission and producing these movements relatively quickly, a crucial consideration in functional validity<sup>[17]</sup>.

Initial reservations in employing TMR included the possibility of creating more nerve pain during the procedure<sup>[17]</sup>. Coapting a nerve that is transected during amputation to a previously intact motor nerve



**Figure 1.** Expanded reconstructive ladder with targeted muscle reinnervation, osseointegration, and vascularized composite allotransplantation. TMR: targeted muscle reinnervation

necessitates creating a proximal motor nerve that is now disconnected and left with no target. However, unlike pure sensory or mixed motor-sensory nerves, these cut pure motor nerves do not form symptomatic neuromas. In fact, contrary to initial reservations, TMR was found to substantially reduce post-amputation pain in upper extremity amputees<sup>[18]</sup>.

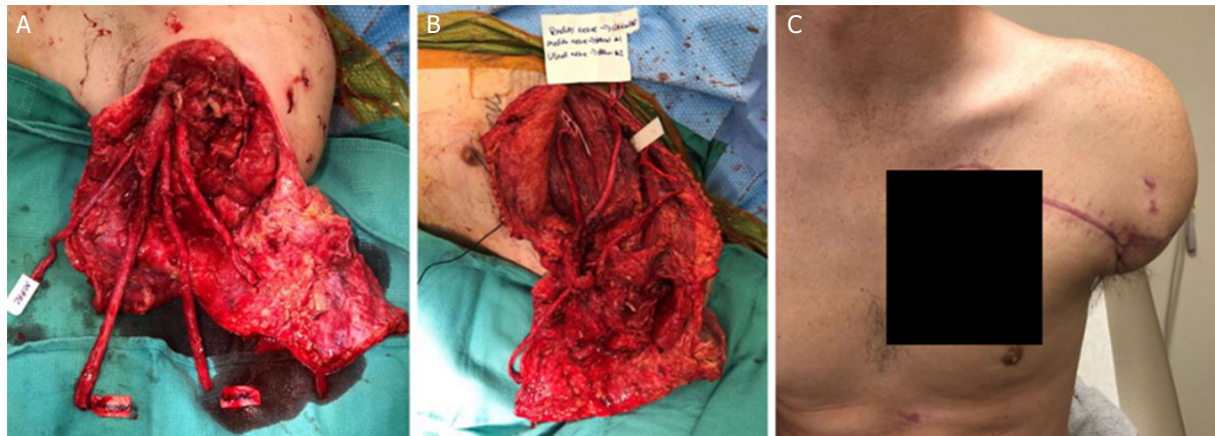
The discovery that TMR had the potential to reduce post-amputation pain drastically expanded its indications. The majority of amputees suffer from chronic pain after amputation that can prevent fitting of a prosthesis. While a state-of-the-art myoelectric prosthesis may not be practical for every amputee, a procedure with the potential to reduce chronic pain ballooned in popularity amongst amputees. With this in mind, surgeons have now turned to employing TMR at the time of amputation to prevent post-amputation pain<sup>[19]</sup>.

Below, we provide an overview of the indications, techniques, and future directions of TMR in upper and lower extremity amputees, both at the time of amputation and as a secondary procedure. With additional experience using TMR and advancements in prostheses, we will continue to see a shift in functional expectations after amputation from both surgeons and amputees alike.

### Upper extremity TMR

The development of upper extremity TMR was primarily driven by the desire for more natural, intuitive prostheses. Loss of a hand or upper extremity is profoundly limiting. While body powered prostheses help fill this functional void, they cannot recapitulate all of the degrees of freedom of a human upper extremity, particularly with higher-level amputations. They also do not allow for simultaneous movements across multiple joints. As such, there was a push to overcome the shortcomings of traditional prosthesis, chiefly funded by the Defense Advanced Research Projects Agency Revolutionizing Prosthetics Program<sup>[20]</sup>.

TMR was an answer to the call for more intuitive, higher capability prosthetic control. By providing a neural interface with the prosthetic using multiple discrete high-amplitude EMG signals, it has exhibited excellent results for real time control of upper extremity myoelectric prostheses for the last decade<sup>[17,21,22]</sup>. The upper extremity amputee now has several commercially available myoelectric prosthetic options to choose from<sup>[21,23]</sup>. Moreover, as signal processing and pattern recognition algorithms continue to improve the neural interface between patient and prosthetic, amputees benefit from more natural prosthetic movement<sup>[24,25]</sup>.



**Figure 2.** Example of targeted muscle reinnervation at the axillary level in a patient with an unsalvageable upper extremity from a drag racing accident: (A) the careful dissection of musculocutaneous, median, ulnar, and radial nerve; (B) the coaptations of these nerves to the motor endpoints of the ipsilateral pectoralis major; and (C) the patient is well healed at three-month follow up with no pain

#### *Upper extremity TMR - technical considerations*

TMR in the upper extremity is commonly performed at one of three levels: axillary (shoulder disarticulation), transhumeral, and transradial. Regardless of the level of amputation, successful TMR for the purposes of myoelectric prosthetic control relies on creating the highest amplitude, most discrete EMG signal possible. This, in turn, depends on both completely disrupting the native innervation to the recipient muscle to provide a good stimulus for nerve ingrowth and performing the nerve coaptation near the motor nerve entry into this muscle to limit ingrowth distance. Recipient motor nerve/muscle units should also be superficialized and separated from one another if possible. This allows easy decoding of EMG signals via surface electrodes, although pattern recognition can overcome signal cross-talk in many cases.

The surgical rationale and specific nerve transfers at all three levels has been previously described in detail<sup>[26-28]</sup>. At the axillary level, this involves denervating the pectoralis major and coapting the musculocutaneous, median, ulnar, and radial nerves to motor endpoints of separated slips of pectoralis [Figure 2]. At the transhumeral level, TMR requires partial preservation of native signals: the radial signal to the long head of triceps and musculocutaneous signal to long head of biceps are left intact. The median nerve is then typically coapted to the short head of biceps, the radial nerve to the lateral head of triceps, and the ulnar nerve to the brachioradialis. TMR at the transradial level is even less prescriptive than in more proximal upper extremity amputations due to the availability of numerous recipient muscles that can easily be superficially relocated<sup>[27]</sup>. However, at the very least, distal targets for the median, ulnar, radial sensory, and lateral antebrachial cutaneous nerves must be created.

As we have become more familiar with upper extremity TMR at our institution, we have increasingly appreciated the intra-operative creativity involved in formulating a reconstructive TMR plan. No two injuries are the same, and they rarely abide by the surgical game-plan, except at the shoulder disarticulation level. We have also found that, even in a busy metropolitan area, finding a physical therapist well familiar with training for myoelectric prostheses can be difficult. Maintaining clear communication with this therapist is critical, as successful prosthetic use depends largely on rehabilitation and monitored feedback.

#### *Upper extremity TMR - future directions*

Future developments in TMR for the upper extremity will continue to work towards more natural myoelectric prosthetic control. OI prostheses stand to stabilize the patient-prosthetic interface, thereby limiting movement at surface electrodes for more reliable EMG directed prosthetic movement<sup>[29]</sup>. Individual

fascicular transfer may allow even greater prosthetic control through the creation of an array of individual EMG signals<sup>[30]</sup>. Finally, while all currently available prostheses rely on visual feedback, future prostheses will integrate a sensory feedback loop to allow more effective patient interaction with their environment<sup>[21]</sup>.

Finally, as new surgical techniques develop, so will indications for TMR versus transfers of existing musculature for prosthetic and pain control. Already, it is possible for metacarpal level amputees to obtain excellent finger function with the starfish procedure, in which existing interosseous muscles are dorsally transposed to create a reliable myoelectric signal<sup>[31]</sup>. In this same patient, a sensory neuroma may be resected and the nerve coapted to a motor nerve to the volar interosseous for pain control. Future success will depend on balancing pain control and function with the most effective surgical technique available.

### Lower extremity TMR

While the use of myoelectric prostheses in the upper extremity is somewhat common, it has been modest in the lower extremity<sup>[32]</sup>. This is likely partially related to the relatively recent introduction of lower extremity TMR when compared to the upper extremity. However, it also appears that sensory and proprioceptive feedback of both the amputated limb/prosthesis and the contralateral unaffected limb play a much more important role in developing an intuitive and natural lower extremity prosthesis. Moreover, in the absence of widespread TMR in the lower extremity, there has been some success in developing myoelectric prostheses that rely on EMG signals from the existing innervated musculature in the amputated limb<sup>[33,34]</sup>. Although TMR in the lower extremity may allow more nuanced control of a myoelectric prosthesis, it is possible to control movement as distal as the ankle even with a transfemoral amputation using pattern recognition from the remaining thigh musculature<sup>[35]</sup>.

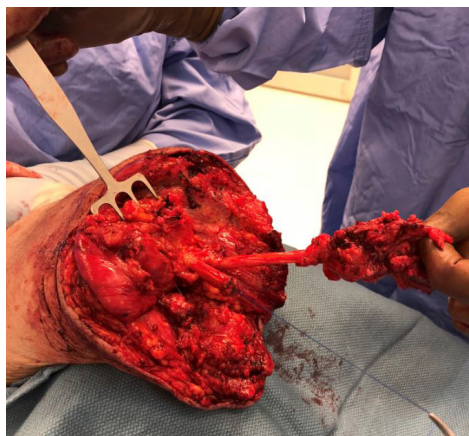
In the absence of widely available lower extremity prostheses that rely on TMR, much of the conversation surrounding lower extremity TMR has focused on treating and preventing nerve-related pain. For decades, traction neurectomy was the standard of care during amputation, which has proven ineffective in both preventing and relieving post-amputation pain<sup>[36]</sup>. For many patients, the decision to amputate is driven by chronic pain, and they are frustrated to find that they trade the pain prior to amputation for new forms of chronic pain after amputation.

This new pain includes pain at their residual limb site, known as residual limb pain (RLP), and painful sensations in their absent extremity, known as phantom limb pain (PLP). RLP is usually due to neuroma formation. These neuromas stem from the terminal sprouts that form at a transected nerve end after injury primarily and are comprised of disorganized, sensory nerve fibers. PLP is thought to be due to sensory-cortical remapping over time and an altered perception of pain. With standard amputation methods, the rates of post-amputation pain were astounding, with over 55% of longstanding amputees suffering from RLP, and the prevalence of PLP after lower extremity amputation ranging as high as 85%<sup>[37-40]</sup>.

Lower extremity TMR was first employed to treat the pain many of these existing amputees had suffered from for years. While several prior methods had achieved moderate success in treating post-amputation nerve pain, such as implanting nerves into bone, veins, or muscle<sup>[41-43]</sup>, these methods do not treat the underlying pathophysiology - they simply provide some mechanical masking of neuromas so they are less likely to result in pain. Conversely, TMR prevents the formation of disorganized nerve growth that results in neuromas by providing a pathway for nerves to grow down and a denervated target to reinnervate.

After its established success as a secondary intervention for post-amputation neuromas, attention shifted to the use of TMR at the time of amputation as a means to prevent pain<sup>[44]</sup>. Several case series have demonstrated excellent results, although efforts at conducting a randomized control trial comparing TMR to standard methods (i.e., implantation into muscle) were thwarted by patient refusal of randomization due





**Figure 3.** Dissection of a large neuroma in the common peroneal nerve of a transfemoral amputee with severe neuroma pain. Note the haphazard organization of the neuroma

to communication between amputees<sup>[45]</sup>. The majority of TMR that we currently perform at our institution is in coordination with vascular and orthopedic surgeons at the time of amputation. This has re-framed amputation from failure on the part of the surgeon and the end of the surgeon-patient relationship to the beginnings of a new collaborative reconstructive relationship that results in less post-amputation pain and the possibility of more advanced prosthetic use.

#### *Lower extremity TMR - technical considerations*

TMR in the lower extremity is commonly performed at the transfemoral level with above the knee amputation (AKA) and transtibial level with below the knee amputation (BKA). In contrast to upper extremity TMR, which can require more creativity, lower extremity TMR is often more formulaic. When performing TMR for the management or prevention of neuroma pain, the specific motor nerve recipients are much less important because they do not have to be superficial or separated from other nearby signals.

In the case of secondary neuroma treatment, the symptomatic nerve is isolated (usually through an incision separate from the amputation site), the neuroma resected, and the fresh nerve end is coapted to a nearby motor nerve recipient [Figure 3]. In the case of primary TMR, each pure sensory and mixed motor/sensory nerve must be coapted directly to a motor nerve. During an AKA, this includes the posterior cutaneous nerve of the thigh, the saphenous nerve, the common peroneal nerve, and the tibial nerve. During a BKA, this includes the tibial nerve, the deep peroneal nerve, the superficial peroneal nerve, and the sural nerves (medial and lateral).

Similar to the upper extremity, many of these initial surgical techniques and much of the continued data for TMR originates from Dumanian and colleagues at Northwestern. They have populated the literature with roadmaps for TMR at both the transfemoral and transtibial levels, including the common locations of frequently found motor nerves that can be used for coaptation<sup>[46,47]</sup>. We found these guides particularly helpful in our initial forays into TMR. Since then, we have realized that finding motor nerves in practice is much more reliant on intraoperative nerve stimulation than was first understood [Figure 4].

When available, we typically use a biphasic nerve stimulator (Checkpoint Surgical; Cleveland, OH) because repeated stimulation with this system does not result in neuronal fatigue. The stimulator is set at a moderate pulse duration and 2 mA for anterograde stimulation or 20 mA for retrograde stimulation. Retrograde stimulation is often particularly useful. In fact, we have found that tracing proximal motor nerve exit locations from a transected nerve is the most expeditious way to find suitable motor nerve



**Figure 4.** Demonstration of intraoperative nerve stimulation to identify appropriate motor nerve recipients

recipients for our transfers. Once suitable recipient nerves are found, performing the nerve coaptations is often the least time consuming and most straightforward portion of the case.

As our experience in lower extremity TMR has matured, we have learned a number of other useful lessons. First, when performing secondary TMR, differentiating between neuroma pain and other chronic pain conditions cannot be overstated. Neuroma pain is isolated to a specific anatomic distribution and relieved with the administration of a block to the offending nerve<sup>[48]</sup>. While the optimal timing for TMR to treat post-amputation pain is unclear, we do know that patient perception of pain is altered due to chronic pain. This global alteration in pain perception is much more difficult to treat than specific neuroma pain and may not be corrected by TMR performed long after the initial amputation. Second, communication with surgical colleagues performing the amputation is paramount. Reconstructive bridges can easily be burned during the amputation. It pays to be present during the first few amputations performed by a surgical colleague and for the surgeon performing the amputation to have a working knowledge of the purpose and requirements of successful TMR. Notably, intraoperative nerve stimulation will prove ineffective after 30-60 min of tourniquet time. Thus, if TMR is performed at the time of amputation, the amputation should be performed without tourniquet or with a short tourniquet run. Finally, while donor and recipient nerves can easily be accessed through a BKA site, this is not the case during an AKA. The patient must be flipped prone for access to the posterior femoral cutaneous nerve of the thigh and the more proximal motor nerve recipients of the posterior thigh musculature. If the patient is not stable enough for an intraoperative position change, a guillotine amputation can be performed and staged TMR can be undertaken at the time of formal amputation/closure, with the patient positioned prone for this second procedure.

#### *Lower extremity TMR - future directions*

While TMR has gained the most traction of any treatment for post-amputation neuroma pain to date, it is worth noting that it is not the first surgical alternative to traction neurectomy, nor is it the only currently popular method<sup>[49]</sup>. A recent meta-analysis noted that any surgical intervention other than traction neurectomy or nerve capping yielded over a 75% success rate for relieving neuroma pain after amputation<sup>[50]</sup>. The current literature is lacking in head-to-head comparisons between TMR and these other methods, most importantly with regenerative peripheral nerve interfaces (RPNI).

RPNI is a progression on the decades-old technique of implanting nerve into muscle that has shown promising results for treatment of neuroma pain<sup>[51]</sup>. Muscle grafts are wrapped around the end of donor

nerves and provide a denervated recipient bed for subsequent reinnervation via a mechanism similar to TMR<sup>[52]</sup>. Feasibly, these nerve/muscle units could also be used to form a neural interface with a myoelectric prosthesis, but current studies have been limited to animal models thus far<sup>[53]</sup>. The main benefit of RPNI is its surgical efficiency because the time consuming process of identifying appropriate recipient motor nerves is omitted. At our institution, we employ RPNI as a backup to TMR in unstable patients or when donor nerves are limited due to scar burden or other unfavorable patient pathology.

Finally, the vast majority of the surgical experience with TMR in the lower extremity is in trauma or oncologic patients. These patients represent a different cohort than the majority of amputees, who suffer from limb loss secondary to diabetes and vascular disease and are much more likely to experience longstanding pre-amputation neuropathy. One of the main unanswered questions is whether TMR is effective for pain relief and prevention in the diabetic vasculopath. We seek to answer this question in the near future at our institution.

## OSSEOINTEGRATION

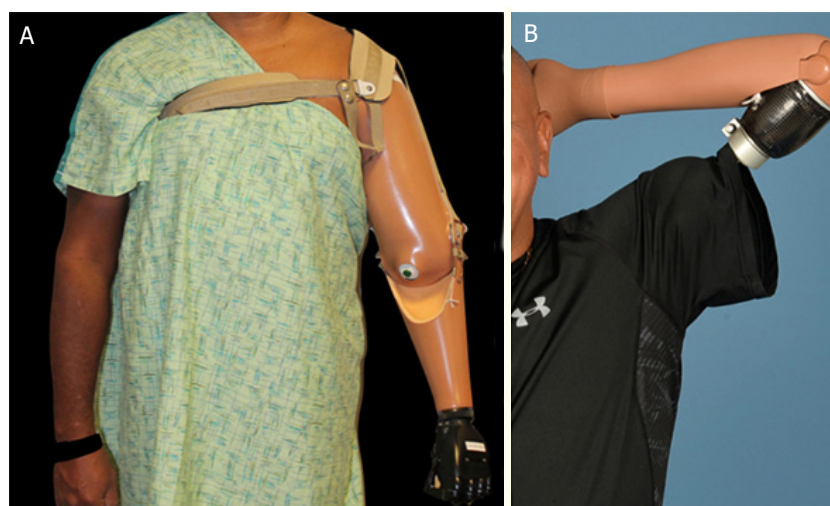
### Background

While TMR offers the potential for markedly improved prosthetic control, the control benefits of the technique can only be realized if the prosthetic is actually used. While this seems to be an obvious prerequisite, at least 35% of upper limb amputees completely abandon use of their prostheses due to socket-related limitations or discomfort<sup>[54]</sup>. Lower limb amputees use their prostheses more consistently, but frequently must endure pain or soft tissue problems to maintain their mobility. In addition, a substantial cohort of patients with limb loss have residual limbs that are too short to be candidates for a conventional, socket-based prosthesis. Even in limbs sufficiently long enough to be fit with a socket, the cylindrical shape of most transhumeral and transfemoral amputations presents challenges with regard to suspension and rotation control that plague conventional liner and socket systems. The additional support straps or harnesses required to adequately support the device further limit range of motion and impart additional difficulty with donning and doffing of the prosthetic. Likewise, efforts to overcome the intrinsic design flaw of socket-based strategies frequently employ closed suction environments and/or occlusive liners that predispose to irritation, breakdown, and soft tissue infection<sup>[55]</sup>.

Direct skeletal attachment of extremity prostheses through OI of a percutaneous implant offers a means to circumvent the limitations imposed by the conventional liner and socket fitting strategy [Figure 5]. OI obviates the need to bear weight or control the prosthetic device through a soft tissue intermediary. This translates into enhanced suspension, finer control, greater ease of use, reduced energy expenditure, and increased range of motion in the immediate proximal joint<sup>[56-58]</sup>.

In addition, OI offers a pathway to successful prosthetic use even in the setting of an insufficient soft tissue envelope or skeletal length that precludes fitting of a socket. In this way, OI expands the reconstructive possibilities and maximizes rehabilitative potential following limb loss.

OI of percutaneous implants for attachment of major limb prostheses has been in limited clinical use for nearly 30 years. The concept of placing titanium implants into living bone was first introduced by Bothe *et al.*<sup>[59]</sup> in 1940. However, the clinical potential of implanted titanium was not fully realized until Per Ingvar Branemark made the serendipitous discovery of bony in-growth while using titanium chambers to study bone microcirculation in a rabbit model<sup>[60]</sup>. The process, which Branemark described as “osseointegration”, served as the foundation for use of titanium implants in dental restoration. OI was first adapted for use in major limb amputees by Rickard Branemark, an orthopedic surgeon and the son of Per Ingvar Branemark, with the first procedure performed in a bilateral transfemoral amputee in 1990<sup>[61]</sup>. Leveraging



**Figure 5.** Pre (A) and post (B) osseointegration demonstrating the improved range of motion and absence of need for suspension straps

experience from several hundred procedures, Branemark has worked to standardize the implant system, surgical technique, and rehabilitation protocol with a program entitled Osseointegrated Prostheses for the Rehabilitation of Amputees (OPRA, Integrum AB). Standardized OPRA protocols are now available for femur, humerus, forearm, and thumb amputees<sup>[61]</sup>. The growing interest in OI is demonstrated by the numerous different osseointegrated implants currently in development or clinical use in multiple centers throughout the world. The Compress Transcutaneous Implant (CTI; Zimmer Biomet), Integral Leg Prosthesis (IPL; OrthoDynamics GmbH), and Osseointegrated Prosthetic Limb (OPL; OrthoDynamics) have all been used in persons with transfemoral and transhumeral amputations<sup>[62]</sup>.

### Safety

Concerns related to infection at the skin penetration site remain the single greatest barrier to widespread adoption of the technique. While circumventing the soft tissue issues produced by weight bearing or controlling a prosthetic through a soft tissue intermediary, percutaneous placement of a permanent implant creates a host of new challenges. The soft tissues of a residual limb are significantly thicker and more mobile than those found in the mouth or head and neck, where OI has previously been employed successfully. The relative motion between the abutment (percutaneous component) and the surrounding soft tissues concentrates stress at the skin-implant interface, leading to inflammation, tissue breakdown, fluid generation (e.g., drainage), and potential infection<sup>[63]</sup>. Additionally, extremity soft tissues are less well vascularized than the intraoral and facial tissues where OI has previously been successful. The rates of superficial soft tissue infection following extremity OI have been reported to be as high as 30%-66%<sup>[64-67]</sup>. However, most superficial infections are successfully treated with oral antibiotic therapy, with a 10-year cumulative risk of deep infection leading to implant extraction reported as less than 10%<sup>[68]</sup>.

### Technical details

While surgical technique varies between implant systems and OI centers, growing experience has identified a universal need to limit the thickness and redundancy of the surrounding soft tissue, in order to minimize motion at the skin penetration site. In the two-stage OPRA procedure, the soft tissues are thinned to the thickness of a full-thickness skin graft, which is affixed to the cortical bone surrounding the percutaneous abutment. In single-stage OPL, IPL, and CTI procedures, the vascularity of the surrounding tissues are preserved to a much greater extent, but the adjacent soft tissue flap is thinned at least to the level of scarp's fascia. These soft tissue management strategies were born out of clinical experience that saw revision surgery for soft tissue redundancy or hypergranulation exceed the need to return to the operating room

for infection-related concerns<sup>[69]</sup>. As the cumulative experience with OI expands and the natural history of the skin penetration site becomes better delineated, the technique for implant placement and soft tissue manipulation is sure to evolve.

### Future directions

Current research efforts aim to improve the safety of OI, while also leveraging the percutaneous abutment as a conduit for prosthetic feedback and control. While OI has been successfully used in the extremity, in the absence of a seal between the implant and surrounding soft tissue, soft tissue integration would dramatically decrease the risk for infection and ameliorate the chronic inflammation currently experienced at the implant interface [Figure 6]. Efforts are currently underway to use bioprinting and tissue engineering to generate a dynamic dermal seal. From the standpoint of neuromuscular integration, the osseointegrated implant and percutaneous abutment serve as a convenient channel through which to directly connect implantable sensors with the prosthetic. Direct connectivity dramatically increases the clinical potential offered by feedback and control strategies that are currently hampered by the need for surface detection, such as regenerative peripheral nerve or agonist–antagonist myoneural interfaces. The enhanced OPRA (e-OPRA), which utilizes a modified abutment screw to allow passage of transcortical wires between implantable electrodes and the terminal device, is already in clinical use in Europe<sup>[70]</sup>.

## EXTREMITY VASCULARIZED COMPOSITE ALLOTRANSPLANTATION

### Background

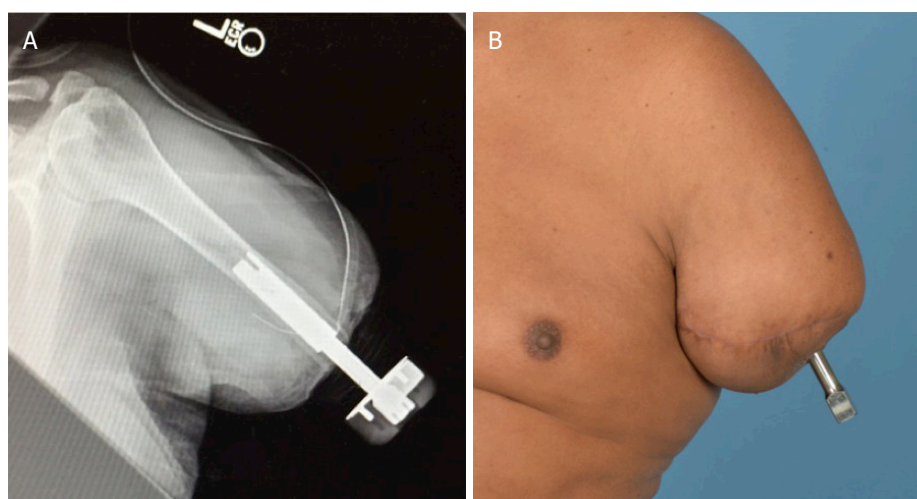
Joseph Murray, an American plastic surgeon, performed the first kidney transplant in the world in 1954 at the Peter Bent Brigham Hospital in Boston, Massachusetts<sup>[71]</sup>. A decade later, the first hand transplantation was performed in Ecuador<sup>[72]</sup>. However, the patient suffered from irreversible rejection and the graft was explanted three weeks later<sup>[72]</sup>. The second hand transplantation was performed in Lyon, France in 1998 but eventually failed due to noncompliance with immunosuppression regimen<sup>[73]</sup>. Although unsuccessful, this case demonstrated the feasibility of the procedure and the importance of compliance/postoperative care. This was followed in January 1999 by the first hand transplantation in the United States, in Louisville, Kentucky<sup>[73,74]</sup>.

The field of extremity VCA quickly gained popularity as replantation data suggested better functional outcomes when compared with revision amputation and prosthetic fitting<sup>[75]</sup>. By 2009, 53 successful hand transplants had been performed worldwide<sup>[76]</sup>. Now, roughly 150 upper limb transplantations in 100 patients have been performed to date at 45 centers worldwide, including those in United States, France, China, Austria, Italy, Spain, Belgium, Poland, Mexico, and Australia<sup>[76-85]</sup>. In 2015, the world's first pediatric bilateral hand-forearm transplantation and, in 2016, the first transatlantic hand transplantation were performed at the University of Pennsylvania under the direction of the senior author (Levin LS)<sup>[78,86]</sup>.

Although it has been advocated as a reasonable pursuit, successful VCA for lower limb amputees have not been reported to date<sup>[87]</sup>. This is mainly due to the fact that, when compared to prosthetic options, the risks, including those associated with surgery and lifelong immunosuppression, outweigh potential benefits. For this reason, the presented discussion on VCA is limited to upper extremity.

Hand and upper-limb transplantation represents the most commonly performed surgery in the growing field of VCA<sup>[88,89]</sup>. The hand transplantation process consists of patient selection, preoperative evaluation/preparation including cadaveric practice runs, the procurement and transplantation procedure, immunosuppression maintenance, and postoperative rehabilitation and follow-up<sup>[88,90]</sup>. Significant progress has been made in the past several decades in extremity VCA, including 3D printing, novel implants, improved imaging techniques [e.g., functional magnetic resonance imaging (MRI)], immunosuppressive regimens, and efficiency of surgical technique<sup>[88]</sup>.





**Figure 6.** Osseointegration radiograph (A) and skin penetration site (B)

Upper extremity VCA faces controversies and challenges that unfold alongside the procedure itself. Policy and regulatory issues strongly influence progress of the field, so much so that transplantation is being performed at overseas centers as part of clinical trials rather than a standard treatment option<sup>[91]</sup>. Preoperatively, patients should be counseled regarding realistic expectations including functional, sensory, and aesthetic ones<sup>[92]</sup>.

### Indications and patient selection

A survey of North American hand surgeons indicates that most support upper extremity VCA for bilateral or dominant below-elbow amputees<sup>[93]</sup>. Specific clinical selection criteria and contraindications developed by the American Society for Reconstructive Transplantation can only be expected to change with maturation of the field and growing reports of functional outcomes with longer follow-up periods<sup>[89,93]</sup>. Patient evaluation is exhaustive and factors including motivation, comorbidities, social support, and psychological profile are critical when deciding if and when to proceed<sup>[14]</sup>. The process involves transplant physician/surgeons, social workers, psychiatrists, and rehabilitative specialists. Upper extremity VCA is most frequently performed for forearm and wrist-level amputations, although above-elbow results are promising and those patients are able to perform many activities of daily living<sup>[94]</sup>. In more distal amputations, extrinsic hand function is possible even without nerve regeneration given the presence of muscles and tendons. More distally, intrinsic hand function and sensation can be restored more quickly given the shorter distances necessary for nerve regeneration.

At our institution, the recipient should be HIV-negative, without any coexisting psychosocial or medical issues, and with a negative cross-match with the donor. Female patients must have a negative pregnancy test. In general, patients should be between the ages of 18 and 65 years old. In fact, just several years ago, it was proposed that VCA should not be extended to pediatric patients given the number of unknowns<sup>[95]</sup>. However, in July 2015, we performed a bilateral hand transplantation on an eight-year old prior kidney transplant recipient with excellent results at four-year follow-up (unpublished data)<sup>[78]</sup>.

### Pediatric hand transplantation

The greater plasticity of the immature brain and longer potential lifetime are potential advantages of VCA in the pediatric patient. Obvious challenges of upper extremity VCA in this young population include informed consent, psychosocial assessment, greater surgical risk in part due to increased technical complexity, lack of assessment tools of objective outcomes, and compliance with rehabilitation/



immunosuppression demands<sup>[77]</sup>. Preoperative evaluation of the pediatric patient includes multiple visits with orthopedic/plastic surgery, transplantation medicine, and occupational/physical therapy<sup>[78,88]</sup>. The child's goals for functional independence are the focus of the occupational therapy assessment<sup>[77]</sup>. Psychosocial aspects are assessed by a child psychologist, pediatric transplantation pharmacist, and a social worker. Informed consent must include a discussion with both the patient and parents regarding all possible risks from the surgery while addressing the unknowns.

The consequences of long-term immunomodulation may become more apparent in this group and some remained concerned for potential learning disabilities and growth impairment<sup>[95]</sup>. It is unclear if pediatric patients will have worse rejection episodes or adverse effects of medications when compared with adult patients, although that has not been a concern in our pediatric VCA patient to date. In addition, unpublished data from our institution suggest that hand-forearm growth in the pediatric patient are as you would expect based on replantation literature and normal growth in the non-transplanted pediatric population.

### **Overseas hand-forearm transplantation**

For the first time in upper extremity VCA history, a successful transatlantic upper extremity VCA was performed at the University of Pennsylvania. Our group was approached by colleagues from Hôpital Européen Georges Pompidou at Paris Descartes University in 2016 to list a European patient for a bilateral hand transplant. The recipient and donor were separated hundreds of miles between France (country of residence) and the United States (VCA team), respectively. The recipient's preoperative assessment was done in France; however, she was unable to have the transplant in her country due to health-system issues. The director of the University of Pennsylvania program (Levin LS) raised support, including financial, for the endeavor and coordinated the team of 30+ specialists and surgeons who would be necessary to perform the transplant. Meanwhile, the patient underwent health screening in France and, simultaneously, coordination with the organ procurement organization was performed to enable a match. Our team calculated precise travel scenarios so that, if donor limbs became available, the recipient patient could begin her travels to Philadelphia in time. Appropriate documentation for travel was ensured much in advance of donor limb availability. In August 2016, the director of the hand transplant program received a call that donor hands were available and the patient then immediately embarked on a 700 mile trip to Philadelphia. The surgery proceeded as expected, lasting nine hours. Aside from the need for minor hematoma evacuation on Postoperative Day 17 for bilateral extremities, the recovery was excellent. French surgeons and specialists with extensive VCA experience then assumed her care upon return, and she returned to the University of Pennsylvania for follow-up eight months later. Her motor and sensory exam has been improving [Figure 7]. Since then, another transatlantic hand transplantation was performed, again in a French recipient, without any event. We were happy to have had such an impact on the quality of life of these overseas patients.

### **Technical considerations**

Checklists are followed closely for all aspects of the operation. In brief, the recipient is prepared first by obtaining peripheral nerve blocks and central venous and large bore intravenous access. General anesthesia is then administered and the residual limbs are dissected under tourniquet control, identifying and tagging all key structures similar to the donor limb preparation. This can be the most difficult part of the operation as tendons, vessels, and nerves are often enveloped in scar tissue. Simultaneously, the donor team is working to prepare and tag vital structures in the transplant extremities. The bones are then prepared with custom cut guides according to the preoperative plan. Once the donor and recipient limbs are fully prepared and structures tagged by the surgical teams, osteosynthesis is performed. Depending on the cold ischemia time and level of transplant, either vascular anastomoses can be performed at this stage or tendon/muscle repairs. We prefer to reperfuse the limbs as soon as possible to limit ischemia-reperfusion injury. This is followed by tendon/muscle repairs and then nerve repairs. Skin closure is performed in a



**Figure 7.** Nearly three-year follow-up of the world's first overseas hand-forearm transplantation

nonconstructive manner. The limbs are photographed, carefully dressed, and splinted. The patient then recovers in the Intensive Care Unit for close monitoring.

### Immunosuppressive protocol

The immunologic profile of hand transplants differs slightly when compared with solid organ transplants due to their composite tissue, including bone, tendon, nerve, muscle, and skin, which is more antigenic<sup>[88]</sup>. That being said, the immunosuppressive therapy is modeled after solid organ transplantation<sup>[96]</sup>. This includes induction therapy of several perioperative doses of polyclonal/monoclonal antibodies such as thymoglobulin along with IV steroids. Maintenance therapy consists of a multidrug regimen of tacrolimus (calcineurin inhibitor), mycophenolate mofetil, and prednisone<sup>[16]</sup>. Alterations to this standard in an attempt to reduce acute rejection episodes have been investigated and applied, including steroid withdrawal, conversion to mammalian target of rapamycin (mTOR) inhibitor sirolimus from tacrolimus, the use of topical steroids, and Belatacept (selective T-cell costimulation blocker)<sup>[79,97-100]</sup>. Switching tacrolimus to sirolimus has been done because of increased creatinine values, resulting in normalization of levels<sup>[99]</sup>. Immunomodulation strategies to create a state of immunologic chimerism in the recipient, such as infusion of donor-derived bone marrow stem cells as part of induction therapy, have been shown to be safe, well-tolerated, and allow for low-dose tacrolimus monotherapy<sup>[101]</sup>. Furthermore, delivery of rapamycin has been shown to promote immunoregulation and survival of the VCA<sup>[102]</sup>.

Consequences of prolonged immunosuppression include opportunistic infections, and medication side effects such as metabolic disorders, nephrotoxicity, neurotoxicity, avascular necrosis, and wound healing issues related to steroid use and neoplasms (e.g., cutaneous and lymphoproliferative disorders). The future of hand transplantation will depend on minimizing side effects of immunosuppression, or perhaps even more ambitious, achieving donor-specific immunologic tolerance and rejection-free maintenance without the use of immunosuppression. Prophylaxis therapy includes antibiotics for 10 days, trimethoprim/sulfamethoxazole to prevent *Pneumocystis carinii* pneumonia, and valganciclovir to prevent cytomegalovirus infection/reactivation for six months<sup>[99]</sup>.

### Postoperative course

Standardized vigilant follow-up is paramount to monitor for signs of surgical complications, acute/chronic rejection, and untoward effects of immunosuppression. This becomes even more critical with the growing transatlantic cases, which require follow-up with a treatment team with expertise in VCA in the area of residence in addition to follow-up with the team that performed and is familiar with the transplant. At six

weeks postoperatively, the patient typically may return to their home residence. We have used FaceTime, Skype, and other Internet-based video platforms to communicate internationally on an as needed basis. In-person visits are coordinated between the patient and the treatment team at the center involved in the transplant and occur every 2-3 months during the first year and every 6-12 months thereafter<sup>[88]</sup>.

Although superficial (e.g., skin) detection of rejection is more easily accomplished than that of deeper structures, the clinical signs of skin rejection are nonspecific. These include findings such as edema, erythema, erythematous maculopapular rash, hair loss, and desquamation at the fingers<sup>[80,103]</sup>. Deep biopsies are rarely performed to diagnose rejection, but one may see lymphocytic infiltrates. Chronic rejection findings are nonspecific, but involve vessel intimal hyperplasia<sup>[104-106]</sup>. Our protocol includes weekly skin biopsies and laboratory tests (renal function, immunosuppression drug concentrations, and complete blood counts) for the first month and subsequent tapering depending on the stability of the drug levels and allograft.

Outcomes following VCA are highly dependent on rehabilitation, which is extensive and can last 2-4 years to optimize cortical reintegration of the transplanted extremity<sup>[107]</sup>. Rehabilitation typically begins anywhere from 12 h to three days after surgery depending on the transplant surgeon preference and is extensive, with physiotherapy, electrostimulation, and occupational therapy components<sup>[88,99]</sup>. Physical therapy exercises include edema management, gentle range of motion exercises, and custom orthosis management. Different splints are used to protect the grafts, avoid retraction, and facilitate the right position of each hand part after extensor and flexor tendon balance. Therapy is extensive during the first year and is usually 4-5 h, five days a week. This regimen is gradually tapered depending on the level of transplant and needs of the patient<sup>[108]</sup>.

Rehabilitation for the pediatric transplant recipient was more difficult due to their attention span, motivations, and emotions. Therapy began six days after transplantation and continued daily for five weeks in acute care, then two weeks in inpatient rehabilitation, followed by ongoing/school therapy. Time for patient-caregiver bonding, child life activities, and rest was set-aside during the therapy days and is as important as the therapy itself. At seven weeks, the patient transitioned to a day hospital program in his community where he received therapy and schooling for five days per week, and then transitioned to outpatient therapy. Therapy is ongoing at four years post-surgery and the exact duration necessary continues to be an area of investigation. Our therapists suggest continuing therapy and minimizing compensatory motor strategies for two years following plateau of sensorimotor function and cortical plasticity. Additional procedures may be necessary during the course of follow-up and this should be discussed preoperatively. These include cosmetic revisions, hardware removal, tendon transfers, and tendon shortening<sup>[99]</sup>.

### VCA outcomes

Standardizing outcome measures remains a challenge for extremity VCA given the variety of surgical protocols, host risk factors, immunosuppressive regimens, and transplant patient/level heterogeneity<sup>[88]</sup>. Moreover, measures and definitions of success are variable. When considering the pediatric patient, there are no validated outcomes measured for hand function. For the adult patients at our transplant center, functional outcome measures include the Sollerman test, a standardized measure of manual dexterity for motor functions; the Disabilities of the Arm, Shoulder and Hand questionnaire; and the Hand Transplant Scoring System<sup>[109]</sup>. We utilized the two-point discrimination test and Semmes-Weinstein monofilament tests for sensation<sup>[108]</sup>. For our pediatric transplant recipient, we used the box and block and nine-hole peg tests that elucidate progress at the functional activity level and efficiency of upper extremity gross motor skills compared with baseline<sup>[88]</sup>. We also used the Functional Independence Measure for Children to assess ability to perform daily activities of living<sup>[88]</sup>.

Structural brain MRI and magnetoencephalography to record neural correlates of sensory responses and hand movement, motor cortex mapping with transcranial magnetic stimulation and motor evoked potentials from intrinsic muscles, and functional MRI (fMRI) before and after transplantation are used to track progress. Psychological and social assessments are made through semi-structured interviews to support coping with transplantation and rehabilitation.

Reported outcomes following hand-forearm transplantation have been promising and follow-up now extends over a decade<sup>[82,99]</sup>. It is well known that more proximal amputations fair poorer with transplantation when compared to distal ones given the distance for regeneration. However, continual yearly improvement is seen in the majority of patients<sup>[92]</sup>. An international registry observed that all transplant recipients develop protective sensibility against pain, 90% regained tactile sensibility, 82.3% achieved discriminative fine sensibility, and 75% reported overall improved quality of life<sup>[16]</sup>. A review of five recipients of bilateral hand allotransplantation performed in Lyon France (Follow-up 3-13 years) demonstrated 100% patient and graft survival, adequate sensorimotor recovery (protective/tactile sensitivity), an ability to perform the majority of daily living activities (e.g., eating, shaving, and using the telephone), and normal social lives<sup>[99]</sup>. In fact, a return of sensation, albeit to varying degrees and timing, can generally be expected in upper extremity VCA patients. Follow-up has also demonstrated normal appearance (i.e., color, temperature, texture, and hair and nail growth) and uneventful bone healing, with normal structure of the recipient and transplanted bones. Motion recovery started at 3-6 months for these patients, with extrinsic recovery allowing the patients to grasp large objects, whereas the intrinsic activity started later (9-12 months), increasing in the first five years post-transplant. Patients experienced at least one episode of acute rejections (range 1-6)<sup>[99]</sup>. The Louisville group reported intrinsic function recovery after at least two years of follow-up in three of five patients<sup>[110]</sup>. The Innsbruck group recently reported on an 18-year experience, with outcomes demonstrating improvement in hand function and sensibility in the first five years and stability thereafter<sup>[100]</sup>. This same group also reported that one of the five patients, a unilateral hand transplant recipient, suffered from recurrent and unmanageable antibody mediated rejections, which eventually led to chronic rejection vasculopathy, necessitating amputation seven years postoperatively. Although bias cannot be excluded based on inherent differences in patient populations, a comparison of prosthetic and upper extremity transplant recipients noted improved quality of life in the transplanted group<sup>[111]</sup>.

Aside from wound healing complications, risks of upper extremity VCA include vessel thrombosis, rejection and possible graft loss, hematoma, and deep venous thrombosis. Aside from side effects of immunosuppression, medication side effects include neurotoxicity and nephrotoxicity. Although graft loss was reported in 22.4% of patients in a 2015 review, graft loss may be estimated at < 1% in patients who are compliant with medications<sup>[112]</sup>. Combined vascularized composite allotransplantation of multiple anatomic areas (e.g., face and hand) has demonstrated an unacceptably high risk of complications, including graft loss and death<sup>[92]</sup>. It is estimated that up to 85% of hand transplant recipients may experience acute rejection within the first year, which manifests as cutaneous lesions<sup>[16,113]</sup>. Acute rejection is typically reversed successfully with intravenous steroids or increasing oral steroid dose. Other complications included transient hyperglycemia, renal toxicity related to immunosuppressive drugs, osteopenia, and infectious complications. Vascular thrombosis, when it occurs, may be successfully treated with embolectomy and bypass procedures. To our knowledge, there have been no reported cases of malignancies<sup>[99,100]</sup>.

## CONCLUSION

OI, TMR, and VCA are just a few ways by which our reconstructive ladder is changing. With regards to prosthetic technology, amputees continue to experience high levels of disability, distress, dependency, and overall dissatisfaction. Bionic limbs still do not replicate the complex function of the upper extremity muscles. It is important to recognize that amputation does not necessarily denote failure, but we have

to find ways to provide these patients with means for obtaining better satisfaction and quality of life postoperatively. Despite the advances in VCA over the last 20 years, there are many challenges that face this discipline including indications for patient selection, minimizing immunosuppressive regimens, standardizing outcome measures, and establishing reliable protocols for monitoring, diagnosing, and managing rejection. The question remains if the risks associated with lifelong immunosuppression justify the non-lifesaving operation. Continued discussions of the advances, successes, and failures will determine how rapidly we are able to improve upon the aforementioned reconstructive options.

## DECLARATIONS

### Acknowledgments

The Authors thank Hansjörg Wyss Foundation for their support.

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Azoury SC, Bauder A, Souza JM, Stranix JT, Othman S, McAndrew C, Tintle SM, Kovach SJ, Levin LS

Performed data acquisition, provided administrative, technical, and material support: Azoury SC, Bauder A, Souza JM, Stranix JT, Othman S, McAndrew C, Tintle SM, Kovach SJ, Levin LS

Reviewed the manuscript for content and grammar/spelling mistakes: Azoury SC, Bauder A, Souza JM, Stranix JT, Othman S, McAndrew C, Tintle SM, Kovach SJ, Levin LS

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Levin LS. The reconstructive ladder. An orthoplastic approach. *Orthop Clin North Am* 1993;24:393-409.
2. Azoury SC, Stranix JT, Kovach SJ, Levin LS. Principles of orthoplastic surgery for lower extremity reconstruction: why is this important? *J Reconstr Microsurg* 2019; Epub ahead of print [PMID: 31454835 DOI: 10.1055/s-0039-1695753]
3. Janis JE, Kwon RK, Attinger CE. The new reconstructive ladder: modifications to the traditional model. *Plast Reconstr Surg* 2011;127:205-12S.
4. Mathes SJ, Nahai F. Classification of the vascular anatomy of muscles: experimental and clinical correlation. *Plast Reconstr Surg* 1981;67:177-87.
5. Gottlieb LJ, Krieger LM. From the reconstructive ladder to the reconstructive elevator. *Plast Reconstr Surg* 1994;93:1503-4.
6. Wong CJ, Niranjana N. Reconstructive stages as an alternative to the reconstructive ladder. *Plast Reconstr Surg* 2008;121:362-3e.



7. Erba P, Ogawa R, Vyas R, Orgill DP. The reconstructive matrix: a new paradigm in reconstructive plastic surgery. *Plast Reconstr Surg* 2010;126:492-8.
8. Zuo KJ, Willand MP, Ho ES, Ramdial S, Borschel GH. Targeted muscle reinnervation. *Plast Reconstr Surg* 2018;141:1447-58.
9. Fracol ME, Janes LE, Ko JH, Dumanian GA. Targeted muscle reinnervation in the lower leg. *Plast Reconstr Surg* 2018;142:541-50e.
10. Dumanian G, Souza J. Surgical techniques for targeted muscle reinnervation. 2013. pp. 21-44.
11. Tintle SM, LeBrun C, Ficke JR, Potter BK. What is new in trauma-related amputations. *J Orthop Trauma* 2016;30:S16-20.
12. Hebert JS, Rehani M, Stiegelmar R. Osseointegration for lower-limb amputation: a systematic review of clinical outcomes. *JBJS Rev* 2017;5:e10.
13. Brånemark RP, Hagberg K, Kulbacka-Ortiz K, Berlin Ö, Rydevik B. Osseointegrated percutaneous prosthetic system for the treatment of patients with transfemoral amputation. *J Am Acad Orthop Surg* 2019;27:e743-51.
14. Levin LS. From autotransplantation to allotransplantation: a perspective on the future of reconstructive microsurgery. *J Reconstr Microsurg* 2018;34:681-2.
15. Carlsen BT, Prigge P, Peterson J. Upper extremity limb loss: functional restoration from prosthesis and targeted reinnervation to transplantation. *J Hand Ther* 2014;27:106-14.
16. Petruzzo P, Dubernard JM. The international registry on hand and composite tissue. *Clin Transpl* 2011;247-53.
17. Kuiken TA, Li G, Lock BA, Lipschutz RD, Miller LA, et al. Targeted muscle reinnervation for real-time myoelectric control of multifunction artificial arms. *JAMA* 2009;301:619-28.
18. Souza JM, Cheesborough JE, Ko JH, Cho MS, Kuiken TA, et al. Targeted muscle reinnervation: a novel approach to postamputation neuroma pain. *Clin Orthop Relat Res* 2014;472:2984-90.
19. Cheesborough JE, Souza JM, Dumanian GA, Bueno RA. Targeted muscle reinnervation in the initial management of traumatic upper extremity amputation injury. *Hand* 2014;9:253-7.
20. Miranda RA, Casebeer WD, Hein AM, Judy JW, Krotkov EP, et al. DARPA-funded efforts in the development of novel brain-computer interface technologies. *J Neurosci Methods* 2014;244:52-67.
21. Mioton LM, Dumanian GA. Targeted muscle reinnervation and prosthetic rehabilitation after limb loss. *J Surg Oncol* 2018;118:807-14.
22. Bowen JB, Wee CE, Kalik J, Valerio IL. Targeted muscle reinnervation to improve pain, prosthetic tolerance, and bioprosthetic outcomes in the amputee. *Adv Wound Care* 2017;6:261-7.
23. Atzori M, Müller H. Control capabilities of myoelectric robotic prostheses by hand amputees: a scientific research and market overview. *Front Syst Neurosci* 2015;9:162.
24. Hargrove LJ, Miller LA, Turner K, Kuiken TA. Myoelectric pattern recognition outperforms direct control for transhumeral amputees with targeted muscle reinnervation: a randomized clinical trial. *Sci Rep* 2017;7:13840.
25. Kuiken TA, Miller LA, Turner K, Hargrove LJ. A comparison of pattern recognition control and direct control of a multiple degree-of-freedom transradial prosthesis. *IEEE J Transl Eng Heal Med* 2016;4:2100508.
26. Gart MS, Souza JM, Dumanian GA. Targeted muscle reinnervation in the upper extremity amputee: a technical roadmap. *J Hand Surg Am* 2015;40:1877-88.
27. Pierrie SN, Gaston RG, Loeffler BJ. Targeted muscle reinnervation for prosthesis optimization and neuroma management in the setting of transradial amputation. *J Hand Surg Am* 2019;44:525.e1-525.e8.
28. Morgan EN, Potter BK, Souza JM, Tintle SM, Nanos GP. Targeted muscle reinnervation for transradial amputation: description of operative technique. *Tech Hand Up Extrem Surg* 2016;20:166-71.
29. Ortiz-Catalan M, Håkansson B, Brånemark R. An osseointegrated human-machine gateway for long-term sensory feedback and motor control of artificial limbs. *Sci Transl Med* 2014;6:257re6.
30. Takagi T, Ogiri Y, Kato R, Kodama M, Yamanoi Y, et al. Selective motor fascicle transfer and neural-machine interface: case report. *J Neurosurg* 2019;1-7.
31. Gaston RG, Bracey JW, Tait MA, Loeffler BJ. A novel muscle transfer for independent digital control of a myoelectric prosthesis: the starfish procedure. *J Hand Surg Am* 2019;44:163.e1-163.e5.
32. Pasquina PF, Perry BN, Miller ME, Ling GSF, Tsao JW. Practice recent advances in bioelectric prostheses. *Neurol Clin Pract* 2015;5:164-70.
33. Hargrove LJ, Simon AM, Young AJ, Lipschutz RD, Finucane SB, et al. Robotic leg control with EMG decoding in an amputee with nerve transfers. *N Engl J Med* 2013;369:1237-42.
34. Hargrove LJ, Young AJ, Simon AM, Fey NP, Lipschutz RD, et al. Intuitive control of a powered prosthetic leg during ambulation: a randomized clinical trial. *JAMA* 2015;313:2244-52.
35. Lund LH, Benson L. Real-time myoelectric control of knee and ankle motions for transfemoral amputees. *JAMA* 2011;305:1542-4.
36. Pet MA, Ko JH, Friedly JL, Smith DG. Traction neurectomy for treatment of painful residual limb neuroma in lower extremity amputees. *J Orthop Trauma* 2015;29:e321-5.
37. Suckow BD, Goodney PP, Nolan BW, Veeraswamy RK, Gallagher P, et al. Domains that determine quality of life in vascular amputees. *Ann Vasc Surg* 2015;29:722-30.
38. Kuffler DP. Coping with phantom limb pain. *Mol Neurobiol* 2018;55:70-84.
39. Ephraim PL, Wegener ST, MacKenzie EJ, Dillingham TR, Pezzin LE. Phantom pain, residual limb pain, and back pain in amputees: results of a national survey. *Arch Phys Med Rehabil* 2005;86:1910-9.
40. Richardson C, Glenn S, Nurmikko T, Horgan M, Fe C. Incidence of phantom phenomena including phantom limb pain 6 months after major lower limb amputation in patients with peripheral vascular disease. *Clin J Pain* 2006;22:353-8.
41. Ducic I, Mesbahi AN, Attinger CE, Graw K. The role of peripheral nerve surgery in the treatment of chronic pain associated with



- amputation stumps. *Plast Reconstr Surg* 2008;121:908-14.
42. Poyntz SA, Dalal M, Hacking N, Fowler S. Peripheral interventions for painful stump neuromas of the lower limb: a systematic review. *Clin J Pain* 2017;34:285-95.
  43. Ives GC, Kung TA, Nghiem BT, Ursu DC, Brown DL, et al. Current state of the surgical treatment of terminal neuromas. *Clin Neurosurg* 2018;83:354-64.
  44. Pet MA, Ko JH, Friedly JL, Mourad PD, Smith DG. Does targeted nerve implantation reduce neuroma pain in amputees? *Clin Orthop Relat Res* 2014;472:2991-3001.
  45. Dumanian GA, Potter BK, Mioton LM, Ko JH, Cheesborough JE, et al. Targeted muscle reinnervation treats neuroma and phantom pain in major limb amputees: a randomized clinical trial. *Ann Surg* 2019;270:238-46.
  46. Agnew SP, Schultz AE, Dumanian GA, Kuiken TA. Targeted reinnervation in the transfemoral amputee: a preliminary study of surgical technique. *Plast Reconstr Surg* 2012;129:187-94.
  47. Fracol ME, Janes LE, Ko JH, Dumanian GA. Targeted muscle reinnervation in the lower leg: an anatomical study. *Plast Reconstr Surg* 2018;142:541-50e.
  48. Curtin C. Pain examination and diagnosis. *Hand Clin* 2016;32:21-6.
  49. Eberlin KR, Ducic I. Surgical algorithm for neuroma management: a changing treatment paradigm. *Plast Reconstr Surg Glob Open* 2018;6:e1952.
  50. Poppler LH, Parikh RP, Bichanich MJ, Rebehn K, Bettlach CR, et al. Surgical interventions for the treatment of painful neuroma: a comparative meta-analysis. *Pain* 2018;159:214-23.
  51. Kubiak CA, Kemp SWP, Cederna PS. Regenerative peripheral nerve interface for management of postamputation neuroma. *JAMA Surg* 2018;153:681-2.
  52. Kung TA, Langhals NB, Martin DC, Johnson PJ, Cederna PS, et al. Regenerative peripheral nerve interface viability and signal transduction with an implanted electrode. *Plast Reconstr Surg* 2014;133:1380-94.
  53. Frost CM, Ursu DC, Flattery SM, Nedic A, Hassett CA, et al. Regenerative peripheral nerve interfaces for real-time, proportional control of a neuroprosthetic hand. *J Neuroeng Rehabil* 2018;15:108.
  54. Biddiss E, Chau T. Upper limb prosthesis use and abandonment: a survey of the last 25 years. *Prosthet Orthot Int* 2007;31:236-57.
  55. Paternò L, Ibrahimi M, Gruppioni E, Menciassi A, Ricotti L. Sockets for limb prostheses: a review of existing technologies and open challenges. *IEEE Trans Biomed Eng* 2018;65:1996-2010.
  56. Hagberg K, Häggström E, Uden M, Brånemark R. Socket versus bone-anchored trans-femoral prostheses: hip range of motion and sitting comfort. *Prosthet Orthot Int* 2005;29:153-63.
  57. Tranberg R, Zügner R, Kärrholm J. Improvements in hip- and pelvic motion for patients with osseointegrated trans-femoral prostheses. *Gait Posture* 2011;33:165-8.
  58. Van De Meent H, Hopman MT, Frölke JP. Walking ability and quality of life in subjects with transfemoral amputation: a comparison of osseointegration with socket prostheses. *Arch Phys Med Rehabil* 2013;94:2174-8.
  59. Bothe, RT, Beaton, KE, Davenport H. Reaction of bone to multiple metallic implants. *Surg Gynecol Obs* 1940;71:598-602.
  60. Brånemark PI. Osseointegration and its experimental background. *J Prosthet Dent* 1983;50:399-410.
  61. Li Y, Brånemark R. Osseointegrated prostheses for rehabilitation following amputation. *Unfallchirurg* 2017;120:285-92.
  62. Zaid MB, O'Donnell RJ, Potter BK, Forsberg JA. Orthopaedic osseointegration: state of the art. *J Am Acad Orthop Surg* 2019;27:e977-85.
  63. Yerneni S, Dhaher Y, Kuiken TA. A computational model for stress reduction at the skin-implant interface of osseointegrated prostheses. *J Biomed Mater Res A* 2012;100:911-7.
  64. Al Muderis M, Lu W, Tettsworth K, Bosley B, Li JJ. Single-stage osseointegrated reconstruction and rehabilitation of lower limb amputees: The Osseointegration Group of Australia Accelerated Protocol-2 (OGAAP-2) for a prospective cohort study. *BMJ Open* 2017;7:e013508.
  65. Aschoff HH, Kennon RE, Keggi JM, Rubin LE. Transcutaneous, distal femoral, intramedullary attachment for above-the-knee prostheses: an endo-exo device. *J Bone Joint Surg Am* 2010;92:180-6.
  66. Muderis MA, Khemka A, Lord SJ, Van De Meent H, Frolke JPM. Safety of osseointegrated implants for transfemoral amputees: a two-center prospective cohort study. *J Bone Joint Surg Am* 2016;98:900-9.
  67. Brånemark R, Berlin Ö, Hagberg K, Bergh P, Gunterberg B, et al. A novel osseointegrated percutaneous prosthetic system for the treatment of patients with transfemoral amputation: a prospective study of 51 patients. *Bone Joint J* 2014;96-B:106-13.
  68. Tillander J, Hagberg K, Berlin Ö, Hagberg L, Brånemark R. Osteomyelitis risk in patients with transfemoral amputations treated with osseointegration prostheses. *Clin Orthop Relat Res* 2017;475:3100-08.
  69. Atallah R, Leijendekkers RA, Hoogbeem TJ, Frölke JP. Complications of bone-anchored prostheses for individuals with an extremity amputation: a systematic review. *PLoS One* 2018;13:e0201821.
  70. Mastinu E, Doguet P, Botquin Y, Hakansson B, Ortiz-Catalan M. Embedded system for prosthetic control using implanted neuromuscular interfaces accessed via an osseointegrated implant. *IEEE Trans Biomed Circuits Syst* 2017;11:867-77.
  71. Delmonico FL. Interview with Dr Joseph Murray. *Am J Transplant* 2002;2:803-6.
  72. Fernandez JGG, Febres-Cordero RG, Simpson RL. The untold story of the first hand transplant: dedicated to the memory of one of the great minds of the ecuadorian medical community and the world. *J Reconstr Microsurg* 2019;35:163-7.
  73. Dubernard JM, Owen E, Herzberg G, Lanzetta M, Martin X, et al. Human hand allograft: report on first 6 months. *Lancet* 1999;353:1315-20.
  74. Breidenbach WC, Gonzales NR, Kaufman CL, Klapheke M, Tobin GR, et al. Outcomes of the first 2 American hand transplants at 8 and 6 years posttransplant. *J Hand Surg Am* 2008;33:1039-47.

75. Graham B, Adkins P, Tsai TM, Firrell J, Breidenbach WC. Major replantation versus revision amputation and prosthetic fitting in the upper extremity: a late functional outcomes study. *J Hand Surg Am* 1998;23:783-91.
76. Thuong M, Petruzzo P, Landin L, Mahillo B, Kay S, et al. Vascularized composite allotransplantation - a Council of Europe position paper. *Transpl Int* 2019;32:233-40.
77. Amaral S, Scott Levin L. Pediatric and congenital hand transplantation. *Curr Opin Organ Transplant* 2017;22:477-83.
78. Amaral S, Kessler SK, Levy TJ, Gaetz W, McAndrew C, et al. 18-month outcomes of heterologous bilateral hand transplantation in a child: a case report. *Lancet Child Adolesc Heal* 2017;1:35-44.
79. Shores JT, Imbriglia JE, Lee WPA. The current state of hand transplantation. *J Hand Surg Am* 2011;36:1862-7.
80. Shores JT, Malek V, Lee WPA, Brandacher G. Outcomes after hand and upper extremity transplantation. *J Mater Sci Mater Med* 2017;28:72.
81. Cavadas PC, Landin L, Ibañez J. Bilateral hand transplantation: result at 20 months. *J Hand Surg Eur Vol* 2009;34:434-43.
82. Weissenbacher A, Pierer G, Gabl M, Ninkovic M, Hautz T, et al. VCA at innsbruck medical university - an update 14 years after the first hand transplantation. *Transplantation* 2014;98:45.
83. Francois CG, Breidenbach WC, Maldonado C, Kakoulidis TP, Hodges A, et al. Hand transplantation: comparisons and observations of the first four clinical cases. *Microsurgery* 2000;20:360-71.
84. Pei G, Gu L, Yu L. A preliminary report of two cases of human hand allograft. *Zhonghua Yi Xue Za Zhi* 2000;80:417-21. (in Chinese)
85. Jones JW, Gruber SA, Barker JH, Breidenbach WC. Successful hand transplantation: one-year follow-up. *N Engl J Med* 2000;343:468-73.
86. Ben-Amotz O, Kruger EA, McAndrew C, Lantieri L, Bozentka D, et al. Logistics in coordinating the first adult transatlantic bilateral hand transplant: lessons learned. *Plast Reconstr Surg* 2018;142:730-5.
87. Carty MJ, Zuker R, Cavadas P, Pribaz JJ, Talbot SG, et al. The case for lower extremity allotransplantation. *Plast Reconstr Surg* 2013;131:1272-7.
88. Mendenhall SD, Brown S, Ben-Amotz O, Neumeister MW, Levin LS. Building a hand and upper extremity transplantation program: lessons learned from the first 20 years of vascularized composite allotransplantation. *Hand (N Y)* 2018.
89. Elliott RM, Tintle SM, Levin LS. Upper extremity transplantation: current concepts and challenges in an emerging field. *Curr Rev Musculoskelet Med* 2014;7:83-8.
90. Colen DL, Carney MJ, Shubinets V, Lanni MA, Liu T, et al. Soft-tissue reconstruction of the complicated knee arthroplasty: principles and predictors of salvage. *Plast Reconstr Surg* 2018;141:1040-8.
91. Magill G, Benedict J, Plock JA, Kronos T, Gorantla VS. Existing and evolving bioethical dilemmas, challenges and controversies in vascularized composite allotransplantation - an international perspective from the broker bioethics working group. *Transplantation* 2019;103:1746-51.
92. Kubiak CA, Etra JW, Brandacher G, Kemp SWP, Kung TA, et al. Prosthetic rehabilitation and vascularized composite allotransplantation following upper limb loss. *Plast Reconstr Surg* 2019;143:1688-701.
93. Mathes DW, Schlenker R, Ploplys E, Vedder N. A survey of north american hand surgeons on their current attitudes toward hand transplantation. *J Hand Surg Am* 2009;34:808-14.
94. Lee WPA, Shores JT, Brandacher G. From auto- to allotransplantation: immunomodulatory protocol for hand and arm transplantation. *J Reconstr Microsurg* 2018;34:683-4.
95. Doumit G, Gharb BB, Rampazzo A, Papay F, Siemionow MZ, et al. Pediatric vascularized composite allotransplantation. *Ann Plast Surg* 2014;73:445-50.
96. Brandacher G, Gorantla VS, Lee WPA. Hand allotransplantation. *Semin Plast Surg* 2010;24:11-7.
97. Grahmmer J, Weissenbacher A, Zelger BG, Zelger B, Boesmueller M, et al. Benefits and limitations of belatacept in 4 hand-transplanted patients. *Am J Transplant* 2017;17:3228-35.
98. Diaz-Siso JR, Fischer S, Sisk GC, Bueno E, Kueckelhaus M, et al. Initial experience of dual maintenance immunosuppression with steroid withdrawal in vascular composite tissue allotransplantation. *Am J Transplant* 2015;15:1421-31.
99. Petruzzo P, Gazarian A, Kanitakis J, Parmentier H, Guigal V, et al. Outcomes after bilateral hand allotransplantation. *Ann Surg* 2015;261:213-20.
100. Messner F, Hautz T. The innsbruck handtransplant program: eighteen years of experience. *Am J Transplant* 2019;19:615.
101. Schneeberger S, Gorantla VS, Brandacher G, Zeevi A, Demetris AJ, et al. Upper-extremity transplantation using a cell-based protocol to minimize immunosuppression. *Ann Surg* 2013;257:345-51.
102. Sutter D, Dzhanova DV, Prost JC, Bovet C, Banz Y, et al. Delivery of rapamycin using in situ forming implants promotes immunoregulation and vascularized composite allograft survival. *Sci Rep* 2019;9:9269.
103. Sarhane KA, Tuffaha SH, Broyles JM, Ibrahim AE, Khalifian S, et al. A critical analysis of rejection in vascularized composite allotransplantation: clinical, cellular and molecular aspects, current challenges, and novel concepts. *Front Immunol* 2013;4:406.
104. Petruzzo P, Lanzetta M, Dubernard JM, et al. The international registry on hand and composite tissue transplantation. *Transplantation* 2010;90:1590-4.
105. Kaufman CL, Ouseph R, Blair B, Kutz JE, Tsai TM, et al. Graft vasculopathy in clinical hand transplantation. *Am J Transplant* 2012;12:1004-16.
106. Schneeberger S, Gorantla VS, Hautz T, Pulikkottil B, Margreiter R, et al. Immunosuppression and rejection in human hand transplantation. *Transplant Proc* 2009;41:472-5.
107. Ninkovic M, Weissenbacher A, Gabl M, Pierer G, Pratschke J, et al. Functional outcome after hand and forearm transplantation: what can be achieved? *Hand Clin* 2011;27:455-65.

108. Severance G, Walsh L. Rehabilitation after bilateral hand transplantation in the quadrimembral patient: review and recommendations. *Tech Hand Up Extrem Surg* 2013;17:215-20.
109. Datta D, Selvarajah K, Davey N. Functional outcome of patients with proximal upper limb deficiency - acquired and congenital. *Clin Rehabil* 2004;18:172-7.
110. Kaufman CL, Breidenbach W. World experience after more than a decade of clinical hand transplantation: update from the Louisville hand transplant program. *Hand Clin* 2011;27:417-21.
111. Salminger S, Sturma A, Roche AD, Hruby LA, Paternostro-Sluga T, et al. Functional and psychosocial outcomes of hand transplantation compared with prosthetic fitting in below-elbow amputees: a multicenter cohort study. *PLoS One* 2016;11:e0162507.
112. Shores JT, Brandacher G, Lee WPA. Hand and upper extremity transplantation. *Plast Reconstr Surg* 2015;135:351-60e.
113. Schneeberger S, Zelger B, Ninkovic M, Margreiter R. Transplantation of the hand. *Transplant Rev* 2005;19:100-7.

Review

Open Access



# Mesh and plane selection: a summary of options and outcomes

Yewande Alimi<sup>1</sup>, Chamilka Merle<sup>1</sup>, Michael Sosin<sup>2</sup>, Marielle Mahan<sup>3</sup>, Parag Bhanot<sup>1</sup>

<sup>1</sup>Department of Surgery, Medstar Georgetown University Hospital, Washington, DC 20007, USA.

<sup>2</sup>NYU Langone Health, The Hansjörg Wyss Department of Plastic Surgery, New York, NY 10016, USA.

<sup>3</sup>Department of Medicine, Medstar Washington Hospital Center, Washington, DC 20010, USA.

**Correspondence to:** Dr. Parag Bhanot, Department of Surgery, Medstar Georgetown University Hospital, 3800 Reservoir Rd NW Washington, DC 20007, USA. E-mail: PXB129@gunet.georgetown.edu

**How to cite this article:** Alimi Y, Merle C, Sosin M, Mahan M, Bhanot P. Mesh and plane selection: a summary of options and outcomes. *Plast Aesthet Res* 2020;7:5. <http://dx.doi.org/10.20517/2347-9264.2019.39>

**Received:** 15 Oct 2019 **First Decision:** 9 Dec 2019 **Revised:** 1 Jan 2020 **Accepted:** 15 Jan 2020 **Published:** 20 Feb 2020

**Science Editor:** Sahil Kuldip Kapur **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

Abdominal wall reconstruction is a relevant and important topic not only in plastic and reconstructive surgery, but in the practice of general surgeons. The ideal anatomic location for mesh placement during the repair of ventral hernias has been debated; however, the most common anatomic locations include onlay, inlay, sublay-retromuscular, sublay-preperitoneal, and sublay-intraperitoneal techniques, as defined by the European Hernia Society. Additionally, the availability of numerous synthetic and biologic meshes on the market provides for several options for the practicing surgeon. In this review, we provide a summary of the available literature of both the ideal mesh plane and the appropriate opportunities to use both synthetic and biologic meshes.

**Keywords:** Ventral hernia repair, mesh, underlay, onlay, inlay, sublay, synthetic mesh, biologic mesh

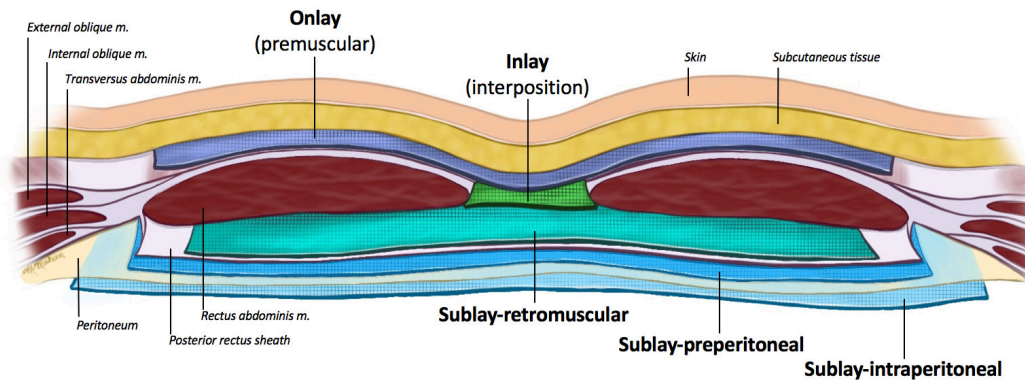
## INTRODUCTION

Defects in the anterior abdominal wall with a resulting ill-defined bulge are both aesthetically displeasing and are associated with musculoskeletal and gastrointestinal dysfunction. The field of abdominal wall reconstruction is a complex topic that comprises a large practice approached by both plastic and reconstructive surgeons and general surgeons. Over one-quarter of all individuals have or will develop a hernia in their lifetime<sup>[1]</sup>. Although the true incidence of incisional hernias is difficult to ascertain,



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1. Abdominal wall demonstrating mesh planes**

approximately 348,000 hernia repairs were performed in the United States in 2006<sup>[2]</sup>. Ventral hernias are an encapsulating term referring to anterior abdominal wall hernias that include the following: epigastric, umbilical, spigelian, parastomal, and most incisional hernias. In the United States alone, over 3.4 billion dollars are spent on the management of hernias<sup>[1]</sup>. The standard approach to ventral hernia repair and the realm of abdominal wall reconstruction is that of repair of the fascial defect with reinforcement of the abdominal wall with mesh. There are many facets to the completion of a ventral hernia repair, including approach to repair, mesh type selection, and mesh plane selection. The approach to the repair can be completed open or through minimally invasive techniques. Presently, minimally invasive techniques utilize both laparoscopic and/or robotic platforms. The selection of the mesh can be that of a prosthetic or biologic variety. Finally, the location in which the selected mesh is placed is crucial to the integrity of the repair. The focus of this review is the latter of these three facets: the choice of mesh and the anatomic location of mesh.

## MESH LOCATION

The ideal anatomic location for mesh placement during the repair of ventral hernias or abdominal wall reconstruction has been debated; however, the most common anatomic locations include: onlay, inlay, sublay-retromuscular, sublay-preperitoneal, and sublay-intraperitoneal [Figure 1]. Numerous single-institution studies, reviews, and meta-analysis have been completed on this topic, still without clear consensus on the ideal location of mesh. The anatomic location of the mesh has an influence on how the mesh is incorporated with the tissues, the tensile strength of the repair and the abdominal wall, and finally the immune reaction between the mesh and the tissue<sup>[3]</sup>. We strive to summarize the advantages and limitations of these locations to make an argument for the ideal mesh plane for ventral hernia repair in abdominal wall reconstruction.

Onlay mesh placement is the placement of mesh on the anterior fascia and is sometimes referred to as a premuscular location of mesh<sup>[4]</sup>. This technique, popularized by Chevrel in 1979, is typically approached in an open fashion with the placement of mesh over the anterior fascia following closure of the fascial defect. The key tenets of this approach include the reapproximation of the linea alba and fixation of mesh to the anterior fascia, which requires the creation of lipocutaneous flaps and the sacrifice of the periumbilical umbilical perforator vessels. The key to the onlay mesh is based on Chevrel's exploration of human cadavers anterior and posterior rectus sheaths. In his 1997 study on cadaveric specimen, Chevrel describes the burst strength of the anterior rectus sheath above the arcuate line to be the strongest portion of the abdominal wall which forms the basis for the onlay approach. He suggests that the strongest reinforcement for the abdominal wall is the combination of the native strength of the anterior rectus sheath in combination with the strength of polypropylene mesh<sup>[5]</sup>.

The inlay (or interposition) technique, as defined by Parker *et al.*<sup>[6]</sup>, is characterized by an approach that places the mesh within the hernia/fascial defect with the mesh fixated to the edges of the neck of the hernia. By definition, the inlay placement of mesh requires a bridging mesh regardless of where the mesh is fixated. If the fascial defect closure is not achieved, this is also considered an interposition mesh. This approach can be completed open, as well as via a minimally invasive approach.

The sublay-retromuscular technique describes mesh placed posterior to the rectus muscle and anterior to the posterior rectus sheath. This plane continues below the arcuate line as the plane between the rectus abdominis muscle and the transversalis fascia<sup>[6]</sup>. This approach was originally described by Rives and Stoppa, and is characterized by opening the rectus sheath and defining the retrorectus plane posterior to the rectus abdominis muscle. The unique characteristics of this repair include the placement of mesh in the well vascularized retrorectus plane. The opening of this plane allows for the medialization and restoration of the linea alba, which results in offloading of tension on the suture line. The posterior fascia is approximated and the mesh is placed anteriorly in the plane between the rectus abdominis muscle and the posterior rectus fascia<sup>[3]</sup>. While this was previously exclusively an open approach as originally described, the sublay-retromuscular approach is now being increasingly performed via a minimally invasive approach. These approaches include combined endoscopic/open procedures as described by Schwarz *et al.*<sup>[7]</sup> in the endoscopic mini/less open sublay repair. Additionally, this sublay-retromuscular approach has now been extensively described as the extended totally extraperitoneal repair, and can be performed both laparoscopically and robotically<sup>[8]</sup>. Belyansky *et al.*<sup>[9]</sup> reported on this novel approach in 2018 and its advantages, including extraperitoneal suture closure of defects, wide mesh coverage in the sublay-retromuscular position with the use of minimal fixation, and an anecdotal appreciation for decreased pain associated with the repair. The sublay-preperitoneal technique describes mesh placement in the plane behind all of the abdominal wall muscles in front of the peritoneum. This technique is more often performed on the robotic platform, given its technical challenge in a laparoscopic approach<sup>[10]</sup>. The sublay-intraperitoneal technique describes mesh placement behind the abdominal wall muscles including the parietal peritoneum<sup>[11]</sup>. If done in an open fashion, the mesh is secured posteriorly to the posterior rectus sheath and the parietal peritoneum of the anterior abdominal. In a minimally invasive approach, both laparoscopically and robotically, the hernia sac is identified and its contents reduced. Although the fascial defect is more often closed in the robotic approach, the defect can also be closed in the laparoscopic approach<sup>[12]</sup>. Regardless of defect closure, the mesh is then secured in place underlying fascia<sup>[13,14]</sup>.

## ADVANTAGES AND CURRENT DATA

The topic of mesh selection has been an ongoing debate in the surgical community and will not serve as the focus of this review. However, regardless of mesh selection, the optimal location remains up for debate. Mesh implantation has been reported with both prosthetic and biologic varieties; however, multiple factors such as hospital contracts, surgeon experience, and cost drive the decision for mesh selection<sup>[13,14]</sup>. In Sosin *et al.*'s<sup>[15]</sup> 2018 meta-analysis of ventral hernia repairs, 6227 patients undergoing ventral hernia repair with mesh were aggregated in a total of 51 studies. The overall recurrence rate for all comers was 8.9% regardless of location of mesh. Notably, there was a statistically significant difference in recurrent rates that was dependent on the location of mesh. The lowest recurrence rate in this meta-analysis was in mesh placed in the sublay-retromuscular plane, with a 5.8% hernia recurrence rate ( $P = 0.023$ ). Recurrence rates in the sublay-intraperitoneal and sublay-preperitoneal (summarily referenced as underlay in Sosin *et al.*'s<sup>[16]</sup> metanalysis) mesh placement were 10.9% and 12.9%, respectively. The highest hernia recurrence rate of 21.6% was observed in patients who underwent an inlay mesh placement<sup>[8,9]</sup>. Additionally, on repeated meta-analysis performed by Holihan *et al.*<sup>[16,17]</sup>, the sublay-retromuscular repair demonstrated a lower risk of recurrence and surgical site infection, when compared to onlay, inlay, and sublay-intraperitoneal or sublay-preperitoneal mesh approaches (range: OR: 0.45-0.79). The sublay-retromuscular repair was given a moderate recommendation of being the best approach when considering recurrence rate and surgical site



infections<sup>[16]</sup>. When evaluating mesh location specific to synthetic versus biologic mesh placement, these distinctions did not remain. Specifically, in all patients who underwent synthetic mesh placement, mesh location was not a statistically significant predictor for recurrence rates ( $P = 0.95$ )<sup>[16]</sup>.

When evaluating overall complication rates, Sosin *et al.*'s<sup>[15]</sup> review highlights similar overall complication rates observed in ventral hernia repairs, which ranged from 32.6% to 39.1%, regardless of mesh location with no statistically significant difference ( $P = 0.738$ ). While the onlay approach is generally considered the least technically challenging approach to mesh placement, it has fallen out of favor due to the reported increased wound and mesh infection complications<sup>[18]</sup> with approximately 7.6% of hernia repairs as of 2018 being performed in this plane. This is compared to greater than 65% of meshes being placed in the sublay-intraperitoneal, preperitoneal plane, or retromuscular plane in their pooled analysis of reported ventral hernia repairs<sup>[15]</sup>. The mean infection rate in the onlay subgroup was 14%. The mean hematoma/seroma complication rate was found to be 17.4%, the highest amongst the four subgroups. However, the differences amongst complications in different mesh planes was not significant. The onlay approach's largest disadvantage is the mesh's direct contact with the environment during revision of the wound, which can lead to the subsequent wound complications observed in these studies<sup>[19]</sup>.

The inlay technique, which requires a bridging mesh, is performed when the fascial defect cannot be closed. Laparoscopic repair was the dominant approach for this mesh placement accounting for 72.6% vs. 27.4% for open repairs. Infection rates in this approach was 12% and mean hematoma/seroma rate was 12.2%, which did not significantly differ among the four techniques. Hernia recurrence was the highest in this subgroup, with a 21.6% hernia recurrence rate. The sublay-retromuscular approach to mesh placement can be achieved both via an open surgical approach or through minimally invasive techniques. The open approach remains the dominant surgical approach in Sosin *et al.*'s<sup>[15]</sup> analysis, with 94% accounting for an open repair. The mean infection rate was 10.4% and mean hematoma/seroma rate was 11%. This subgroup had the lowest rate of hernia recurrence, at only 5.8% ( $P = 0.023$ ). The closure of the rectus muscles over prosthetic mesh in a well vascularized plane has proven to result in decreased wound infection rates. The sublay-intraperitoneal technique was achieved both laparoscopically (63%) and through an open surgical technique (37%). The mean infection rate in this group was the highest in Sosin *et al.*'s<sup>[15]</sup> analysis, at 17.7%. This compares to only 10.2% in the sublay-retromuscular cohort; however, in this analysis, these were not found to be statistically significant. Mean hematoma/seroma rate was recorded as 11.5%. Hernia recurrence in this group was 10.9%, the second lowest rate based on anatomic mesh placement<sup>[15]</sup>. These data are summarized in Table 1. These data corroborate previously reported outcomes by Holihan *et al.*<sup>[16]</sup>, who found the lowest odds of developing a surgical site infection in those with a sublay-retromuscular approach (OR: 0.449; 95%CI: 0.12-1.16) when compared to onlay mesh placement. The sublay-intraperitoneal or sublay-preperitoneal was almost double the odds (OR: 0.878; 95%CI: 0.29-1.99). Notably, infection rates are significantly different when evaluating open versus laparoscopic approach. This is demonstrated in Table 2. In Gokcal *et al.*'s<sup>[10]</sup> single institution comparison of robotic preperitoneal and intraperitoneal ventral hernia repair, perioperative outcomes at three months were similar. Extremely short-term outcomes at three weeks demonstrated higher surgical site occurrences in the intraperitoneal cohort when compared to the preperitoneal cohort (14% vs. 5.3%,  $P = 0.042$ ).

## MESH SELECTION

Mesh selection is a multifaceted dilemma based on what is familiar to the surgeon, what is available to the surgeon based on institutional contracts and cost, and the approach to repair selected. However, at the core of selection are the properties of the mesh and these in general fall into two categories: biologic and synthetic. Similar to the lack of strong consensus on the optimal location for mesh placement, there remains lack of strong consensus on what type of mesh to use. While there is general consensus on the

**Table 1. Review of outcomes by mesh plane location<sup>[15]</sup>**

Outcomes	Onlay	Inlay	Sublay (Retromuscular)	Sublay (Intraperitoneal/Preperitoneal)	P value
Infection	14.0%	12.0%	10.2%	17.7%	0.276
Seroma/Hematoma	17.4%	12.2%	11.0%	11.5%	0.288
Mesh removal	0.3%	0.3%	0.5%	1.1%	0.346
Recurrence	12.9%	21.6%	5.8%	10.9%	0.023
Mortality	0.3%	0.3%	0.5%	0.5%	0.929
Overall complication	38.6%	39.1%	32.6%	37.8%	0.738

**Table 2. Review of outcomes by mesh plane location and surgical approach (open vs. laparoscopic)<sup>[15]</sup>**

Outcomes	Onlay	Inlay	Sublay (Retromuscular)	Sublay (Intraperitoneal/Preperitoneal)	P value
Open					
Seroma/Hematoma	22.1%	10.7%	11.0%	7.8%	0.016*
Infection	9.6%	20.9%	12.1%	17.8%	0.121
Recurrence	9.9%	25.4%	6.7%	10.9%	0.020*
Overall complication	36.2%	51.5%	37.0%	37.7%	0.529
Laparoscopic					
Seroma/Hematoma	N/A	10.7%	3.3%	3.5%	0.044*
Infection	N/A	1.3%	0.1%	2.8%	0.605
Recurrence	N/A	10.0%	0.1%	4.2%	0.041*
Overall complication	N/A	24.1%	6.2%	17.8%	0.738

\*Denotes a statistically significant difference. N/A: Not applicable

use of a reinforcement material in the management of ventral/incision hernias, the composition of the reinforcement continues to be debated.

### Synthetic mesh

Permanent synthetic mesh is the most commonly used reinforcement material in clinical practice. Sosin *et al.*'s<sup>[15]</sup> recent review of the literature demonstrated 68.5% of reviewed cases utilizing synthetic mesh, while only 31.5% of cases were performed with biologic mesh. Kingsnorth *et al.*<sup>[20]</sup> reported as high as 90% of cases being performed with synthetic material. The breadth of synthetic mesh available on the market is vast and the products listed here, while not comprehensive, demonstrate the wide variety of products available [Table 3]. These meshes vary in their composition and can be further classified as permanent or bioabsorbable. The advantages of both types of synthetic mesh when compared to biologic mesh are their low cost. Although permanent synthetic mesh in general has the overall lowest cost and lowest recurrence rates, they are not recommended in grossly contaminated and infected fields and reportedly have higher rates of infection, discomfort, and adhesions encountered in re-operative fields. Synthetic meshes are marketed by several manufacturers and are usually made of polypropylene, expanded polytetrafluoroethylene, or polyethylene terephthalate polyester.

Bioabsorbable meshes were devised as an alternative to synthetic meshes offering a safer side effect profile in a contaminated field. They are made of the following materials and marketed under a variety of names by different manufacturers: polyglactin, polyglycolic acid, polyglycolic acid/trimethylene carbonate, poly-4-hydroxybutyrate, and polyglycolide/polylactide/trimethylene carbonate. These degradable materials vary particularly in the time in which they degrade with products such as Polyglactin (Vicryl, Ethicon, USA) degrading in merely 1-3 months while, e.g., Phasix (Bard Davol Inc., USA) is a slowly resorbable mesh biosynthetic mesh with both the biocompatibility and resorbability of a biological mesh and the mechanical strength of a synthetic mesh. The drawback of these materials is the paucity of long-term data demonstrating efficacy with comparable recurrence rates. There are protocols in the pipeline looking at biosynthetics, such as Phasix (Bard Davol Inc., USA), and its long-term outcomes in Ventral Hernia Working Group (VHWG) Grade 3 wounds<sup>[21]</sup>.

**Table 3. Sample of synthetic mesh products by manufacture<sup>[24]</sup>**

Synthetic mesh product name	Manufacture
Prolene	Boston Scientific
Parietex	Medtronic
Polutetrafluoroethylene	W. L. Gore & Associates
Seri	Sofregan Medical Inc.
Marlex	CR Bard
Sepramesh	CR Bard
Proceed	Ethicon
ProGrip	Medtronic
Prolite	Atrium Medical Corp.

**Table 4. Ventral hernia working group classification system for surgical site occurrence risk<sup>[22]</sup>**

VHWG Grade	Characteristics
1. Low risk	Low risk of complications No history of wound infections
2. Co-morbid	Smoker Obese Diabetic Immunosuppressed COPD
3. Potentially contaminated	Previous wound infection Stoma present Violation of the gastrointestinal tract
4. Infected	Infected mesh Septic dehiscence

VHWG: Ventral Hernia Working Group; COPD: chronic obstructive pulmonary disease

In 2010, the VHWG developed a grading system for surgeons to use to determine the complexity of the case with regards to risk of surgical site occurrence (SSO). At that time, this novel grading system created a framework by which a surgeon could assess the risk of SSO based on both the patient's comorbidities and the characteristics of the hernia to be repaired<sup>[22]</sup>. While this grading system does not take into account the size of the hernia defect or if the defect is the result of a recurrence, it created a uniform system by which to categorize wounds based on their SSO risk and provided recommendations as to which mesh types may be appropriate based on these categories of risk [Table 4]<sup>[22]</sup>. With this grading system in mind, the VHWG recommends the use of a prosthetic reinforcement material in the case of all incisional/ventral hernias regardless of whether the midline fascia is reapproximated or not<sup>[22]</sup>. The working group concludes that synthetic mesh should be used in hernias without gross contamination, or Grade 1 categorized patients. In Sosin *et al.*'s<sup>[15]</sup> review, there were notable differences in complications, infections, and the formation of seroma/hematoma in the placement of synthetic mesh in varying planes, as described in Table 5. Recurrence rates and occurrence of mesh removal were statistically similar regardless of mesh plane.

### Biologic mesh

Biologic mesh development occurred because of the need for a material that was believed to heal by tissue ingrowth as opposed to scar formation and encapsulation, which potentially would allow its utilization in an infected or contaminated field. Products on the market are in general created with a decellularized human, porcine, or bovine scaffold, whether dermis, pericardium, or intestinal mucosa. The extracellular collagen matrix is thought to encourage incorporation of the surrounding tissue by ingrowth of the fibrocollagenous tissue and blood vessels<sup>[23]</sup>. Table 6 lists the most commonly used biologic meshes currently on the market<sup>[24]</sup>. While many of the meshes perform in a similar manner, the unique qualities within these meshes include some chemical modifications to create cross-links in the collagen fibers, while others are xenogenic, and some are allogenic. Some have reported that the cross-linking nature of the meshes help to prevent degradation and increase the durability of the product and the repair; however, they

**Table 5. Review of outcomes by mesh plane location with the use of synthetic mesh<sup>[15]</sup>**

Synthetic mesh	Onlay	Inlay	Sublay (Retromuscular)	Sublay (Intraperitoneal/Preperitoneal)	P value
Infection	7.6%	1.3%	10.3%	2.6%	0.008
Seroma/Hematoma	19.7%	10.8%	9.5%	3.5%	0.003
Mesh removal	0.0%	0.3%	0.8%	0.5%	0.594
Recurrence	4.7%	5.7%	5.2%	4.4%	0.952
Overall complication	31.8%	19.1%	31.3%	16.6%	0.022

**Table 6. Biologic mesh types<sup>[24]</sup>**

Biologic mesh types	Component
Alloderm	Human dermis
Allomax	Human dermis
Collamend	Porcine dermis
Fortagen	Porcine intestine collagen
Peramcol	Cross-linked porcine dermis collagen
Periguard	Bovine pericardium
Strattice	Non-cross-linked porcine dermis collagen
SurgiMend	Bovine dermis
Surgisis	Porcine intestine collagen
Tutopas	Bovine pericardium
Veritas	Bovine pericardium
XenMatriX	Porcine dermis

can cause a heightened foreign body reaction and early inflammatory response<sup>[24]</sup>. Others have reported that the crosslinking can contribute to the lack of integration of the mesh with the surrounding tissue with resultant encapsulation of the mesh and may result in decreased tensile strength<sup>[25]</sup>. Non-crosslinked products, such as Strattice (Medtronic Inc., Dublin, Ireland), are reported to demonstrate fewer adhesions and complications, when compared to the cross-linked products, such as Permacol (Medtronic Inc., Dublin, Ireland). The human acellular dermal matrices, such as Alloderm (Allergan Plc, Dublin, Ireland), have been shown to have higher failure rates including eventration secondary to higher elastin content<sup>[26]</sup>.

While there are no randomized control trials comparing synthetic versus biologic mesh in VHWG Grade > 1, the VHWG recommends the use of biologic mesh in incisional hernias with VHWG Grade 4, which describes a wound that was involved with infected mesh or a septic dehiscence. There are ample studies suggesting the increased rate of reoperation and need for removal of mesh due to additional mesh infections when placing synthetic mesh in a grossly contaminated or infected field. In the setting of a potentially contaminated field, or VHWG 3, the VHWG advises against the use of synthetic mesh and acknowledges there may be benefit to the use of a biologic prosthesis. In general, no strong recommendations exist for the absolute use of specific biologic prosthesis; however, Liang *et al.*<sup>[27]</sup>, reiterated the need for randomized control trials comparing synthetic, biologic, and bioabsorbable meshes to provide clarity on their respective uses<sup>[22]</sup>. Nonetheless, many experts avoid the routine use of biologic mesh in clean cases and reserve its use in the setting of high risk patients and grossly contaminated cases<sup>[27]</sup>. In our practice, we limit its use to the grossly contaminated field. Sosin *et al.*'s<sup>[15]</sup> review demonstrates overall unfavorable outcomes occurred when compared to synthetic meshes. Plane selection with the use of biologic mesh were similar in overall complications, except in the occurrence of hematomas/seromas in which the underlay location of mesh resulted in the lowest occurrence of hematomas/seromas [Table 7]. However, when looking at the occurrence of overall infections in the synthetic mesh group compared to that of the biologic mesh group, plane for plane, there is an overwhelmingly higher occurrence of wound infection in the biologic cohort when compared to the synthetic cohort. This may be explained by the general use of synthetic mesh in a clean operative field compared to that of biologic mesh. Nonetheless, even in the preferred sublay-intraperitoneal plane, there was as high as 19.2% infection occurrence with the use of biologic mesh compared to only 2.6% in the synthetic mesh cohort.

**Table 7. Review of outcomes by mesh plane location with the use of biologic mesh<sup>[15]</sup>**

Biologic mesh	Onlay	Inlay	Sublay (Retromuscular)	Sublay (Intraperitoneal/Preperitoneal)	P value
Infection	21.3%	23.3%	18.1%	19.2%	0.814
Seroma/Hematoma	32.3%	10.8%	11.2%	8.0%	< 0.001
Mesh removal	0.0%	0.0%	1.6%	0.5%	0.359
Recurrence	28.6%	29.1%	11.6%	11.2%	0.016
Overall complication	57.3%	54.2%	40.9%	40.9%	0.197

## CONCLUSION

Half a million patients undergo ventral hernia repair annually in the United States as a result of incisional hernias, failed repairs, *de novo* abdominal wall defects, and abdominal catastrophes. Although the standard approach of hernia repair has been well studied, the ideal anatomic location of mesh placement is still highly debated. Sosin *et al.*'s<sup>[15]</sup> systematic review of mesh placement found that anatomic location can change outcomes in hernia recurrence. Analysis of 51 articles showed that, of the four mesh techniques, namely onlay, interposition, sublay-retromuscular, and sublay-preperitoneal/sublay-peritoneal, the sublay-retromuscular approach is associated with the lowest recurrence rate, whereas the interposition technique is associated with the highest recurrence rate. There was no statistical difference in other complication rates among the four groups, which included postoperative infection, hematoma/seroma formation, mesh explantation, and mortality. Overwhelmingly, the inlay placement of mesh is the least favored and should be avoided if possible. In the minimally invasive approach, both robotically and laparoscopically, the sublay-preperitoneal/sublay-intraperitoneal repair has proven very useful with similar perioperative complications and recurrence rates. In regards to mesh selection, in accordance with the recommendations of the VHWG, we recommend that all ventral hernias be reinforced with mesh regardless of whether the midline fascia can be reapproximated<sup>[22]</sup>. While strong recommendations for the use of synthetic versus biologic mesh are unclear in patients with VHWG Grades 2 and 3, biologic mesh's benefit is clear in grossly contaminated wounds and synthetic mesh is recommended in VHWG Grade 1 patients.

## DECLARATIONS

### Authors' contributions

Conceived the concept of the review and are the primary authors of the manuscript: Alimi Y, Bhanot P  
 Performed significant writing and review of the final manuscript: Merle C  
 Provided feedback, essential data gathering and review of the manuscript: Sosin M  
 Provided principal figures in the article: Mahan M  
 All authors read and approved the final manuscript.

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared no conflicts of interest. Dr. Parag Bhanot is a consultant to Allergan.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

**Copyright**

© The Author(s) 2020.

**REFERENCES**

1. Bedewi MA, El-Sharkawy MS, Al Boukai AA, Al-Nakshabandi N. Prevalence of adult paraumbilical hernia. Assessment by high-resolution sonography: a hospital-based study. *Hernia* 2012;16:59-62.
2. Colavita PD, Walters AL, Tsipline VB, Belyansky I, Lincourt AE, et al. The regionalization of ventral hernia repair: occurrence and outcomes over a decade. *Am Surg* 2013;79:693-701.
3. Timmermans L, de Goede B, van Dijk SM, Kleinrensink GJ, Jeekel J, et al. Meta-analysis of sublay versus onlay mesh repair in incisional hernia surgery. *Am J Surg* 2014;207:980-8.
4. Haskins IN, Voeller GR, Stoikes NF, Webb DL, Chandler RG, et al. Onlay with adhesive use compared with sublay mesh placement in ventral hernia repair: was chevreul right? An Americas hernia society quality collaborative analysis. *J Am Coll Surg* 2017;224:962-70.
5. Rath AM, Zhang J, Chevrel JP. The sheath of the rectus abdominis muscle: an anatomical and biomechanical study. *Hernia* 1997;1:139-42.
6. Parker SG, Wood CPJ, Sanders DL, Windsor ACJ. Nomenclature in abdominal wall hernias: is it time for consensus? *World J Surg* 2017;41:2488-91.
7. Schwarz J, Reinhold W, Bittner R. Endoscopic mini/less open sublay technique (EMILOS)-a new technique for ventral hernia repair. *Langenbecks Arch Surg* 2017;402:173-80.
8. Cox TC, Pearl JP, Ritter EM. Rives-Stoppa incisional hernia repair combined with laparoscopic separation of abdominal wall components: a novel approach to complex abdominal wall closure. *Hernia* 2010;14:561-7.
9. Belyansky I, Daes J, Radu VG, Balasubramanian R, Zahir HR, et al. A novel approach using the enhanced-view totally extraperitoneal (eTEP) technique for laparoscopic retromuscular hernia repair. *Surg Endosc* 2018;32:1525-32.
10. Gokcal F, Morrison S, Kudsi OY. Short-term comparison between preperitoneal and intraperitoneal onlay mesh placement in robotic ventral hernia repair. *Hernia* 2019;23:957-67.
11. Muysoms F, Campanelli G, Champault GG, DeBeaux AC, Dietz UA, et al. EuraHS: the development of an international online platform for registration and outcome measurement of ventral abdominal wall hernia repair. *Hernia* 2012;16:239-50.
12. Chelala E, Baraké H, Estievenart J, Dessily M, Charara F, et al. Long-term outcomes of 1326 laparoscopic incisional and ventral hernia repair with the routine suturing concept: a single institution experience. *Hernia* 2016;20:101-10.
13. Nobaek S, Rogmark P, Petersson U, Hu SB, Sun P, et al. Incisional hernia: complications & quality of life. *Hernia* 2015;19 (Suppl 1):S51-6.
14. Criss CN, Petro CC, Krpata DM, Seafler CM, Lai N, et al. Functional abdominal wall reconstruction improves core physiology and quality-of-life. *Surgery* 2014;156:176-82.
15. Sosin M, Nahabedian MY, Bhanot P. The perfect plane: a systematic review of mesh location and outcomes, update 2018. *Plast Reconstr Surg* 2018;142:S107-16.
16. Holihan JL, Hannon C, Goodenough C, Goodenough C, Flores-Gonzalez JR, et al. Ventral hernia repair: a meta-analysis of randomized controlled trials. *Surg Infect* 2017;18:647-58.
17. Holihan JL, Nguyen DH, Nguyen MT, Mo J, Kao LS, et al. Mesh location in open ventral hernia repair: a systematic review and network meta-analysis. *World J Surg* 2016;40:89-99.
18. Köckerling F, Lammers B. Open intraperitoneal onlay mesh (IPOM) technique for incisional hernia repair. *Front Surg* 2018;5:66.
19. Korenkov M, Paul A, Sauerland S, Campanelli G, Champault GG, et al. Classification and surgical treatment of incisional hernia. *Langenbecks Arch Surg* 2001;386:65-73.
20. Sanders D, Kingsnorth A. The modern management of incisional hernias. *BMJ* 2012;344:e2843.
21. van Rooijen MMJ, Jairam AP, Tollens T, Jørgensen LN, de Vries Reilingh TS, et al. A post-market, prospective, multi-center, single-arm clinical investigation of Phasix™ mesh for VHWG grade 3 midline incisional hernia repair: a research protocol. *BMC Surg* 2018;18:104.
22. Breuing K, Butler CE, Ferzoco S, Franz M, Hultman CS, et al. Incisional ventral hernias: review of the literature and recommendations regarding the grading and technique of repair. *Surgery* 2010;148:544-58.
23. Slater NJ, van der Kolk M, Hendriks T, van Goor H, Bleichrodt RP. Biologic grafts for ventral hernia repair: a systematic review. *Am J Surg* 2013;205:220-30.
24. Rastegarpour A, Cheung M, Vardhan M, Ibrahim MM, Facs CEB, et al. Surgical mesh for ventral incisional hernia repairs: understanding mesh design. *Plast Surg (Oakv)* 2016;24:41-50.
25. Jin J, Rosen MJ, Blatnik J, McGee MF, Williams CP, et al. Use of acellular dermal matrix for complicated ventral hernia repair: does technique affect outcomes? *J Am Coll Surg* 2007;205:654-60.
26. Huntington CR, Cox TC, Blair LJ, Schell S, Randolph D, et al. Biologic mesh in ventral hernia repair: outcomes, recurrence, and charge analysis. *Surgery* 2016;160:1517-27.
27. Liang MK, Holihan JL, Itani K, Alawadi ZM, Flores Gonzalez JR, et al. Ventral hernia management: expert consensus guided by systematic review. *Ann Surg* 2017;265:80-9.



Original Article

Open Access



# Indocyanine green lymphangiography-guided liposuction in breast cancer-related lymphedema treatment - patient selection and technique

Hin-Lun Liu, Melody Man-Kuen Wong, Joseph Hon-Ping Chung

Division of Plastic and Reconstructive Surgery, Department of Surgery, The University of Hong Kong, Queen Mary Hospital, Hong Kong SAR, China.

**Correspondence to:** Dr. Hin-Lun Liu, Division of Plastic and Reconstructive Surgery, Department of Surgery, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong SAR, China. E-mail: lawrencehliu@gmail.com

**How to cite this article:** Liu HL, Wong MMK, Chung JHP. Indocyanine green lymphangiography-guided liposuction in breast cancer-related lymphedema treatment - patient selection and technique. *Plast Aesthet Res* 2020;7:6. <http://dx.doi.org/10.20517/2347-9264.2019.62>

**Received:** 27 Nov 2019 **First Decision:** 13 Jan 2020 **Revised:** 19 Jan 2020 **Accepted:** 7 Feb 2020 **Published:** 20 Feb 2020

**Science Editor:** Xiao Long **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

**Aim:** The rationale of using liposuction to treat lymphedema is that the chronic inflammatory process of lymphedema results in fat hypertrophy. The authors describe the technique of indocyanine green lymphangiography-guided liposuction, its rationale, and our patient selection criteria for better clinical outcomes.

**Methods:** Thirty-two patients underwent liposuction for breast cancer-related lymphedema. Indocyanine green lymphangiography was performed prior to liposuction. For patients without linear and splash patterns in indocyanine green lymphangiography, circumferential liposuction was performed liberally. For patients who had linear or splash patterns, liposuction was not performed at regions with remaining functional lymphatic vessels. Outcomes were assessed using circumferential reduction rate.

**Results:** At a mean follow-up of  $24.5 \pm 6.5$  months, all (100%) patients had a reduction in limb circumferences after liposuction. The mean circumference reduction rate was  $67.6\% \pm 27.9\%$ .

**Conclusion:** Liposuction is a valuable treatment for breast cancer-related lymphedema. We believe patients with fat predominant lymphedema are the best candidates for liposuction.

**Keywords:** Lymphedema, breast cancer-related lymphedema, liposuction, indocyanine green lymphangiography



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



## INTRODUCTION

Although lymphedema starts off as lymph stasis, the disease is not purely obstructive in nature. The stasis of protein rich lymphatic fluid in the subcutaneous plane causes infiltration of immune cells and activation of inflammatory cascade, which in turn lead to chronic inflammation of the subcutaneous plane. The end results of chronic inflammation include fat hypertrophy and fibrosis of lymphatic vessels and connective tissue<sup>[1-6]</sup>.

Physiological operations, e.g., lymphaticovenous anastomosis (LVA) and vascularized lymph node transfer (VLNT), aim at the restoration of normal lymphatic flow<sup>[7-9]</sup>. However, there is no evidence that these physiological type operations can reverse fat hypertrophy. Therefore, excisional treatment is mandatory to achieve a significant limb size reduction in patients with fat hypertrophy.

Liposuction, debulking excision, and Charles operation are the commonly performed excisional operations. Debulking excision mainly tackles excess skin fold, which is usually seen in lower-limb lymphedema. The operation also leaves long linear scars on the limb. Charles operation is almost only performed for patients with late stage, fibrotic lower-limb lymphedema. Therefore, the only sensible option of excisional surgery for upper-limb lymphedema is liposuction.

In our institute, both physiological and excisional operations are available for lymphedema patients. We consider liposuction is a valuable tool for breast cancer-related lymphedema (BCRL). In this article, patient selection and the indocyanine green (ICG) lymphangiography-guided liposuction technique are explained.

## METHODS

### Patients and patient selection

Institutional review board approval was obtained for this study. For the patient selection, only two groups of BCRL patients were included for liposuction. The first group was those who had fatty phase of BCRL; these patients may or may not have received prior surgical treatment of lymphedema. The second group was the good responders after receiving VLNT in our institute.

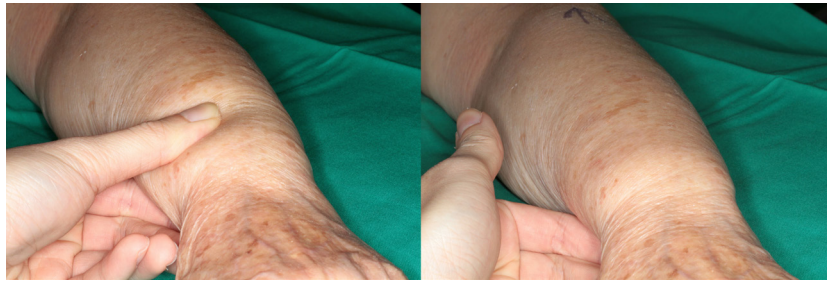
Detailed history taking and physical examination were performed during clinic consultation. Special attention was paid to the softness and the nature of edema of the patient's diseased limb. Patients with soft limb and non-pitting edema are the best candidates for liposuction [Figure 1]. At the other end of the spectrum, patients with tense, fibrotic limb and severe pitting edema were excluded from liposuction.

For patients who were worked up for liposuction, bioimpedance analysis (BIA) was performed as a baseline measurement before operation. Liposuction was only offered to patients who had BIA value < 50.

### Patients

Between January 2015 and December 2018, 32 consecutive patients with BCRL were included in this study. All patients had unilateral upper-limb lymphedema, i.e., the total number of upper limbs treated was 32. The mean age was  $63.4 \pm 8.7$  years (range, 39-72 years). All 32 patients had axillary dissection. Thirty patients received adjuvant radiotherapy. The mean duration of lymphedema was  $10.4 \pm 7.4$  years (range, 6-20 years). The International Society of Lymphology's staging system was adopted in this series<sup>[10]</sup>. Twenty-seven patients had Stage II disease and five patients had late Stage II disease.

Twenty (62.5%) patients had fatty phase lymphedema and none of them had received prior lymphedema surgery. Twelve (37.5%) patients were the good responders of VLNT. The mean time interval between VLNT and liposuction was  $13.1 \pm 2.3$  months (range, 11-15 months).



**Figure 1.** Physical examination can provide useful information on the degree of fluid retention in the edematous limb. In this patient with left upper-limb breast cancer-related lymphedema, her limb was soft and there was no indentation after pressing on the skin. These findings signified that the limb was fat predominate. Patients with fatty phase of lymphedema are the ideal candidates for liposuction



**Figure 2.** A: Indocyanine green-lymphangiography was performed before liposuction; B: The indocyanine green lymphangiography findings were marked on the skin. This patient had a linear pattern at the volar aspect of forearm. Liposuction was not performed in a 2-cm region around the linear pattern (red circle)

### ICG lymphangiography guided liposuction

After general anesthesia, ICG lymphangiography was performed by injecting 5 mg/mL ICG solution to the second and fourth web-spaces and the volar aspect of wrist subdermally, 0.1 mL to each spot. A near infrared camera system was used to capture the ICG signal 30 min after injection. The four patterns of lymphangiography findings, i.e., linear, splash, stardust, and diffuse patterns, were then marked on the skin with a permanent marker pen [Figure 2].

For patients without linear and splash patterns over the edematous limb, circumferential liposuction of the whole upper limb was performed. For patients who still had functional lymphatic vessels in the limb, i.e., linear or splash patterns shown in ICG lymphangiography, liposuction was not performed at the regions of remaining lymphatic vessels. We preferred to have a 2-cm liposuction-free zone around the marked linear and splash patterns.

Usually, for average build patients, two skin incisions, around 1 cm each, over the medial and lateral aspects of elbow sufficed for the access of liposuction cannula to the whole upper limb. An extra skin incision may be made at the lateral aspect of wrist for access to the distal forearm. Tumescence suction-assisted liposuction was subsequently performed [Figure 3].

### Postoperative care

Bandaging was applied to the operated limb immediately after liposuction. During the hospital stay, patients and their family members were educated on limb bandaging by our physiotherapists. Patients were usually discharged on Postoperative Day 2. Follow-up was arranged at two weeks after operation. Patients were advised to perform limb bandaging daily for at least four weeks.



**Figure 3.** The same patient as in Figure 1: suction-assisted liposuction was performed until the overlying skin could be lifted with ease

### Clinical measurement

Limb circumferences of both upper limbs were measured before operation and at a six-month intervals after operation. The circumferences were measured at five levels, i.e., mid-palm, wrist, 10 cm above the wrist, elbow, and 10 cm above the elbow. Readings were taken twice to reduce measurement error.

The circumference difference before and after liposuction was expressed in terms of reduction rate. The numerator was: (pre-liposuction lymphedema limb circumference - pre-liposuction healthy limb circumference) - (post-liposuction lymphedema limb circumference - postop healthy limb circumference). The denominator was: (pre-liposuction lymphedema limb circumference - pre-liposuction healthy limb circumference).

### Statistical analysis

All values are reported as mean  $\pm$  standard deviation. All statistical analyses were performed with IBM SPSS Version 22.0 (IBM Corp., Armonk, NY).

## RESULTS

The mean volume of aspirated fat was  $1137 \pm 126$  mL (range, 1000-1400 mL). At a mean follow-up of  $24.5 \pm 6.5$  months (range, 12-46 months), all (100%) patients had a reduction in limb circumferences [Figure 4]. Throughout the follow-up period, 24 (75%) patients had progressive reduction and reached a plateau in limb size, while eight (25%) patients had fluctuation of limb circumference. Of these eight patients, three patients were those with fatty phase of lymphedema and five were the good responders after VLNT.

The mean circumference reduction rates were  $40.2\% \pm 29.3\%$  (range, 35.4%-50.8%) at mid-palm,  $36.5\% \pm 31.2\%$  (range, 30.3%-45.7%) at wrist,  $57.7\% \pm 22.4\%$  (range, 41.6%-71.3%) at 10 cm above the wrist,  $70.1\% \pm 25.5\%$  (range, 68.2%-76.5%) at elbow, and  $85.7\% \pm 27.8\%$  (range, 73.2%-92.3%) at 10 cm above the elbow. The overall mean circumference reduction rate was  $67.6\% \pm 27.9\%$  (range, 30.3%-92.3%).

## DISCUSSION

The rationale of treating lymphedema with liposuction is the fat hypertrophy caused by chronic inflammatory process<sup>[5,6]</sup>. In lymphedema, the increase in limb size is contributed by the fluid component due to lymphatic obstruction and the fatty component due to chronic inflammation. However, not every patient has the same proportion of fluid retention and fat hypertrophy. We believe that fluid predominant lymphedema is more commonly encountered in the daily practice of most lymphedema practitioners. Therefore, the International Society of Lymphology has devised its staging system according to the nature





**Figure 4.** The same patient as in Figure 1: (A) clinical photo before liposuction; and (B) clinical photo 40 months after liposuction. The limb size was maintained with only regular use of Class I compression sleeve. When liposuction is performed in the right patients, i.e., patients with fatty phase of lymphedema, only low maintenance is required for the control of limb size

of fluid retention<sup>[10]</sup>.

On the other hand, there are patients who have predominately fat hypertrophy. Some lymphedema surgeons called it the fatty phase of lymphedema. This entity is not well documented in textbooks and literature. It also does not fit into any modern lymphedema staging system. In the fatty phase of lymphedema, the limb is often soft without pitting edema. BIA value is usually less than 40. We also observed that this fatty phase of lymphedema is more likely to be associated with severe brachial plexus neuropathy in BCRL patients.

In theory, physiological operations such as LVA and VLNT aim at restoring normal lymphatic flow, which in turn decrease the fluid retention<sup>[7-9]</sup>. These operations are not effective in reducing the fat content of limb. Dr. Brorson *et al.*<sup>[11]</sup> has advocated liposuction since late 1990s<sup>[11,12]</sup>. He and his team treated lymphedema with circumferential liposuction. Preoperative ICG markings were not documented in his studies. Patients need to wear long-term pressure garments after operation lifelong.

Dr. Brorson's liposuction protocol is difficult to follow in our locality. The main reason is the high maintenance after surgery, i.e., regular physiotherapy and long-hour pressure garment. In Hong Kong, the hot and humid weather during summertime discourages the regular use of pressure garment and frequent limb bandaging. Moreover, from our earlier experience when liposuction was performed more liberally for upper-limb lymphedema patients, we noticed the outcome of liposuction for fluid predominant lymphedema, i.e., patients with pitting, tense limb, and high BIA value, is less satisfactory. In the short term, the wound usually remained tense and firm for a longer postoperative period. In the long term, the limb size reduction was not as significant as for patients with limbs that contained less fluid. A relatively higher maintenance, i.e., more intense physiotherapy and more regular use of pressure garment, is also required to keep the reduced limb size.

To utilize liposuction as a treatment and at the same time knowing that the compliance of pressure garment and physiotherapy will be low after operation, we have to tighten the patient selection criteria



to achieve better clinical results and allow patients to have a relatively low maintenance of limb after operation.

We consider patients with fatty phase of lymphedema as the best surgical candidates. The second-best group includes the patients who responded well after VLNT. The response after VLNT was assessed clinically with limb circumference measurement and pitting edema detection. BIA and traditional lymphoscintigraphy were also performed after VLNT. The effect of liposuction in these two patient groups is usually more long lasting. The limbs can stay soft and the reduction in limb size can remain for a longer time.

During the follow-up period, eight patients had fluctuation in limb circumferences. There were overall reductions in limb size after liposuction. However, there was an upward trend of limb circumferences in the middle of the follow-up period. The possible explanations include: (1) inadequate effort of postoperative physiotherapy; or (2) recurrence of fat hypertrophy.

Up until now, there is only one published paper from Campisi *et al.*<sup>[13]</sup> describing lymphatic vessel sparing liposuction. More than 100 lymphedema patients were included in this study. Prior to suction-assisted liposuction, lymphatic mapping with ICG and blue dye was performed. The group reported that there was a significant volume reduction after liposuction. The immediate postoperative ICG lymphangiography also confirmed no lymphatic complications. However, the imaging findings were not listed in detail.

There is no good evidence in the literature to support that: (1) functional lymphatic vessels can be successfully preserved with this maneuver; and (2) the preserved functional lymphatic vessels can improve the long-term results after liposuction. To prove these two points valid, it is necessary to compare the pre- and postoperative (at least mid-term) ICG lymphangiography findings and perform a case-control study to compare the long-term surgical outcomes between liposuction-only group and the liposuction with ICG lymphangiography one. In our opinion, it is sensible to preserve as many remaining functional lymphatic vessels as possible for better lymphatic function. The preserved functional lymphatic vessels can also be used for future surgery such as LVA.

In conclusion, Liposuction is a valuable treatment for BCRL. The role of ICG lymphangiography in improving clinical results and long-term outcomes has yet to be proven by further study.

## DECLARATIONS

### Authors' contributions

Contributed equally to the drafting of manuscript, data collection and analysis; Liu HL, Wong MMK, Chung JHP

### Availability of data and materials

Data were strictly obtained from medical records, in accordance with the privacy policy and code of ethics at our institutions.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

## Ethical approval and consent to participate

The study was approved by the University of Hong Kong Institutional Review Board (IRB reference: UW 14-098).

## Consent for publication

Not applicable.

## Copyright

© The Author(s) 2020.

## REFERENCES

1. Zampell JC, Yan A, Elhadad S, Avraham T, Weitman E, et al. CD4(+) cells regulate fibrosis and lymphangiogenesis in response to lymphatic fluid stasis. *PLoS One* 2012;7:e49940.
2. Ghanta S, Cuzzone DA, Torrisi JS, Albano NJ, Joseph WJ, et al. Regulation of inflammation and fibrosis by macrophages in lymphedema. *Am J Physiol Heart Circ Physiol* 2015;308:H1065-77.
3. Ly CL, Kataru RP, Mehrara BJ. Inflammatory manifestations of lymphedema. *Int J Mol Sci* 2017;18:E171.
4. Koshima I, Kawada S, Moriguchi T, Kajiura Y. Ultrastructural observations of lymphatic vessels in lymphedema in human extremities. *Plast Reconstr Surg* 1996;97:397-405.
5. Brorson H, Ohlin K, Olsson G, Nilsson M. Adipose tissue dominates chronic arm lymphedema following breast cancer: an analysis using volume rendered CT images. *Lymphat Res Biol* 2006;4:199-210.
6. Hoffner M, Peterson P, Månsson S, Brorson H. Lymphedema leads to fat deposition in muscle and decreased muscle/water volume after liposuction: a magnetic resonance imaging study. *Lymphat Res Biol* 2018;16:174-81.
7. Koshima I, Inagawa K, Urushibara K, Moriguchi T. Supermicrosurgical lymphaticovenular anastomosis for the treatment of lymphedema in the upper extremities. *J Reconstr Microsurg* 2000;16:437-42.
8. Becker C, Assouad J, Riquet M, Hidden G. Postmastectomy lymphedema: long-term results following microsurgical lymph node transplantation. *Ann Surg* 2006;243:313-5.
9. Liu HL, Pang SY, Lee CC, Wong MM, Chung HP, et al. Orthotopic transfer of vascularized groin lymph node flap in the treatment of breast cancer-related lymphedema: clinical results, lymphoscintigraphy findings, and proposed mechanism. *J Plast Reconstr Aesthet Surg* 2018;71:1033-40.
10. International Society of Lymphology. The diagnosis and treatment of peripheral lymphedema: 2013 consensus document of the international society of lymphology. *Lymphology* 2013;46:1-11.
11. Brorson H, Svensson H. Complete reduction of lymphoedema of the arm by liposuction after breast cancer. *Scand J Plast Reconstr Surg Hand Surg* 1997;31:137-43.
12. Damstra RJ, Voesten HG, Klinkert P, Brorson H. Circumferential suction-assisted lipectomy for lymphoedema after surgery for breast cancer. *Br J Surg* 2009;96:859-64.
13. Campisi CC, Ryan M, Boccardo F, Campisi C. Fibro-lipo-lymph-aspiration with a lymph vessel sparing procedure to treat advanced lymphedema after multiple lymphatic-venous anastomoses: the complete treatment protocol. *Ann Plast Surg* 2017;78:184-90.

Review

Open Access



# Abdominal wall procedures: the benefits of prehabilitation

Nathan Knapp<sup>1</sup>, Breanna Jedrzejewski<sup>2</sup>, Robert Martindale<sup>1</sup>

<sup>1</sup>Division of Gastrointestinal and General Surgery Department of Surgery, Oregon Health and Science University, Portland, OR 97239, USA.

<sup>2</sup>Division of Plastic and Reconstructive Surgery, Department of Surgery, Oregon Health and Science University, Portland, OR 97239, USA.

**Correspondence to:** Dr. Nathan Knapp, Department of Surgery, Oregon Health and Science University, 3181 S.W. Sam Jackson Park Rd, Portland, OR 97239, USA. E-mail: knappn@ohsu.edu

**How to cite this article:** Knapp N, Jedrzejewski B, Martindale R. Abdominal wall procedures: the benefits of prehabilitation. *Plast Aesthet Res* 2020;7:7. <http://dx.doi.org/10.20517/2347-9264.2019.69>

**Received:** 6 Dec 2019 **First Decision:** 6 Feb 2020 **Revised:** 11 Feb 2020 **Accepted:** 12 Feb 2020 **Published:** 21 Feb 2020

**Science Editor:** Alexander F. Mericli **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

Prehabilitation for abdominal wall procedures provides an opportunity to further modify patient risk factors for surgical complications. It includes interventions that optimize nutrition, glycemic control, functional status, and utilization of the patient's microbiome pre-, intra-, and postoperatively. Through a multidisciplinary and anticipatory approach to patients' existing co-morbidities, the physiological stress of surgery may be attenuated to ultimately minimize perioperative morbidity in the elective setting. With increasing data to support the efficacy of prehabilitation in optimizing surgical outcomes and decreasing hospital length of stay, it is incumbent on the surgeon to employ these practices in elective abdominal wall reconstruction. Further research on the effects of prehabilitation interventions will help to shape and inform protocols that may be implemented beyond abdominal wall procedures in an effort to continually improve best practices in surgical care.

**Keywords:** Prehabilitation, perioperative optimization, abdominal wall reconstruction, minimize co-morbidities

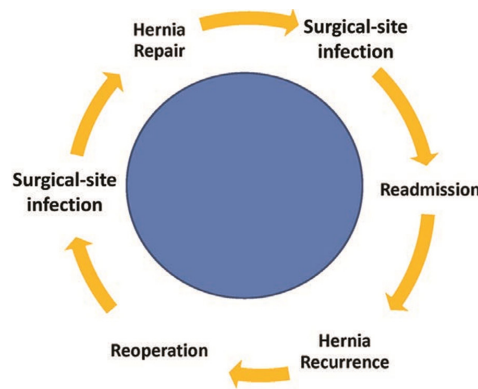
## INTRODUCTION

Achieving optimal surgical outcomes for ventral hernia repairs (VHRs) is inherently challenging. Patients who require complex reconstruction of the abdominal wall are commonly overweight, deconditioned, malnourished with or without sarcopenia, and are often chronically infected/inflamed in the setting of the previously placed synthetic mesh. Most patients in need of reconstruction have had prior repairs/



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** The vicious hernia cycle<sup>[10]</sup>

recurrences or have other significant comorbidities affecting their surgical fitness. Optimizing surgical outcomes and minimizing perioperative morbidity in this patient population requires careful preparation and planning.

## EPIDEMIOLOGY

The burden of VHR and abdominal wall reconstruction (AWR) is increasing not only with regards to incidence but also in the case complexity, contributing to overall higher rates of complications<sup>[1,2]</sup>. While infection remains the most common postoperative complication, the issue of hernia recurrence is arguably the most commonly discussed and used to monitor the success of an outcome<sup>[3-6]</sup>. Following each subsequent repair, the risk of recurrence is linear to and directly related to the number of repairs<sup>[7]</sup>. The financial burden for complications status post hernia surgery are significant: patients with recurrent hernias constitute a minority (15%) of the AWR patient population, yet account for half of the total spending for hernia surgery<sup>[1]</sup>. Recurrent hernia patients tend to be older with more significant medical comorbidities, and are associated with higher hospital and post-discharge health care costs such as readmissions, emergency department visits, *etc.* The magnitude of increased financial burden is likely under-reported as other expenses are more difficult to capture and quantify, including skilled nursing facilities, long-term acute care, wound care, home health services, and hospital readmissions to hospitals other than that of the primary procedure<sup>[8]</sup>. Perioperative surgical site occurrence (infection, seroma, and wound ischemia/dehiscence) increases the risk of hernia recurrence at least three-fold<sup>[5]</sup>. Surgical site infection (SSI) has been shown not only to be independently associated with an increased rate of SSI at subsequent operation in an otherwise clean wound bed, but also to act as a marker of increased case complexity<sup>[9]</sup>. A vicious cycle often develops whereby a ventral herniorrhaphy can lead to an unfortunate pattern of bacterial infection, hernia recurrence, reoperation, and hospital readmission [Figure 1]<sup>[10]</sup>. With an increasing emphasis placed on readmission to determine reimbursement, this cycle looms even larger on the minds of hernia surgeons<sup>[11]</sup>. Therefore, the surgeon should consider optimization of any and all factors that can promote optimal patient recovery.

## THE METABOLIC EFFECTS OF SURGERY

Large hernia repairs and AWR result in considerable surgical stress that induce a predictable sequence of metabolic and physiologic changes in the patient. Further evaluation of these metabolic changes highlights areas for intervention that may allow the patient to respond to the stress with a more favorable physiologic state in the perioperative period. Immediately following surgical incision, the body initiates a response on multiple levels, including the neuroendocrine system, the sympathetic system, and the hypothalamic-pituitary axis. This concert of effects leads the body to tilt toward a catabolic state to provide a metabolic substrate for mounting an acute phase response to the surgical trauma.

**Table 1. Surgeon modifiable risks for preventing complications**

Preoperative	Immediate perioperative	Postoperative
Glycemic control	Skin prep selection	Resistance exercise/early ambulation
Smoking cessation	Antibiotics	Glycemic control
Nutrition	Glycemic control	High protein intake
- Metabolic prep	Hyperoxygenation	Early enteral feeding
- Carbohydrate loading	Drapes/wound protectors	Microbiome
Clearing <i>S. Aureus</i>	MIS surgery	- Probiotics
Weight loss		- Limiting antibiotics
Prehabilitation		Minimize narcotics
- Cardiac/pulmonary conditioning		
- Resistance exercise		

MIS: Minimally Invasive Surgery

Activation of the sympathetic pathway induces a hyperglycemic state via gluconeogenesis and glycogenolysis. Simultaneously, a surge in stress hormones including cortisol, glucagon, prolactin, and growth hormone mediated by the hypothalamic-pituitary axis contributes to insulin resistance and therefore an inability for the body to correct hyperglycemia. In the acute perioperative period, persistent hyperglycemia inhibits immune function and thus surgical recovery by driving catabolic changes via cortisol and glucagon, translating to breakdown of skeletal muscle, loss of lean body mass, and significant deconditioning. While a patient's preoperative physical fitness and young age may also compensate for proteolysis, fat metabolism primarily serves to minimize protein breakdown by mobilizing glycerol and fatty acids for energy usage. However, increased insulin levels and tissue insulin resistance present in times of stress yield a relative decrease in adipose breakdown. Recent literature demonstrates that immune-related nutrients such as glutamine and arginine may be depleted postoperatively and that their replacement may improve surgical outcomes<sup>[12]</sup>. While the effects on the modulation and attenuation of the inflammatory response to the catabolic effects of surgery by omega-3 fatty acids [eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA)] are well documented, recent data suggest that they also serve as a substrate for production of specialized pro-resolving molecules (SPMs). SPMs not only accelerate the resolution of inflammation, decrease post-surgical pain, and enhance the function of macrophages and neutrophils in bacterial killing and clearance, but they do so without increasing the inflammatory state in the process<sup>[13,14]</sup>. Thus, micronutrient supplementation with vitamins may be warranted in patients who are unable to resume a balanced enteral diet in the days following surgery.

## PREOPERATIVE MODIFIABLE RISK FACTORS

The preoperative preparation and optimization serve to acknowledge and modify risk factors that may negatively impact surgical outcomes. Table 1 summarizes the factors that are reviewed in this review.

### Obesity

Over 60% of AWRs are performed on obese patients<sup>[15]</sup> and obesity increases the risk of numerous complications, including seroma, dehiscence, fistula, infections, reoperation, and thromboembolic events. Numerous studies by bariatric surgeons confirm the high incidence of incisional hernias as well as increased rates of wound infections in the obese patient population<sup>[16]</sup>. The reduction of postoperative incisional hernias and wound complications with laparoscopic gastric bypass motivated development of the technique<sup>[17]</sup>. However, the risk of hernia recurrence has been shown to positively correlate with increased body mass index (BMI) regardless of the type of repair performed<sup>[18-20]</sup>. While excess weight must be addressed with patients desiring hernia repair, it is not feasible to expect all hernia patients to achieve ideal weight prior to an operation. We have found that hernia recurrence and surgical site occurrence rates are prohibitively high in patients with a BMI > 50. Therefore, at our institution, elective repairs for patients with BMI > 50 are not performed unless they present with acute concern for bowel compromise.



Weight loss counseling should be a routine component of preoperative visits for those patients with BMI > 35. This counseling involves review of specific dietary modifications, exercise regimen, dietician consult, and establishment of realistic weight loss goals. A reasonable rate of weight loss entails 0.5 kg or one pound per week with a 15-30-pound deficit over 3-6 months. Even with the support of a multidisciplinary clinical team, successful weight loss is greatly variable. Should the patient not meet weight loss goals with dietician support, the date of surgery may be postponed and a referral may be placed to bariatric surgery for evaluation.

In cases where the patient elects to proceed with a weight loss operation, the literature remains split regarding timing of hernia repair. A study using NSQIP data for all VHRs showed an increased risk of infection at 30 days with concurrent VHR and bariatric surgery (sleeve gastrectomy or Roux-en-Y); however, the increased risk did not exceed that expected of dual procedures<sup>[21]</sup>. Thus, the authors of the review advocated for a combined approach to minimize the morbidity of two otherwise separate procedures. We would agree that, with a relatively small ventral hernia in a patient undergoing a laparoscopic sleeve gastrectomy, the benefit of concurrent repair would outweigh separate anesthetic events. However, in our experience, patients undergoing a gastric bypass or who require AWR have improved outcomes after they experience the full scope of benefit from bariatric surgery, including, but not limited to, metabolic, endocrine, and hormonal changes, weight distribution, cardiopulmonary enhancement, and increased mobility. In general, we recommend waiting until the patient's weight has plateaued (typically 18-24 months post-bariatric surgery), and then scheduling a definitive hernia repair 3-4 months later.

### Smoking

Tobacco smoking widely increases the risk of postoperative complications in most procedures, and hernia repair is without exception<sup>[22-25]</sup>. A recent study using NSQIP data examined 30-day outcomes in patients undergoing elective hernia repairs and showed that current smokers were at increased risk of reoperation, readmission, death, wound, and pulmonary complications<sup>[26]</sup>. Several studies examining the effects of smoking have found an increase in wound infection rate after hernia surgery and have identified smoking as an independent risk factor for the development of incisional hernia after abdominal surgery<sup>[23,27,28]</sup>. Smoking has a multifactorial detrimental effect on wound healing due to its reduction of oxygen tension levels in the blood and tissue, disruption of microvasculature, and alteration in surgical site collagen deposition<sup>[29-31]</sup>. VHR and AWR involve several components that may compromise wound healing and promote infection such as undermined skin flaps, myofascial advancement flaps, mesh products, reduction of chronically incarcerated hernia contents, and other concurrent gastrointestinal operations such as fistula take-downs. These factors are compounded with problems associated with active tobacco use, further motivating smoking cessation prior to surgery. Establishing the timing of the "last" cigarette is key as smoking cessation at least one month prior to an operation has been shown to reduce the risk of complications<sup>[25]</sup>. A prospective trial showed that infection rates of compliant patients quickly approach those of nonsmokers after four weeks of abstinence<sup>[25]</sup>. A systemic review and meta-analysis confirmed the benefit of smoking cessation on postoperative outcomes and showed that the magnitude of the benefit rises significantly with each week of cessation up to the four-week mark<sup>[32]</sup>. While the debate continues regarding nicotine replacement in the preoperative setting due to concern for vasoconstriction and impaired healing, several studies maintain it has no impact on surgical outcomes<sup>[29,33]</sup>.

For all patients who desire elective complex VHR at our institution, we require a minimum of 30 days smoking cessation preoperatively with allowance for nicotine replacement formulations as needed. Urine cotinine (metabolite of nicotine with a longer half-life) is checked at least 2 weeks prior to surgery to allow rescheduling in case of positive testing. Of note, the use of nicotine-replacement products can result in a positive urine cotinine test. If there is serious concern about a patient's ongoing smoking status, a urine anabasine level can be checked, which is an alkaloid only present in tobacco and not in any replacement products<sup>[34]</sup>.

### Glycemic control in perioperative period

Glycemic control pre-, intra-, and postoperatively has been proven essential for reducing complications in elective surgery, particularly infection<sup>[35-37]</sup>. Hyperglycemia has been shown to have numerous adverse effects at the cellular level including altered chemotaxis, phagocytosis, pseudopod formation, and oxidative burst, all of which prevent neutrophils from functioning optimally<sup>[38]</sup>. In diabetic patients or those with suspected hyperglycemia, glycemic control should be measured with hemoglobin A1c (HbA1c), which gives an indication of glycemic control over the previous 2-3 months. While a goal HbA1c of 6.5% is ideal, the risk of infection rises significantly at values > 7.5%<sup>[35]</sup>. Those patients with difficulty in achieving a HbA1c below 7.5% warrant additional education and assistance from an endocrinologist, diabetic nutritionist, and/or diabetes nurse educator.

In the early 2000s, a large randomized controlled trial (RCT) demonstrated that tight glucose control (80-110 mg/dL) resulted in a decrease in ICU and surgical patient mortality giving rise to the popularity of strict glucose regulation<sup>[39]</sup>. In the years after this study, the risks of hypoglycemia and its complications were found to outweigh the benefits of meticulous glucose protocol (80-110 mg/dL)<sup>[40]</sup>. Currently, perioperative blood sugar control in both diabetic and non-diabetic patients should aim for 120-160 mg/dL to minimize complication risks<sup>[40-42]</sup>. Postoperative hyperglycemia remains a significant risk factor for the development of surgical site occurrences; it has been reported that even one episode of serum glucose of > 200 mg/dL increases the risk of wound dehiscence<sup>[37,43]</sup>. Strict protocols for preventing hyperglycemia and glycemic interventions have effectively reduced rates of hyperglycemia and improved outcomes<sup>[43,44]</sup>.

### Sarcopenia

Sarcopenia refers to a combination of muscle atrophy and replacement by fibrosis or adipose<sup>[45]</sup>. This degenerative loss of muscle mass is most strongly associated with aging and is commonly a component of underlying pathologic processes such as cancer or liver disease. It may also occur in relatively healthy individuals if they are obese and inactive. Compared to sarcopenia in non-obese patients, sarcopenia in obesity is associated with a decrease in overall survival<sup>[46]</sup>. Sarcopenia is quantified using computed tomography by measuring a cross-sectional muscle area ( $\text{cm}^2/\text{m}^2$ ) of the paraspinal muscles at the L3 level and comparing the values to sex-specific cutoffs<sup>[45,47]</sup>. The presence of sarcopenia in surgical and critical care patients has been shown to be a predictor of poor outcomes such as surgical site occurrence, length of stay (LOS), and need for rehabilitation<sup>[48-53]</sup>. Increased ventilator dependence and overall mortality were seen in elderly trauma patients found to be sarcopenic<sup>[49]</sup>. Some retrospective data with VHR patients show an association of sarcopenia with increased postoperative complications and hernia recurrences<sup>[54]</sup>, whereas other preliminary reviews of prospective data fail to show a significant correlation<sup>[55]</sup>. The true role of sarcopenia in AWR and VHR requires further investigation, but methods to preserve and improve lean body mass would likely have a positive impact on patient outcomes<sup>[56]</sup>.

### Conditioning and prehabilitation

It has been widely accepted that poor physical fitness is associated with poor surgical outcomes. While surgical risk calculators use biometric variables and laboratory data from the NSQIP database to estimate 30-day perioperative risks, quantifying functional status might be a better predictive tool<sup>[57]</sup>. Reddy *et al.*<sup>[58]</sup> found that time to complete a stair climb in a preoperative setting was strongly associated with complication rates after abdominal surgery. The stress of this exercise likely simulates the physiologic demand induced in surgery and may help triage patients for fitness optimization. This concept, known as preconditioning or prehabilitation, serves to improve functional status leading up to an elective operation utilizing a multidisciplinary approach that includes psychological, physical, and nutritional interventions. Numerous studies have been completed over the past decade to investigate the utility of prehabilitation and demonstrate improved preoperative functional capacity<sup>[59]</sup>, rate of return to preoperative function after abdominal surgery<sup>[60]</sup>, and reduction of complication rates in elective abdominal aortic aneurysm repair<sup>[61]</sup>.

Given significant heterogeneity in the surgical diseases being studied and the specifics of the prehabilitation programs, there is some variability in conclusions and no large-scale evidence of one program exists to support its use<sup>[62]</sup>. Liang *et al.*<sup>[63]</sup> completed the first RCT of prehabilitation in VHR patients in 2018. They showed that the prehabilitation group (which consisted of a multidisciplinary consultation with a nutritionist, physical therapist, hernia navigator, weekly group meetings, and daily goals checklists for diet and exercise) were more likely to be without hernia or other complications at one month. A recent study identified surgical prehabilitation as an independent predictor of five-year disease-free survival in patients with stage III colorectal cancer<sup>[64]</sup>.

## Nutrition

The literature well-establishes that poor nutritional status translates into higher rates of postoperative complications and adverse outcomes for patients undergoing elective surgery<sup>[65]</sup>. Despite knowledge of this, the surgeon buy-in regarding preoperative nutritional optimization remains lackluster. Few major centers have organized programs to evaluate and manage preoperative nutritional status. Successfully identifying and intervening on nutritionally replete patients in the preoperative setting has potential to significantly decrease complications, length of stay, and readmissions based on multiple RCTs<sup>[66-68]</sup>.

Undernourished patients may be identified through one of several simple screening tools. Nutritional Risk Screening 2002 and Nutrition Risk in Critically Ill (NUTRIC score) are both validated systems that project risk of impairment caused by the metabolic stress of the clinical condition<sup>[69]</sup>. NUTRIC was initially calculated from six variables: age, APACHE II score, SOFA score, number of comorbidities, days from hospital to ICU admission, and IL-6. The current NUTRIC score has excluded IL-6 and remains validated<sup>[70]</sup>. It is important to remember these scores are risk assessment scores and not nutritional indicators.

The complexity of a patient's nutritional evaluation exceeds a single laboratory value. While albumin and prealbumin have historically been used as markers of nutritional status, they lack both the sensitivity and specificity for detection of malnutrition. During an inflammatory state, the production of these visceral proteins is decreased, making the relevance of the absolute values of these proteins even more limited after the onset of illness. There are still reliable data demonstrating that low preoperative albumin levels are associated with increased postoperative complications, but it is not clear that malnutrition is definitively linked to hypoalbuminemia<sup>[71]</sup>.

Adequate energy intake (both total calories and protein) is clearly important for postoperative recovery, and enteral feeding should begin as soon as possible for nearly all surgical patients. For patients in the hospital and recovering from the stress of major surgery, data from interventions on elderly and critically ill patients show that resistance exercise combined with protein goals of 1.5-2.5 g/kg/day optimizes preservation of muscle mass and functional status<sup>[72-77]</sup>.

A more interesting and proactive concept is the use of preoperative nutritional strategies. Preoperative immune and metabolic modulation gained traction following a series of data by Braga *et al.*<sup>[78-80]</sup> and Gianotti *et al.*<sup>[81]</sup> in the early 2000s. They demonstrated reduction of complications, LOC, and total cost of hospitalization with delivery of a specific "immune-enhancing" formula for five days prior to operation. This "immune-enhancing" formulation contained supplemental amounts of omega-3 fatty acids (DHA and EPA), arginine, and nucleotides. The benefit of this formula was demonstrated in both well-nourished and undernourished patients. Although the complete range of mechanisms has not been elucidated, several animal models and clinical studies propose improvement of protein kinetics, wound healing, lymphocyte function, M1 to M2 macrophage conversion (transitioning macrophages from pro-inflammatory and microbicidal functions to more extracellular matrix building and wound healing functions), and blood flow

via nitric oxide vasodilation with arginine supplementation<sup>[12,13,82-84]</sup>. Omega-3 fatty acids/fish oils dampen the metabolic response to stress, decrease inflammation, regulate bowel motility via vagal efferents, and stimulate the resolution of the inflammatory response by the endogenous production of SPMs<sup>[12,13,82,85,86]</sup>. Several large meta-analyses in the past decade have added support to the use of perioperative metabolic manipulation. This concept has been shown to be beneficial not in the perioperative period but also when given only preoperatively with essentially preparing the host for the metabolic insult of surgery. The overall conclusions from these studies are that immune-enhancing formulations (more so than other nutritional regimens) lead to decreased overall infections, a reduction in hospital LOS, a decrease in overall complication rate<sup>[87-90]</sup>, and one study even reporting a decrease in mortality<sup>[91]</sup>.

Another area of metabolic manipulation that has been explored is preoperative carbohydrate loading, which has shown usefulness mostly in reducing perioperative hyperglycemia/insulin resistance<sup>[92,93]</sup>. In a standard protocol, patients consume a 300-mL isotonic clear beverage with 50 g of complex carbohydrate three hours prior to surgery to decrease insulin resistance in the perioperative period. The original carbohydrate loading studies administered the isotonic formulations the night prior to surgery and the morning of surgery with the concept of maximally loading the myocardium, liver, and muscle with glycogen. Subsequent studies have shown that the carbohydrate loading the night before surgery is not necessary<sup>[94]</sup>. Reported outcomes with this regimen include: no increased risk of aspiration, decreased postoperative insulin resistance, maintenance of muscle strength, decreased patient anxiety, and possibly decreased LOS but no major difference in major clinical significant outcomes such as reduced infections or length of stay<sup>[95-97]</sup>. While the European Society for Clinical Nutrition and Metabolism consensus guidelines for surgical nutrition endorses carbohydrate loading<sup>[98,99]</sup>, further studies are needed to better elucidate quantity and optimal timing of intervention.

### **Skin preparation, antibiotics, and the microbiome**

The literature suggests that acute changes in the host microbiome may alter metabolism on a systemic level. A majority of surgeons and hospitals instruct patients to shower with chlorhexidine gluconate soap the night prior to and the morning of surgery. A Cochrane Database review in 2015 summarizing seven studies and over 10,000 patients showed that, while they reported a decrease in skin bacterial colonization, there was no reduction of surgical-site infections with use of chlorhexidine compared to other agents<sup>[100]</sup>. Furthermore, a study using prospectively collected data in VHR patients actually suggested the use of pre-hospital chlorhexidine scrub increases the risk of infection<sup>[101]</sup>. While preoperative bathing can certainly reduce bacteria counts on the skin, it does not clearly translate into positive impacts on surgical outcomes. It may disrupt normal skin flora and therefore remove the competitive inhibition that usually prevents pathogenic bacteria from proliferating. These antibacterial soaps destroy not only pathogenic bacteria but also commensal strains<sup>[102]</sup>. However, more research is necessary before making any definitive changes to standard of care. Our program has eliminated the night before surgery chlorhexidine showers as we believe that the elimination of normal skin flora for long periods before surgery allows potential pathogens to colonize.

The data on the choice of skin preparation in the operating room are more conclusive and stem from two major trials. A prospective trial by Swenson *et al.*<sup>[103]</sup> with over 3200 patients demonstrated that iodine skin preparation was superior to chlorhexidine preparations. Then, a prospective randomized trial was published reporting that chlorhexidine was superior to iodine<sup>[104]</sup>. Swenson and Sawyer<sup>[105]</sup> then reanalyzed the data from both studies and concluded that the decreased infection rate was related to the alcohol in preparations. Duraprep and Cloraprep had similar infection risk, whereas the iodine preparation without alcohol was associated with higher surgical site infections (SSI) rates.

*Staphylococcus aureus* is the most common culprit in postoperative surgical infections and the rate of chronic colonization in the patient population is rising. Several studies have been conducted to investigate

the utility of decolonization prior to a planned operation with significant beneficial results. A randomized control trial including over 6000 patients evaluated infection rates in those pretreated for 5 days with twice-daily nasal mupirocin and daily chlorhexidine showers to a placebo group<sup>[106]</sup>. The results showed a 44% decrease in postoperative *S. aureus* infections in the treated group. Several other prospective trials with the implementation of a prescreening and eradication protocol showed similar reductions in infections in patients undergoing elective orthopedic operations<sup>[107]</sup>. The logistics of screening and subsequently treating these patients need streamlining, but it is clearly cost-effective if performed according to a protocol.

According to joint guidelines developed by several professional surgical and pharmacist societies, prophylactic antibiotics (a first-generation cephalosporin) should be administered within the first hour before incision to decrease surgical-site infection in patients undergoing routine VHR<sup>[108]</sup>. Specifically, antibiotic administration should occur as close to incision as possible according to a recent large study using NSQIP data<sup>[109]</sup>. Antibiotics should be re-dosed during the operation, if necessary, taking into account the half-life of the drug, blood loss, and the use of cell saver. If planned, or inadvertent, violation of the colon occurs during the operation, additional antimicrobial coverage is warranted to cover for Gram-negative species and anaerobes (commonly second-generation cephalosporin or a carbapenem). The BMI of the patient must also be taken into consideration, as many of these VHR patients are obese and therefore require higher than standard doses of antibiotics to reach effective levels. One large survey showed that only 66% of patients with a BMI > 30 received adequate prophylactic antibiotic doses<sup>[110,111]</sup>. Retrospective and anecdotal literature support continued postoperative antibiotics in the presence of surgical drains, but no high quality or Level 1 data validate this practice<sup>[112]</sup>. It is important to remain cognizant regarding the drawbacks of prolonged antibiotics use with respect to alteration of the gut microbiome and potential development of antibiotic-associated diarrhea and *Clostridium difficile*. While the exact ideal duration of antibiotics continues to be debated, prospective studies of prophylactic antibiotics support discontinuation upon skin closure<sup>[113-116]</sup>.

The gut microbiome has been shown to play a key role in the human stress response to critical illness<sup>[117-121]</sup>. When healthy and diverse, the microbiome supports symbiosis, homeostasis, and gut barrier function. The gut microbiome is affected by numerous factors that often arise in this patient population, including administration of broad-spectrum antibiotics, proton-pump inhibitors, vasopressors, and opioids, as well as decreases in luminal nutrient delivery and even changes to the exposed partial pressure of oxygen if the bowel is opened. Probiotics (live microorganisms which confer beneficial effects to the host when given in sufficient quantities)<sup>[122]</sup> and prebiotics (food ingredients which are largely non-digestible fibers that induce the growth of beneficial microorganisms in the colon) have emerged as potential treatments to help reduce postoperative infections by supporting a healthy gut microbiome. Several randomized controlled trials using pro- and prebiotics have been conducted in various surgical patient populations<sup>[123]</sup> in an effort to prevent specific infections, e.g., MRSA<sup>[124]</sup>. Numerous high quality meta-analyses make it clear that the use of pro- and prebiotics lowers the rates of SSIs, urinary tract infections, and sepsis<sup>[125-128]</sup>.

### **Enhanced recovery after surgery, opioid reduction, anxiety, and miscellaneous**

Enhanced Recovery After Surgery (ERAS) protocols were first developed in patients undergoing colorectal surgery, but are now used widely throughout surgical specialties. ERAS protocol has resulted in shorter hospitalizations, reduced complication rates, lower readmissions, and lower healthcare costs<sup>[129-131]</sup>. Having a protocolized and multidisciplinary approach to the care of complex patients, such as AWR patients, in the pre-, intra-, and postoperative settings is clearly the best strategy for success.

Intraoperative wound protectors in abdominal surgery are employed to protect the wound edges from bacterial contamination and to minimize mechanical trauma. Several clinical trials have been performed to investigate their role in preventing SSIs with some success<sup>[132-134]</sup>. Plastic adhesive skin barriers used to



prevent contamination are popular with some surgeons, but current data show no real impact on the rate of SSIs in general surgery<sup>[135]</sup>. The impact of surgical drains in the presence of synthetic mesh during AWR has been largely debated; however, a retrospective study provided evidence that their use does not increase SSI and may be protective against surgical site occurrences such as seroma<sup>[136]</sup>. Supplemental oxygenation in the perioperative period has been studied in colorectal surgery with two landmark studies showing a benefit by reducing SSIs<sup>[137,138]</sup>. A meta-analysis favored supplemental oxygen protocols in higher-risk populations<sup>[139]</sup>; however, there are no studies specific to AWR.

Another difficult topic in open abdominal surgery is pain control. Multimodal pain control with both pharmacological and non-pharmacological techniques are continuously being revisited to find the optimal regimen. Pain, and therefore pain control, is very subjective and has to be approached on an individual basis. Common pharmacological modalities include systemic opioids, local or regional blocks, central neuraxial infusions, acetaminophen, non-steroidal anti-inflammatory drugs, gamma-aminobutyric acid analogs, and beta-blockers to name a few<sup>[140]</sup>. Several non-pharmacological techniques such as acupuncture, music therapy, and hypnosis have mixed evidence regarding efficacy. The role of preoperative anxiety on postoperative experience is often overlooked and may be an avenue for improvement. A meta-analysis of 54 studies showed an association between preoperative anxiety and postoperative pain and analgesia requirements<sup>[141]</sup>. In addition to psychological preparation, proper education, and open communication of risks, benefits, and expectations prior to surgery, music therapy may be an additional strategy to help ease anxiety<sup>[142]</sup>. Music likely shifts the patient attention and aids in cognitive coping. One study showed that patients report lower pain scores when exposed to music in the post-anesthesia care unit<sup>[143]</sup> and a meta-analysis showed music leads to reduced anxiety in mechanically ventilated patients, as evidenced by lower respiratory rates and systolic blood pressures, and may even reduce sedative and analgesia requirements<sup>[144]</sup>.

## CONCLUSION

As the incidence and complexity of VHR and AWR continues to rise, so does the importance of addressing all adjustable elements to achieve optimal outcomes. Identifying and intervening on these modifiable risk factors in the pre-, intra-, and immediately postoperative period is key to consistent success. It could certainly be argued that outcomes for these increasingly complex cases are less dependent on operative technique and more dependent on prehabilitation, addressing patient comorbidities preoperatively, adequate glucose control, focus on proper nutrition, and awareness of the microbiome.

## DECLARATIONS

### Authors' contributions

Participated in accumulation of data, literature review, writing and editing the manuscript: Knapp N, Jedrzejewski B, Martindale R

The authors have had equal contributions to this article.

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

Dr's Knapp and Jedrzejewski have no conflicts of interest. Dr. Martindale has no direct conflicts of interest in this manuscript or subject matter but remains a consultant for Bard and Allergan.

## Ethical approval and consent to participate

Not applicable.

## Consent for publication

The authors give consent for publication and release copyright issues.

## Copyright

© The Author(s) 2020.

## REFERENCES

1. Poulouse BK, Beck WC, Phillips SE, Sharp KW, Nealon WH, et al. The chosen few: disproportionate resource use in ventral hernia repair. *Am Surg* 2013;79:815-8.
2. Poulouse BK, Shelton J, Phillips S, Moore D, Nealon W, et al. Epidemiology and cost of ventral hernia repair: making the case for hernia research. *Hernia* 2012;16:179-83.
3. Hawn MT, Gray SH, Snyder CW, Graham LA, Finan KR, et al. Predictors of mesh explantation after incisional hernia repair. *Am J Surg* 2011;202:28-33.
4. Merkow RP, Ju MH, Chung JW, Hall BL, Cohen ME, et al. Underlying reasons associated with hospital readmission following surgery in the United States. *JAMA* 2015;313:483-95.
5. Sanchez VM, Abi-Haidar YE, Itani KMF. Mesh infection in ventral incisional hernia repair: incidence, contributing factors, and treatment. *Surg Infect (Larchmt)* 2011;12:205-10.
6. Höer J, Lawong G, Klinge U, Schumpelick V. Factors influencing the development of incisional hernia. A retrospective study of 2,983 laparotomy patients over a period of 10 years. *Chirurg* 2002;73:474-80.
7. Flum D, Horvath k, Koepsell T. Have outcomes of incisional hernia repair improved with time?: a population-based analysis. *Ann Surg* 2003;237:129-35.
8. Plymale MA, Ragulojan R, Davenport DL, Roth JS. Ventral and incisional hernia: the cost of comorbidities and complications. *Surg Endosc* 2017;31:341-51.
9. Tastaldi L, Petro CC, Krpata DM, Alkhatib H, Fafaj A, et al. History of surgical site infection increases the odds for a new infection after open incisional hernia repair. *Surgery* 2019;166:88-93.
10. Holihan JL, Alawadi Z, Martindale RG, Roth JS, Wray CJ, et al. Adverse events after ventral hernia repair: the vicious cycle of complications. *J Am Coll Surg* 2015;221:478-85.
11. Leppin AL, Gionfriddo MR, Kessler M, Brito JP, Mair FS, et al. Preventing 30-day hospital readmissions: a systematic review and meta-analysis of randomized trials. *JAMA Intern Med* 2014;174:1095-107.
12. Desborough JP. The stress response to trauma and surgery. *Br J Anaesth* 2000;85:109-17.
13. Serhan CN. Discovery of specialized pro-resolving mediators marks the dawn of resolution physiology and pharmacology. *Mol Aspects Med* 2017;58:1-11.
14. Serhan CN. Treating inflammation and infection in the 21st century: new hints from decoding resolution mediators and mechanisms. *FASEB J* 2017;31:1273-88.
15. Berger RL, Li LT, Hicks SC, Davila JA, Kao LS, et al. Development and validation of a risk-stratification score for surgical site occurrence and surgical site infection after open ventral hernia repair. *J Am Coll Surg* 2013;217:974-82.
16. Sugeran HJ, Kellum Jr JM, Reines HD, DeMaria EJ, Newsome HH, et al. Greater risk of incisional hernia with morbidly obese than steroid-dependent patients and low recurrence with prefascial polypropylene mesh. *Am J Surg* 1996;171:80-4.
17. Puzifferri N, Austrheim-Smith IT, Wolfe BM, Wilson SE, Nguyen NT. Three-year follow-up of a prospective randomized trial comparing laparoscopic versus open gastric bypass. *Ann Surg* 2006;243:181-8.
18. Desai KA, Razavi SA, Hart AM, Thompson PW, Losken A. The effect of BMI on outcomes following complex abdominal wall reconstructions. *Ann Plast Surg* 2016;76:S295-7.
19. Lin HJ, Spoerke N, Deveney C, Martindale R. Reconstruction of complex abdominal wall hernias using acellular human dermal matrix: a single institution experience. *Am J Surg* 2009;197:599-603.
20. Sauerland S, Korenkov M, Kleinen T, Arndt M, Paul A. Obesity is a risk factor for recurrence after incisional hernia repair. *Hernia* 2004;8:42-6.
21. Spaniolas K, Kasten KR, Mozer AB, Sippey ME, Chapman WHH, et al. Synchronous ventral hernia repair in patients undergoing bariatric surgery. *Obes Surg* 2015;25:1864-8.
22. Sorensen LT, Hemmingsen U, Kallehave F, Wille-Jørgensen P, Kjaergaard J, et al. Risk factors for tissue and wound complications in gastrointestinal surgery. *Ann Surg* 2005;241:654-8.
23. Finan KR, Vick CC, Kiefe CI, Neumayer L, Hawn MT. Predictors of wound infection in ventral hernia repair. *Am J Surg* 2005;190:676-81.
24. Sørensen LT, Hemmingsen UB, Kirkeby LT, Kallehave F, Jørgensen LN. Smoking is a risk factor for incisional hernia. *Arch Surg* 2005;140:119-23.
25. Sorensen LT, Karlsmark T, Gottrup F. Abstinence from smoking reduces incisional wound infection: a randomized controlled trial. *Ann Surg* 2003;238:1-5.
26. DeLancey JO, Blay Jr E, Hewitt DB, Engelhardt K, Bilimoria KY, et al. The effect of smoking on 30-day outcomes in elective hernia repair. *Am J Surg* 2018;216:471-4.

27. Sørensen LT, Hørby J, Friis E, Pilsgaard B, Jørgensen T. Smoking as a risk factor for wound healing and infection in breast cancer surgery. *Eur J Surg Oncol* 2002;28:815-20.
28. Yang GP, Longaker MT. Abstinence from smoking reduces incisional wound infection: a randomized, controlled trial. *Ann Surg* 2003;238:6-8.
29. Jensen JA, Goodson WH, Hopf HW, Hunt TK. Cigarette smoking decreases tissue oxygen. *Arch Surg* 1991;126:1131-4.
30. Knuutinen A, Kokkonen N, Risteli J, Vähäkangas K, Kallioinen M, et al. Smoking affects collagen synthesis and extracellular matrix turnover in human skin. *Br J Dermatol* 2002;146:588-94.
31. Sørensen LT, Toft BG, Rygaard J, Ladelund S, Paddon M, et al. Effect of smoking, smoking cessation, and nicotine patch on wound dimension, vitamin C, and systemic markers of collagen metabolism. *Surgery* 2010;148:982-90.
32. Mills E, Eyawo O, Lockhart I, Kelly S, Wu P, et al. Smoking cessation reduces postoperative complications: a systematic review and meta-analysis. *Am J Med* 2011;124:144-54.e8.
33. Lindstrom D, Azodi OS, Wladis A, Tønnesen H, Linder S, et al. Effects of a perioperative smoking cessation intervention on postoperative complications: a randomized trial. *Ann Surg* 2008;248:739-45.
34. Benowitz NL, Hukkanen J, Jacob P 3rd. Nicotine chemistry, metabolism, kinetics and biomarkers. *Handb Exp Pharmacol* 2009;29-60.
35. Dronge AS, Perkal MF, Kancir S, Concato J, Aslan M, et al. Long-term glycemic control and postoperative infectious complications. *Arch Surg* 2006;141:375-80.
36. Kwon S, Thompson R, Dellinger P, Yanez D, Farrohi E, et al. Importance of perioperative glycemic control in general surgery: a report from the Surgical Care and Outcomes Assessment Program. *Ann Surg* 2013;257:8-14.
37. Won EJ, Lehman EB, Geletzk AK, Tangel MR, Matsushima K, et al. Association of postoperative hyperglycemia with outcomes among patients with complex ventral hernia repair. *JAMA Surg* 2015;150:433-40.
38. Turina M, Fry DE, Polk Jr HC. Acute hyperglycemia and the innate immune system: clinical, cellular, and molecular aspects. *Crit Care Med* 2005;33:1624-33.
39. Van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, et al. Intensive insulin therapy in critically ill patients. *N Engl J Med* 2001;345:1359-67.
40. Finfer S, Liu B, Chittock DR, Norton R, Myburgh JA, et al. Hypoglycemia and risk of death in critically ill patients. *N Engl J Med* 2012;367:1108-18.
41. Ramos M, Khalpey Z, Lipsitz S, Steinberg J, Panizales MT, et al. Relationship of perioperative hyperglycemia and postoperative infections in patients who undergo general and vascular surgery. *Ann Surg* 2008;248:585-91.
42. Ata A, Lee J, Bestle SL, Desemone J, Stain SC. Postoperative hyperglycemia and surgical site infection in general surgery patients. *Arch Surg* 2010;145:858-64.
43. Endara M, Masden D, Goldstein J, Gondek S, Steinber J, et al. The role of chronic and perioperative glucose management in high-risk surgical closures: a case for tighter glycemic control. *Plast Reconstr Surg* 2013;132:996-1004.
44. DeSantis AJ, Schmeltz LR, Schmidt K, O'Shea-Mahler E, Rhee C, et al. Inpatient management of hyperglycemia: the northwestern experience. *Endocr Pract* 2005;12:491-505.
45. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European working group on sarcopenia in older people. *Age Ageing* 2010;39:412-23.
46. Prado CMM, Lieffers JR, McCargar LJ, Reiman T, Sawyer MB, et al. Prevalence and clinical implications of sarcopenic obesity in patients with solid tumours of the respiratory and gastrointestinal tracts: a population-based study. *Lancet Oncol* 2008;9:629-35.
47. Narici MV, Maffulli N. Sarcopenia: characteristics, mechanisms and functional significance. *Br Med Bull* 2010;95:139-59.
48. Lieffers JR, Bathe OF, Fassbender K, Winget M, Baracos VE. Sarcopenia is associated with postoperative infection and delayed recovery from colorectal cancer resection surgery. *Br J Cancer* 2012;107:931-6.
49. Moisey LL, Mourtzakis M, Cotton BA, Premji T, Heyland DK, et al. Skeletal muscle predicts ventilator-free days, ICU-free days, and mortality in elderly in ICU patients. *Crit Care* 2013;17:R206.
50. Montano-Loza AJ, Meza-Junco J, Prado CMM, Lieffers JR, Baracos VE, et al. Muscle wasting is associated with mortality in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2012;10:166-73, 173.e1.
51. Okumura S, Kaido T, Hamaguchi Y, Fujimoto Y, Kobayashi A, et al. Impact of the preoperative quantity and quality of skeletal muscle on outcomes after resection of extrahepatic biliary malignancies. *Surgery* 2016;159:821-33.
52. Pisitsak C, Lee JGH, Boyd JH, Coxson HO, Russell JA, et al. Increased ratio of visceral to subcutaneous adipose tissue in septic patients is associated with adverse outcome. *Crit Care Med* 2016;44:1966-73.
53. Weig T, Milger K, Langhans B, Janitz S, Sisis A, et al. Core muscle size predicts postoperative outcome in lung transplant candidates. *Ann Thorac Surg* 2016;101:1318-25.
54. Barnes LA, Li AY, Wan DC, Momeni A. Determining the impact of sarcopenia on postoperative complications after ventral hernia repair. *J Plast Reconstr Aesthet Surg* 2018;71:1260-8.
55. Siegal SR, Guimaraes AR, Lasarev MR, Martindale RG, Orenstein SB. Sarcopenia and outcomes in ventral hernia repair: a preliminary review. *Hernia* 2018;22:645-52.
56. Deutz NEP, Ashurst I, Ballesteros MD, Bear DE, Cruz-Jentoft AJ, et al. The underappreciated role of low muscle mass in the management of malnutrition. *J Am Med Dir Assoc* 2019;20:22-7.
57. Bilimoria KY, Liu Y, Paruch JL, Zhou L, Kmiecik TE, et al. Development and evaluation of the universal ACS NSQIP surgical risk calculator: a decision aid and informed consent tool for patients and surgeons. *J Am Coll Surg* 2013;217:833-42.e1-3.
58. Reddy S, Contreras CM, Singletary B, Bradford TM, Waldrop MG, et al. Timed stair climbing is the single strongest predictor of perioperative complications in patients undergoing abdominal surgery. *J Am Coll Surg* 2016;222:559-66.
59. Minnella EM, Awasthi R, Gillis C, Fiore Jr JF, Liberman AS, et al. Patients with poor baseline walking capacity are most likely to improve their functional status with multimodal prehabilitation. *Surgery* 2016;160:1070-9.

60. Li C, Carli F, Lee L, Charlebois P, Stein B, et al. Impact of a trimodal prehabilitation program on functional recovery after colorectal cancer surgery: a pilot study. *Surg Endosc* 2013;27:1072-82.
61. Barakat HM, Shahin Y, Khan JA, McCollum PT, Chetter IC. Preoperative supervised exercise improves outcomes after elective abdominal aortic aneurysm repair: a randomized controlled trial. *Ann Surg* 2016;264:47-53.
62. Hijazi Y, Gondal U, Aziz O. A systematic review of prehabilitation programs in abdominal cancer surgery. *Int J Surg* 2017;39:156-62.
63. Liang MK, Bernardi K, Holihan JL, Cherla DV, Escamilla R, et al. Modifying risks in ventral hernia patients with prehabilitation: a randomized controlled trial. *Ann Surg* 2018;268:674-80.
64. Trépanier M, Minnella EM, Paradis T, Awasthi R, Kaneva P, et al. Improved disease-free survival after prehabilitation for colorectal cancer surgery. *Ann Surg* 2019;270:493-501.
65. Martindale RG, McClave SA, Vanek VW, McCarthy M, Robert P, et al. Guidelines for the provision and assessment of nutrition support therapy in the adult critically ill patient: society of critical care medicine and american society for parenteral and enteral nutrition: executive summary. *Crit Care Med* 2009;37:1757-61.
66. Jie B, Jiang ZM, Nolan MT, Zhu SN, Yu K, et al. Impact of preoperative nutritional support on clinical outcome in abdominal surgical patients at nutritional risk. *Nutrition* 2012;28:1022-7.
67. Johansen N, Kondrup J, Plum LM, Bak L, Nørregaard P, et al. Effect of nutritional support on clinical outcome in patients at nutritional risk. *Clin Nutr* 2004;23:539-50.
68. Starke J, Schneider H, Alteheld B, Stehle P, Meier R. Short-term individual nutritional care as part of routine clinical setting improves outcome and quality of life in malnourished medical patients. *Clin Nutr* 2011;30:194-201.
69. Heyland DK, Dhaliwal R, Jiang X, Day AG. Identifying critically ill patients who benefit the most from nutrition therapy: the development and initial validation of a novel risk assessment tool. *Crit Care* 2011;15:R268.
70. Rahman A, Hasan RM, Agarwala R, Martin C, Day AG, et al. Identifying critically-ill patients who will benefit most from nutritional therapy: further validation of the "modified NUTRIC" nutritional risk assessment tool. *Clin Nutr* 2016;35:158-62.
71. Daley J, Khuri SF, Henderson W, Hur K, Gibbs JO, et al. Risk adjustment of the postoperative mortality rate for the comparative assessment of the quality of surgical care: results of the national veterans affairs surgical risk study. *J Am Coll Surg* 1997;185:328-40.
72. Bauer J, Biolo G, Cederholm T, Cesari M, Cruz-Jentoft AJ, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. *J Am Med Dir Assoc* 2013;14:542-59.
73. Churchward-Venne TA, Tieland M, Verdijk LB, Leenders M, Dirks ML, et al. There are no nonresponders to resistance-type exercise training in older men and women. *J Am Med Dir Assoc* 2015;16:400-11.
74. Deutz NEP, Bauer JM, Barazzoni R, Biolo G, Boirie Y, et al. Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group. *Clin Nutr* 2014;33:929-36.
75. Fiatarone MA, O'Neill EF, Ryan ND, Clements KM, Solares GR, et al. Exercise training and nutritional supplementation for physical frailty in very elderly people. *NEJM* 1994;330:1769-75.
76. Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the health, aging, and body composition (health ABC) study. *Am J Clin Nutr* 2008;87:150-5.
77. Witard OC, McGlory C, Hamilton DL, Phillips SM. Growing older with health and vitality: a nexus of physical activity, exercise and nutrition. *Biogerontology* 2016;17:529-46.
78. Braga M, Gianotti L, Nespoli L, Radaelli G, Di Carlo V. Nutritional approach in malnourished surgical patients: a prospective randomized study. *Arch Surg* 2002;137:174-80.
79. Braga M, Gianotti L, Vignali A, Di Carlo V. Preoperative oral arginine and n-3 fatty acid supplementation improves the immunometabolic host response and outcome after colorectal resection for cancer. *Surgery* 2002;132:805-14.
80. Braga M, Gianotti L, Vignali A, Schmid A, Nespoli L, et al. Hospital resources consumed for surgical morbidity: effects of preoperative arginine and omega-3 fatty acid supplementation on costs. *Nutrition* 2005;21:1078-86.
81. Gianotti L, Braga M, Nespoli L, Radaelli G, Beneduce A, et al. A randomized controlled trial of preoperative oral supplementation with a specialized diet in patients with gastrointestinal cancer. *Gastroenterology* 2002;122:1763-70.
82. Dalli J, Serhan C. Macrophage proresolving mediators-the when and where. *Microbiol Spectr* 2016;4.
83. Zhu X, Pribis JP, Rodriguez PC, Morris Jr SM, Vodovotz Y, et al. The central role of arginine catabolism in T-cell dysfunction and increased susceptibility to infection after physical injury. *Ann Surg* 2014;259:171-8.
84. MacLeod AS, Mansbridge JN. The innate immune system in acute and chronic wounds. *Adv Wound Care (New Rochelle)* 2016;5:65-78.
85. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. *Nature* 2014;510:92-101.
86. Serhan CN, Levy BD. Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. *J Clin Invest* 2018;128:2657-69.
87. Drover JW, Dhaliwal R, Weitzel L, Wischmeyer PE, Ochoa JB, et al. Perioperative use of arginine-supplemented diets: a systematic review of the evidence. *J Am Coll Surg* 2011;212:385-99.
88. Marimuthu K, Varadhan KK, Ljungqvist O, Lobo DN. A meta-analysis of the effect of combinations of immune modulating nutrients on outcome in patients undergoing major open gastrointestinal surgery. *Ann Surg* 2012;255:1060-8.
89. Adiamah A, Skořepa P, Weimann A, Lobo DN. The impact of preoperative immune modulating nutrition on outcomes in patients undergoing surgery for gastrointestinal cancer: a systematic review and meta-analysis. *Ann Surg* 2019;270:247-56.
90. Osland E, Hossain MB, Khan S, Memon MA. Effect of timing of pharmaconutrition (immunonutrition) administration on outcomes of elective surgery for gastrointestinal malignancies: a systematic review and meta-analysis. *JPEN J Parenter Enteral Nutr* 2014;38:53-69.
91. Mazaki T, Ishii Y, Murai I. Immunoenhancing enteral and parenteral nutrition for gastrointestinal surgery: a multiple-treatments meta-analysis. *Ann Surg* 2015;261:662-9.
92. Awad S, Lobo DN. Metabolic conditioning to attenuate the adverse effects of perioperative fasting and improve patient outcomes. *Curr Opin Clin Nutr Metab Care* 2012;15:194-200.
93. Gianotti L, Biffi R, Sandini M, Marrelli D, Vignali A, et al. Preoperative oral carbohydrate load versus placebo in major elective abdominal

- surgery (PROCY): a randomized, placebo-controlled, multicenter, Phase III trial. *Ann Surg* 2018;267:623-30.
94. Awad S, Varadhan KK, Ljungqvist O, Lobo DN. A meta-analysis of randomised controlled trials on preoperative oral carbohydrate treatment in elective surgery. *Clin Nutr* 2013;32:34-44.
95. Fearon KCH, Ljungqvist O, Von Meyenfeldt M, Revhaug A, Dejong CHC, et al. Enhanced recovery after surgery: a consensus review of clinical care for patients undergoing colonic resection. *Clin Nutr* 2005;24:466-77.
96. Soop M, Nygren J, Myrenfors P, Thorell A, Ljungqvist O. Preoperative oral carbohydrate treatment attenuates immediate postoperative insulin resistance. *Am J Physiol Endocrinol Metab* 2001;280:576-83.
97. Smith MD, McCall J, Plank L, Herbison GP, Soop M, et al., Preoperative carbohydrate treatment for enhancing recovery after elective surgery. *Cochrane Database Syst Rev* 2014;CD009161.
98. Amer MA, Smith MD, Herbison GP, Plank LD, McCall JL. Network meta-analysis of the effect of preoperative carbohydrate loading on recovery after elective surgery. *Br J Surg* 2017;104:187-97.
99. Weimann A, Braga M, Carli F, Higashiguchi T, Hübner M, et al. ESPEN guideline: Clinical nutrition in surgery. *Clin Nutr* 2017;36:623-50.
100. Webster J, Osborne S. Preoperative bathing or showering with skin antiseptics to prevent surgical site infection. *Cochrane Database Syst Rev* 2015;CD004985.
101. Prabhu AS, Krpata DM, Phillips S, Huang L, Haskins IN, et al. Preoperative chlorhexidine gluconate use can increase risk for surgical site infections after ventral hernia repair. *J Am Coll Surg* 2017;224:334-40.
102. Wong VW, Martindale RG, Longaker MT, Gurtner GC. From germ theory to germ therapy: skin microbiota, chronic wounds, and probiotics. *Plast Reconstr Surg* 2013;132:854e-61e.
103. Swenson BR., Hedrick TL, Metzger R, Bonatti H, Pruett TL, et al. Effects of preoperative skin preparation on postoperative wound infection rates: a prospective study of 3 skin preparation protocols. *Infect Control Hosp Epidemiol* 2009;30:964-71.
104. Darouiche RO, Wall Jr MJ, Itani KMF, Otterson MF, Webb AL, et al. Chlorhexidine-alcohol versus povidone-iodine for surgical-site antisepsis. *N Engl J Med* 2010;362:18-26.
105. SwensonBR, Sawyer RG. Importance of alcohol in skin preparation protocols. *Infect Control Hosp Epidemio* 2010;31:977.
106. Bode LGM, Kluytmans JAJW, Wertheim HFL, Bogaers D, Vandenbroucke-Grauls CMJE, et al. Preventing surgical-site infections in nasal carriers of staphylococcus aureus. *N Engl J Med* 2010;362:9-17.
107. KimDH, Spencer M, Davidson SM, Li L, Shaw JD, et al. Institutional prescreening for detection and eradication of methicillin-resistant Staphylococcus aureus in patients undergoing elective orthopaedic surgery. *J Bone Joint Surg Am* 2010;92:1820-6.
108. BratzlerDW, Dellinger EP, Olsen KM, Perl TM, Auwaerter PG, et al. Clinical practice guidelines for antimicrobial prophylaxis in surgery. *Am J Health Syst Pharm* 2013;70:195-283.
109. Koch CG, Li L, Hixson E, Tang A, Gordon S, et al. Is it time to refine? An exploration and simulation of optimal antibiotic timing in general surgery. *J Am Coll Surg* 2013;217:628-35.
110. Freeman JT, Anderson DJ, Hartwig MG, Sexton DJ. Surgical site infections following bariatric surgery in community hospitals: a weighty concern? *Obes Surg* 2011;21:836-40.
111. Hanley MJ, Abernethy DR, Greenblatt DJ. Effect of obesity on the pharmacokinetics of drugs in humans. *Clin Pharmacokinet* 2010;49:71-87.
112. Wong A, Lee S, Nathan NS, Wang F, Hansen S, et al. Postoperative prophylactic antibiotic use following ventral hernia repair with placement of surgical drains reduces the postoperative surgical-site infection rate. *Plast Reconstr Surg* 2016;137:285-94.
113. Osmon DR. Antimicrobial prophylaxis in adults. *Mayo Clin Proc* 2011;75:98-109.
114. Berbari EF, Osmon DR, Lahr B, Eckel-Passow JE, Tsaras G, et al. The Mayo prosthetic joint infection risk score: implication for surgical site infection reporting and risk stratification. *Infect Control Hosp Epidemiol* 2012;33:774-81.
115. Bratzler DW, Houck PM; Workgroup SIPGW. Antimicrobial prophylaxis for surgery: an advisory statement from the national surgical infection prevention project. *Am J Surg* 2004;189:395-404.
116. Suehiro T, Hirashita T, Araki S, Matsumata T, Tsutsumi S, et al. Prolonged antibiotic prophylaxis longer than 24 hours does not decrease surgical site infection after elective gastric and colorectal surgery. *Hepato-Gastroenterology* 2008;55:1636-9.
117. Hayakawa M, Asahara T, Henzan N, Murakami H, Yamamoto H, et al. Dramatic changes of the gut flora immediately after severe and sudden insults. *Dig Dis Sci* 2011;56:2361-5.
118. Klingensmith NJ, Coopersmith CM. The gut as the motor of multiple organ dysfunction in critical illness. *Crit Care Clin* 2016;32:203-12.
119. Krezalek MA, DeFazio J, Zaborina O, Zaborin A, Alverdy JC, et al. The shift of an intestinal “microbiome” to a “pathobiome” governs the course and outcome of sepsis following surgical injury. *Shock* 2016;45:475-82.
120. McClaveSA, Lowen CC, Martindale RG. The 2016 ESPEN arvid wretling lecture: the gut in stress. *Clin Nutr* 2018;37:19-36.
121. Turne JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* 2009;9:799-809.
122. FAO/WHO Expert Consultation. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. 2001.
123. Kotzampassi K, Stavrou G, Damoraki G, Georgitsi M, Basdanis G, et al. A four-probiotics regimen reduces postoperative complications after colorectal surgery: a randomized, double-blind, placebo-controlled study. *World J Surg* 2015;39:2776-83.
124. Sikorska H, Smoragiewicz W. Role of probiotics in the prevention and treatment of methicillin-resistant Staphylococcus aureus infections. *Int J Antimicrob Agents* 2013;42:475-81.
125. Wu XD, Xu W, Liu MM, Hu KJ, Sun YY, et al. Efficacy of prophylactic probiotics in combination with antibiotics versus antibiotics alone for colorectal surgery: a meta-analysis of randomized controlled trials. *J Surg Oncol* 2018;117:1394-404.
126. Arumugam S, Lau CSM, Chamberlain RS. Probiotics and synbiotics decrease postoperative sepsis in elective gastrointestinal surgical patients: a meta-analysis. *J Gastrointest Surg* 2016;20:1123-31.
127. Lytvyn L, Quach K, Banfield L, Johnston BC, Mertz D, et al. Probiotics and synbiotics for the prevention of postoperative infections following abdominal surgery: a systematic review and meta-analysis of randomized controlled trials. *J Hosp Infect* 2016;92:130-9.



128. Chowdhury AH, Adiamah A, Kushairi A, Varadhan KK, Krznaric Z, et al. Perioperative probiotics or synbiotics in adults undergoing elective abdominal surgery: a systematic review and meta-analysis of randomized controlled trials. *Ann Surg* 2019.
129. Ljungqvist O, Scott M, Fearon KC. Enhanced recovery after surgery: a review. *JAMA Surg* 2017;152:292-8.
130. Burgess LC, Wainwright TW. What is the evidence for early mobilisation in elective spine surgery? a narrative review. *Healthcare (Basel)* 2019;7.
131. Stein MJ, Frank SG, Lui A, Zhang T, Zhang J, et al. Ambulatory latissimus dorsi flap breast reconstruction: a prospective cohort study of an enhanced recovery after surgery (ERAS) protocol. *J Plast Reconstr Aesthet Surg* 2019;72:1950-55.
132. Reid K, Pockney P, Draganic B, Smith SR. Barrier wound protection decreases surgical site infection in open elective colorectal surgery: a randomized clinical trial. *Dis Colon Rectum* 2010;53:1374-80.
133. Horiuchi T, Tanishima H, Tamagawa K, Matsuura I, Nakai H, et al. Randomized, controlled investigation of the anti-infective properties of the Alexis retractor/protector of incision sites. *J Trauma* 2007;62:212-5.
134. Gaines S, Luo JN, Gilbert J, Zaborina O, Alverdy JC, et al. Optimum operating room environment for the prevention of surgical site infections. *Surg Infect (Larchmt)* 2017;18:503-7.
135. Webster J, Alghamdi A. Use of plastic adhesive drapes during surgery for preventing surgical site infection. *Cochrane Database Syst Rev* 2015;CD006353.
136. Krpata DM, Prabhu AS, Carbonell AM, Haskins IN, Phillips S, et al. Drain placement does not increase infectious complications after retromuscular ventral hernia repair with synthetic mesh: an AHSQC analysis. *J Gastrointest Surg* 2017;21:2083-89.
137. Greif R, Akça O, Horn EP, Kurz A, Sessler DI, et al. Supplemental perioperative oxygen to reduce the incidence of surgical-wound infections. *N Engl J Med* 2000;342:161-7.
138. Belda FJ, Aguilera L, Asunción JG, Alberti J, Vicente R, et al. Supplemental perioperative oxygen and the risk of surgical wound infection: a randomized controlled trial. *JAMA* 2005;294:2035-42.
139. Al-Niaim A, Safdar N. Supplemental perioperative oxygen for reducing surgical site infection: a meta-analysis. *J Eval Clin Pract* 2009;15:360-5.
140. Tan M, Law LS, Gan TJ. Optimizing pain management to facilitate enhanced recovery after surgery pathways. *Can J Anaesth* 2015;62:203-18.
141. Sobol-Kwapinska M, Babel P, Plotek W, Stelcer B. Psychological correlates of acute postsurgical pain: a systematic review and meta-analysis. *Eur J Pain* 2016;20:1573-86.
142. Stamenkovic DM, Rancic NK, Latas MB, Neskovic V, Rondovic GR, et al. Preoperative anxiety and implications on postoperative recovery: what can we do to change our history. *Minerva Anesthesiol* 2018;84:1307-17.
143. Nilsson U, Rawal N, Enqvist B, Unosson M. Analgesia following music and therapeutic suggestions in the PACU in ambulatory surgery: a randomized controlled trial. *Acta Anaesthesiol Scand* 2003;47:278-83.
144. Bradt J, Dileo C. Music interventions for mechanically ventilated patients. *Cochrane Database Syst Rev* 2014;12:CD006902.

Original Article

Open Access



# Comparison of adipose particle size on autologous fat graft retention in a rodent model

Xiaonan Yang<sup>1,2,#</sup>, Francesco M. Egro<sup>1,#</sup>, Taraneh Jones<sup>3</sup>, W. Vincent Nerone<sup>1</sup>, Michael Yousefpour<sup>1</sup>, Jeffrey A. Gusenoff<sup>1</sup>, J. Peter Rubin<sup>1,4</sup>, Lauren E. Kokai<sup>1,4</sup>

<sup>1</sup>Department of Plastic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA.

<sup>2</sup>No.16 Department of Plastic Surgery, Plastic Surgery Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100730, China.

<sup>3</sup>Department of Chemical Engineering, University of Pittsburgh, Pittsburgh, PA 15213, USA.

<sup>4</sup>McGowan Institute for Regenerative Medicine, Pittsburgh, PA 15219, USA.

#Authors contributed equally.

**Correspondence to:** Dr. Lauren E. Kokai, Department of Plastic Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA. E-mail: kokail@upmc.edu

**How to cite this article:** Yang X, Egro FM, Jones T, Nerone WV, Yousefpour M, Gusenoff JA, Rubin JP, Kokai LE. Comparison of adipose particle size on autologous fat graft retention in a rodent model. *Plast Aesthet Res* 2020;7:8.  
<http://dx.doi.org/10.20517/2347-9264.2019.63>

**Received:** 27 Nov 2019 **First Decision:** 17 Jan 2020 **Revised:** 3 Feb 2020 **Accepted:** 14 Feb 2020 **Published:** 27 Feb 2020

**Science Editor:** Jian-Xing Song **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

**Aim:** Unpredictable retention outcomes remain a significant issue in autologous fat grafting procedures. Liposuction cannula variation leads to variability in fat particle size. Recent data suggest that the size of fat particles is closely related to graft healing outcomes; however, this remains a point of contention due to potential confounding variables such as tissue trauma with harvest. The aim of this study was to compare autologous fat grafting outcomes with variable fat particle sizes in an animal model which isolated fat particle size as the primary experimental variable. The overall goal of this work was to determine if reducing fat particle size is an effective method for enhancing graft retention in autologous fat grafting.

**Methods:** The range of fat particle diameter harvested by four common liposuction cannulas was quantified to define relevant small and large particle target diameters. To determine if particle size impacted nutrient and oxygen permeability, small and large particles were incubated *in vitro* in a spinner flask with an abundance of culture media and vascular endothelial growth factor secretion was measured with enzyme-linked immunosorbent assay. Finally, small and large fat grafts were prepared from subcutaneous mouse fat pads and grafted in syngeneic Balb/CJ mice. Weight and volume retention were evaluated at 1, 4, 8, and 12 weeks. Histological analysis with Masson's trichrome and perilipin



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



immunofluorescent staining was performed. Real-time quantitative polymerase chain reaction was performed for adipogenic, inflammatory and apoptotic genes.

**Results:** The range of fat particle diameters harvested with four commonly used cannulas was 2-7 mm. *In vitro* studies showed that 5-7-mm particles had significantly increased VEGF secretion normalized to weight, indicating increased tissue hypoxia in these particles compared to 2-4-mm particles. Surprisingly, in vivo comparison in two unique studies showed 2-4-mm and 5-7-mm fat particles had comparable graft retention ( $P = 0.5329$ ). Masson's trichrome staining revealed increased extracellular matrix and fibrosis in the 5-7-mm particle group ( $P = 0.0115$ ). Adipocyte survival with perilipin demonstrated comparable viability. Gene expression showed large particles experienced increased inflammation and apoptosis at one week after grafting, but overall there were no significant differences between groups.

**Conclusion:** The ideal fat particle size should be large enough to contain adequate mesenchyme while not so thick as to preclude imbibition. This study suggests that, despite changes in hypoxia and VEGF levels, differing fat particles (2-4-mm and 5-7-mm) can achieve similar graft retention.

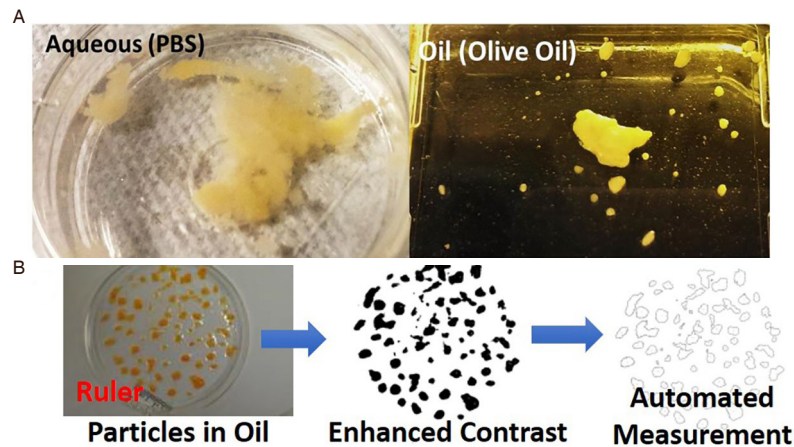
**Keywords:** Fat grafting, particle size, lipofilling, lipoharvesting, adipocyte viability, clinical translation

## INTRODUCTION

Autologous adipose tissue transfer, or fat grafting, is a common procedure in aesthetic and reconstructive plastic surgery with approximately 90,000 patients treated each year in the US alone<sup>[1]</sup>. Fat grafting allows permanent tissue volume augmentation and correction of contour irregularities in a minimally invasive and safe manner<sup>[2]</sup>. However, studies have demonstrated unpredictable and unsatisfactory graft retention in some patients that can range between 40% and 90%<sup>[3]</sup>. Thus, significant efforts have been undertaken to understand the biology of fat grafting and optimize each step of the fat grafting process from end-to-end (harvesting, processing, and injection)<sup>[4-9]</sup>.

In modern liposuction, excess adipose tissue is extracted through a hollow-bore cannula, typically between 12 and 14 G, with variable numbers and sizes of holes. The resulting lipoaspirate product is a suspension of solid adipose tissue particles in liquid comprised of tumescent fluid, blood, and oil from lysed cells. From this lipoaspirate, additional processing steps may be added to remove fluid and oil, ultimately producing a collection of fat particles that are then grafted into the volume void. It is well known in the literature that fat grafts depend on diffusion of oxygen and nutrients from the recipient bed until new blood vessels are formed<sup>[10,11]</sup>. According to work by Khouri *et al.*<sup>[12]</sup>, when fat particles are very large (greater than 0.16 cm in radius), oxygen and nutrient diffusion to the interior particle core is insufficient to maintain tissue viability resulting in central necrosis and potentially oil cyst formation. These oil cysts are eventually resorbed through macrophage clearance, resulting in overall graft tissue loss and inferior long-term fat grafting outcomes.

The predominate theory in fat graft preparation is therefore to minimize particle size to as small as possible to reduce central necrosis and ultimately increase graft retention. However, other groups have challenged this assertion, hypothesizing that small cannula diameters increase shear stress in tissue, thereby damaging viability and increasing graft resorption<sup>[13,14]</sup>. In two unique studies, Erdim *et al.*<sup>[15]</sup> and Kirkham *et al.*<sup>[16]</sup> demonstrated that large diameter cannulas lead to higher graft retention, improved adipocyte survival, and less inflammatory infiltrate and fibrosis. In another comparative study in rabbits, fat was harvested with a regular 4.5-mm, 1-mL syringe with a sharp steel sleeve, excised, and cut into 1-mg pieces and aspirated with a 2-mm harvesting cannula, and long-term retention was equal in all groups<sup>[17]</sup>. Therefore, the impact of fat particle size on survival and resorption outcomes remains controversial.



**Figure 1.** A: comparison of fat particles dispersed in aqueous solution (PBS) or Oil (Olive Oil) showing increased definition of particle boundary in lipophilic solutions; B: adipose particle measurements were obtained by imaging a minimum of 50 particles dispersed in oil with a ruler included in each photograph. Images were then processed with ImageJ to increase particle contrast and the maximum distance between the particle center and the edge was determined. PBS: phosphate-buffered saline

The aim of this study was to compare autologous fat grafting outcomes with variable fat particle sizes in an animal model, which isolated fat particle size as the primary experimental variable. We elected to use a syngeneic mouse model to isolate the variable of fat particle size from confounding factors such as human donor tissue variability, trauma from tissue harvest, ischemic time, and recipient tissue bed vascularity. Our overall study goal was to clarify if smaller fat particles result in better fat grafting outcomes in terms of graft retention, histological architecture, adipocyte viability, and neovascularization.

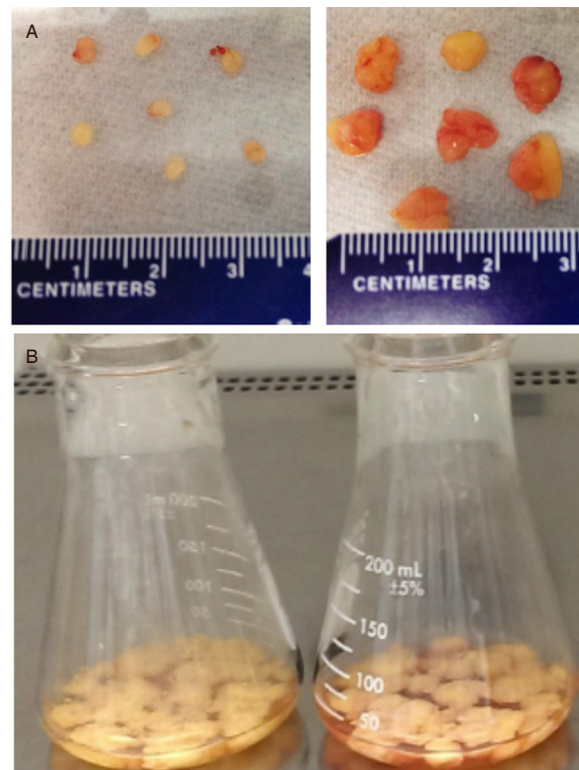
## METHODS

### *In vitro* analysis of human adipose particle size and hypoxia

#### *Lipoaspirate fat particle size analysis*

For the purposes of this study, the range of “typical” fat parcel size was determined by measuring the diameters of adipose particles produced by four commonly used harvesting cannulas for fat grafting procedures: 15-cm Coleman-bucket (two opposing holes) aspiration cannula (Mentor Texas L.P., TX, USA #COL-ASPI), Khouri 12-hole harvesting cannula [Marina Medical Inc. FL, USA; #800-205 (12-hole design)], Mercedes tip 3-hole cannula (Grams Medical Inc., Calif., USA), and Shippert cannula (Shippert Medical Co., USA). To obtain lipoaspirate, full thickness skin was obtained as discarded tissue from three different elective body contouring procedures under University of Pittsburgh IRB exemption (PRO13090506). The skin was divided into four even areas with a surgical marker and evenly infused with 0.9% NaCl solution. Cannulas were connected to a 20-mL syringe and negative pressure was applied by hand. At least 10 mL of adipose particles were obtained with each cannula type in unique tissue segments.

To measure the size of fat parcels, lipoaspirate was washed with phosphate buffered saline to remove oil and blood and then tissue particles were dispersed in olive oil and photographed for automated particle analysis [Figure 1A]. Olive oil was selected as the solution of choice after comparing the resolution of particle boundaries in hydrophobic and hydrophilic (PBS) solutions and selecting for the one providing the sharpest particle boundary. Glass calibration beads (QAQC Lab/Coffee Laboratory, White Stone, VA. Cat #s 600 ZSICSA-2.00, 600 ZSICSA-3.35) of known diameter were used to validate the technique. High resolution images containing at least 50 particles adjacent to a ruler were taken with a Nikon camera. ImageJ was used to enhance image contrast, and particle diameter analysis was performed by measuring the greatest particle diameter [Figure 1B].



**Figure 2.** *Ex vivo* study with adipose particles and VEGF secretion. Small (2-4 mm) and large (5-7 mm) particles were prepared from whole adipose tissue using surgical scissors and a sterile ruler. Each particle was assessed by weight to ensure consistency. Particles were submerged in sterile cell culture media in a flask and stirred continuously with a stir bar in a humidified CO<sub>2</sub> incubator. VEGF: vascular endothelial growth factor

#### *Ex vivo quantification of particle hypoxia*

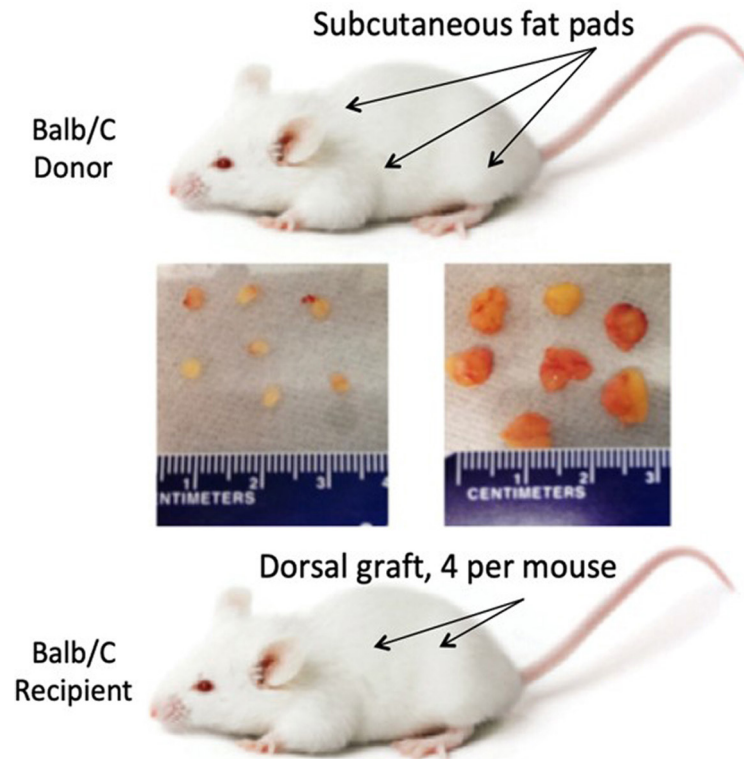
After determining the typical diameter range of fat parcels obtained with four common fat grafting aspiration cannulas, we artificially created particles of the lower end of the size range (2-4 mm) and upper range (5-7 mm), representing “small” and “large” particles, respectively. To determine if the 5-7-mm particle group incurred oxygen diffusion limitations and increased hypoxia compared to the 2-4-mm group, we cultured particles for seven days in conditions with free access to glucose and oxygen and measured vascular endothelial growth factor secretion<sup>[18,19]</sup> [Figure 2A]. Full thickness skin samples were obtained from a single donor after body contouring surgery under IRB exemption and placed in a biosafety cabinet for processing. Pieces of adipose tissue for each desired size range were prepared using surgical scissors and subsequently placed into sterile flasks with vented caps with 1:1 (volume) ratio of culture media to fat particles. The total weight of adipose tissue and volume of media in all flasks was equal [Figure 2B]. Particles were constantly stirred inside of a 37 °C incubator, at 5% CO<sub>2</sub>. Half of the media was refreshed every other day up to seven days. Media concentration of vascular endothelial growth factor (VEGF) was measured with enzyme-linked immunosorbent assay (ELISA) according to manufacturer’s instructions.

#### ***In vivo analysis of fat particle survival in mouse autograft model***

##### *Animals*

All animal experiments were performed under approved protocols by the University of Pittsburgh Institutional Animal Care and Use Committee (Protocol# 12080782). In total, 47 four-week-old female Balb/CJ (The Jackson Laboratory, ME, USA) mice were used: 18 mice for fat grafting and 17 for fat harvesting. All animals were housed under controlled environmental conditions with a 12-h/12-h light/dark cycle. Standard laboratory chow and sterilized water were provided ad libitum.





**Figure 3.** Animal study design schematic. Subcutaneous fat pads were harvested from Balb/C mice. Fat particles with two diameter sizes were prepared with sterile surgical instruments and then immediately syngeneically-transplanted into dorsal subcutaneous spaces via small skin incisions, two points per side in one mouse. At least  $n = 10$  particles were included in each experimental group at each timepoint

#### *Fat particles preparation and transplantation*

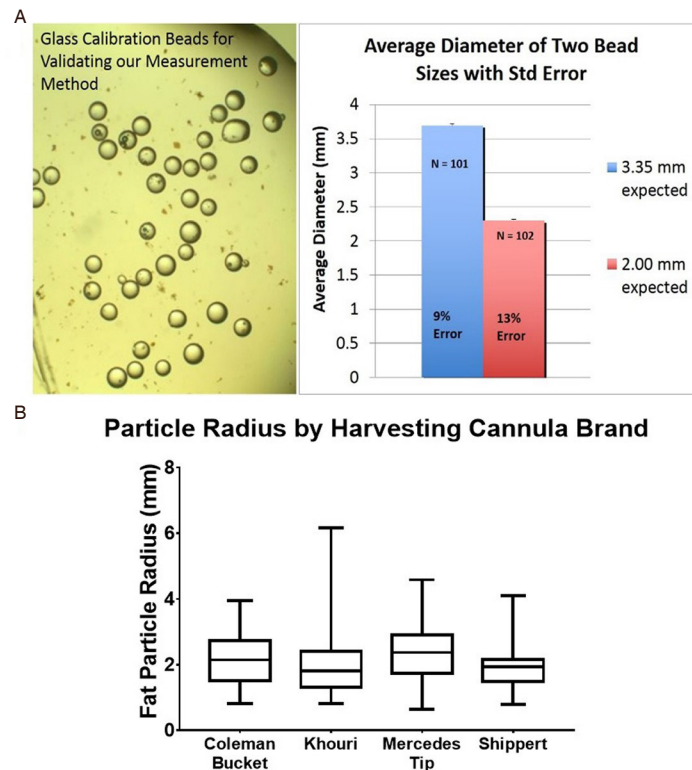
Animals were weighed and anesthetized with isoflurane inhalation. The surgical team comprised of two operators, one who harvested fat particles and a second who immediately implanted grafts, minimizing ischemic time and maintaining consistency across the study [Figure 3]. Inguinal and axillary subcutaneous fat pads were harvested in a biosafety cabinet with sterile instruments and immediately weighed with a microelectronic balance (Sartorius, Data Weighing Systems Inc., IL, USA). Simultaneously, the recipient site in a unique, but genetically identical anesthetized mouse was prepared by making a small skin incision on the dorsal flank with scissors and preparing a subcutaneous pocket by opening scissor blades. Immediately after graft preparation, the tissue particles were placed individually into the recipient site and the incision was closed with skin glue (Vetbond, No. NC9604126; Fisher Scientific). A single dose of analgesics, 5 mg/kg ketoprofen (Fort Dodge Animal Health, Overland Park, KS), was administered into the nuchal subcutis.

#### *Evaluation of grafted fat particles*

At defined study timepoints (1, 4, 8, and 12 weeks), animals were weighed, euthanized by CO<sub>2</sub>, and fat particles were explanted, weighed, and measured by gas volume displacement using an AccuPyc II 1340 gas Pycnometer (Micrometrics, Norcross, GA). After measurement, grafts were immediately submerged in 10% neutral buffered formalin for at least 24 h, processed, and embedded in paraffin for sectioning.

#### *Histological analysis*

Formalin fixed, paraffin embedded samples were sectioned to 6- $\mu$ m sections affixed to charged slides. Masson's trichrome and perilipin immunofluorescent staining were performed on the explants ( $n = 6$ ) at 1-, 4-, 8-, and 12-week timepoints. The sections were observed under a Keyence microscope (Keyence Corp.,



**Figure 4.** Comparison of fat particle radii from typical harvesting cannulas. Mean  $\pm$  SD of at least 50 particles from three unique donors are presented

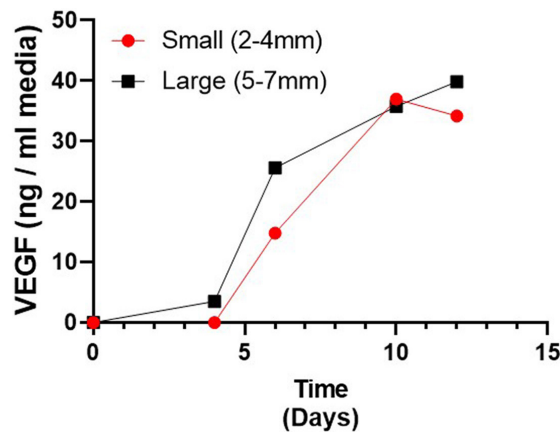
IL, USA). The percentage of area of extracellular matrix and fibrosis was evaluated using Image J (National Institutes of Health, Bethesda, MD).

#### *Real-time quantitative polymerase chain reaction*

Real-time quantitative polymerase chain reaction (qRT-PCR) was used to compare the relative expression of genes related to adipocyte function [fatty acid binding protein 4 (FABP4)]<sup>[20]</sup>, inflammation [tumor necrosis factor (TNF $\alpha$ ) and interleukin 1 (IL1)]<sup>[21]</sup> and cell stress [glutathione peroxidase (GPX1)]<sup>[22-24]</sup> and Caspase 3 (CASP3)<sup>[25]</sup>. To isolate RNA, graft particles were immediately snap frozen in liquid nitrogen after extraction and stored at -80 °C until processing. Frozen samples were thawed on ice and transferred to a sterile petri dish and cut into very small pieces, approximately the size of a grain of rice. Tissue was transferred to a 5-mL round bottom tube with 1-mL RNeasy Lysis Buffer (RLT) plus (Qiagen) buffer and placed immediately on ice until homogenization with a probe homogenizer (Bio-Gen PRO200). RNA was then extracted with a Qiagen RNeasy MinElute kit according to manufacturer's instructions. RNA quantity and purity were assessed using a plate-reader (Tecan Infinite M200) at 260-nm/280-nm ratio. complementary DNA was reverse transcribed using 25-ng/ $\mu$ L RNA with Moloney Murine Leukemia Virus Reverse Transcriptase (200 U/ $\mu$ L). qRT-PCR was performed with the following primers (all Thermo Fisher): FABP4 (Mm00445878\_m1); GPX1 (Mm00492427\_m1), CASP3 (Mm01195085\_m1), TNF (Mm00438653\_m1), and IL1a (Mm04336046\_m1). Relative expression was determined by  $\Delta\Delta$ Ct from GAPDH housekeeping gene.

#### *Statistical analysis*

All data are presented as mean and standard deviation (SD) for all groups. The *t*-test was performed to compare groups at single timepoints or differences between two samples in a group at various timepoints with GraphPad Software Prism (GraphPad Software, Inc. San Diego, CA). Statistically significant differences



**Figure 5.** Measurement of VEGF concentration in ex vivo culture media with ELISA. Data are presented as mean ( $n = 2$  independent biologic replicates per group) with no statistical comparison performed due to low sample size. VEGF: vascular endothelial growth factor ELISA: enzyme-linked immunosorbent assay

were determined using  $P$ -value less than 0.05. Statistical modeling of intra-animal reproducibility was performed in consultation with the University of Pittsburgh Department of Statistics.

## RESULTS

### *In vitro* analysis of human adipose particle size and hypoxia

#### *Liposuction cannulas and harvested fat particles analysis*

Semi-automated measurement of particle size was verified using glass calibration beads. The results from glass particles demonstrated that the method was consistent, with 8% and 6.5% standard deviation for 3.35- and 2-mm particles, respectively, and the method was able to separate particle diameter differences of 1.35-mm diameter with statistical significance [Figure 4A].

The radius of adipose particles harvested with the Bucket, Khouri, Mercedes Tip, and Shippert cannulas were  $3.03 \pm 0.78$ ,  $1.98 \pm 0.92$ ,  $2.31 \pm 0.85$ , and  $1.9 \pm 0.59$  mm, respectively [Figure 4B]. The range of fat particles diameters was approximately 2-7 mm [Figure 2]; therefore, the range was divided into two groups for future studies, with small (2-4 mm) and large (5-7 mm) sized particles.

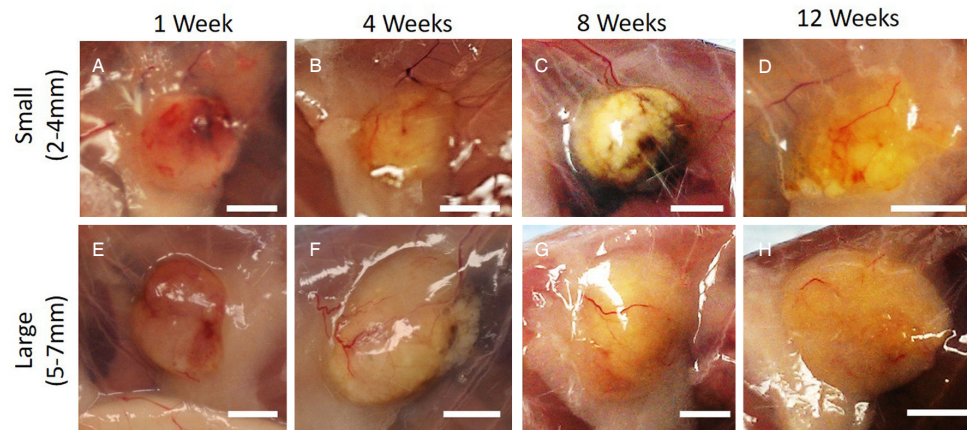
#### *VEGF expression in ex vivo model*

An *ex vivo* study was conducted with fat particles of variable diameter to evaluate the impact of fat particle size on oxygen diffusion limitation and tissue hypoxia. Adipose tissue obtained during panniculectomy was minced with surgical scissors into small and large particle sizes and cultured in equal total amounts in cell culture media. VEGF is an important growth factor for particle revascularization and is expressed by adipose due to hypoxia. Quantification of VEGF concentration in media with ELISA showed increased expression in both particle groups every consecutive day for seven days of culture [Figure 5]. VEGF media concentration was significantly higher in 5-7-mm group compared to the 2-4-mm group at Days 4 and 6 ( $5928.73 \pm 2572.74$  pg/mL vs.  $1507.18 \pm 313.16$  pg/mL and  $23,950.61 \pm 2946.86$  pg/mL vs.  $15,126.37 \pm 3846.77$  pg/mL, respectively,  $P < 0.01$ ), suggesting that larger particles experience increased hypoxia compared to smaller particles.

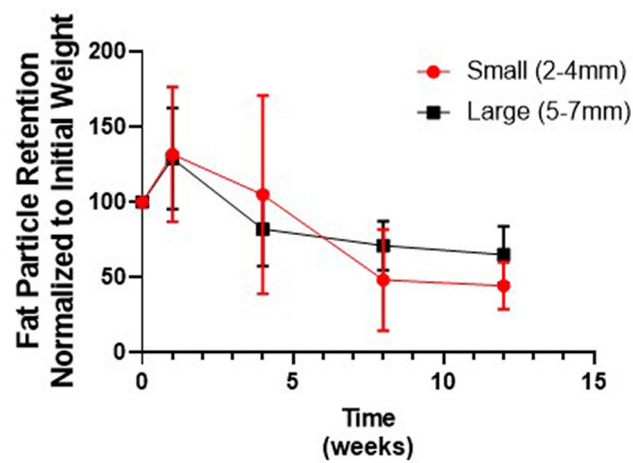
### *In vivo* analysis of fat particle survival in mouse autograft model

#### *Fat particles preparation and transplantation*

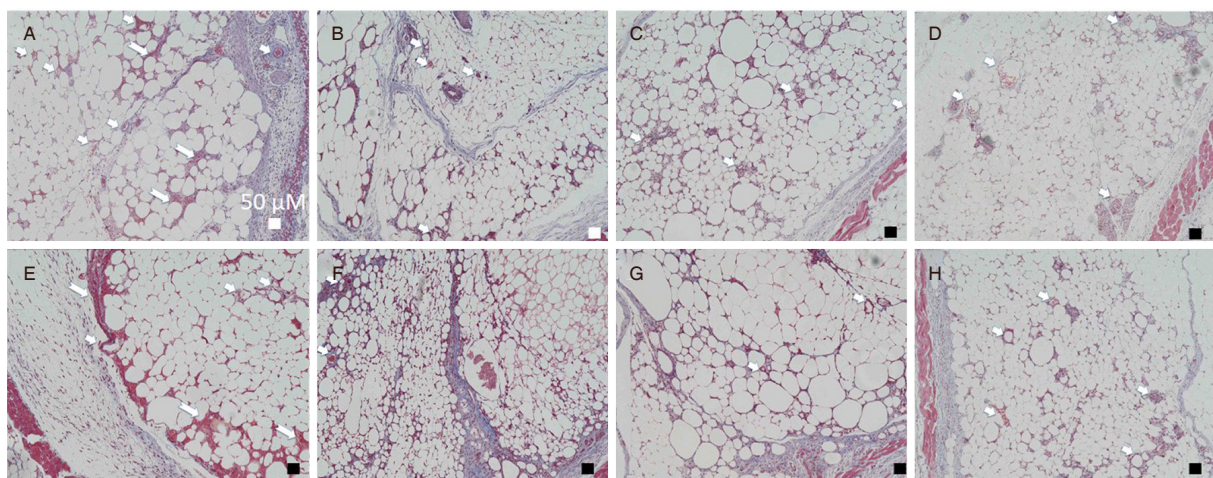
All experimental animals receiving fat grafts survived well without complications and all surgical incision wounds healed normally. At each timepoint, grafted particles were easily discernable in the subcutaneous



**Figure 6.** Macroscopic views of grafted fat particles of 2-4 mm (A-D) and 5-7 mm (E-H) group at 1, 4, 8, and 12 weeks, respectively, after surgery

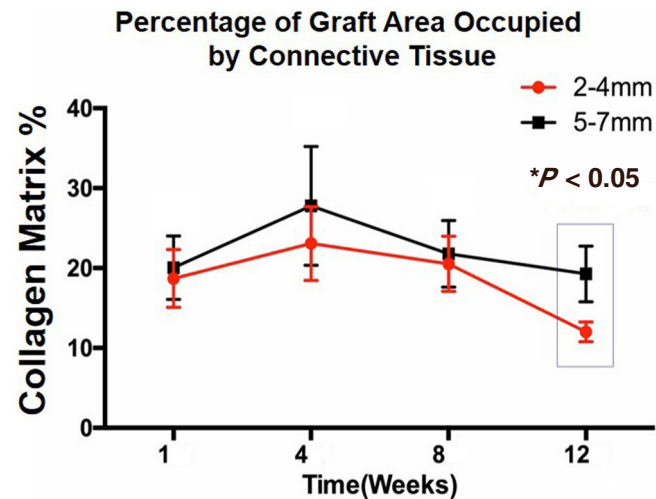


**Figure 7.** Retention of fat particles normalized to starting weight. Data are presented as the mean percentage of weight retention  $\pm$  SD, with 10 replicates per group, pooled from two complete experimental replicates



**Figure 8.** Masson's trichrome staining of explanted fat particles. Fat particles of 2-4 mm (A-D) and 5-7 mm (E-H) groups at 1, 4, 8, and 12 weeks, respectively, after surgery. Small arrows, vessels; Long-tailed arrows, fibrosis. Scale bar, 50  $\mu$ m





**Figure 9.** Analysis of fat particle cross-sectional area comprised of connective tissue at each experimental timepoint. Analysis performed by measuring blue collagen as a percentage of total particle area. \* $P < 0.05$  at 12 weeks

space and were encapsulated in thin membranes of fibrous septae [Figure 6A-H]. At one week, fat parcels in both small and large particle groups demonstrated bleeding, potentially indicating more permeable blood vessels and ongoing inflammation<sup>[26]</sup>. At later timepoints, angiogenesis and blood vessel stabilization were observed in both particle groups. Considering the small size of fat particles, retention was calculated in terms of weight, as we could measure this with an analytic balance with greater sensitivity than available methods to measure volume. For each sample, the percent retention was determined by normalizing the explantation weight to the initial weight of each particle recorded before grafting. There was no significant difference in particle retention at any timepoint for 2-4- and 5-7-mm particles [Figure 7].

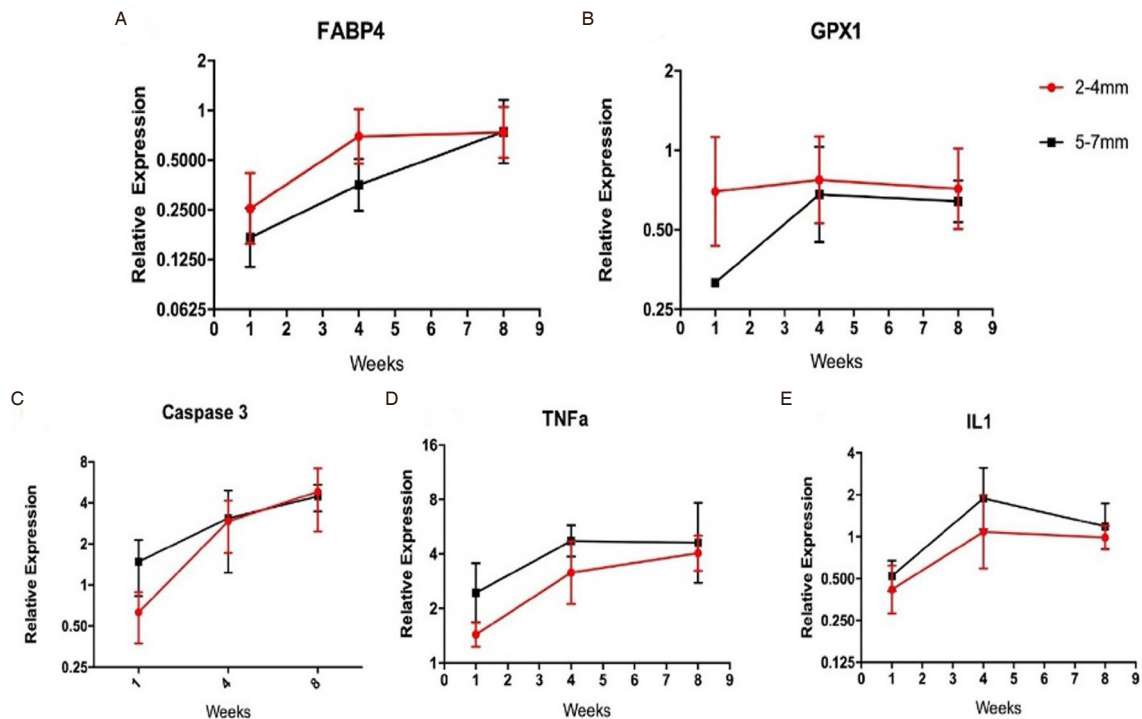
#### *Histologic assessment of grafted fat particle in mice*

Masson's trichrome staining revealed morphological changes of connective tissue components including extracellular matrix and fibrosis over time with interesting differences in adipocyte sizes between small and large particles [Figure 8A-H]. In general, adipocyte necrosis was observed by four weeks, as evident by large spaces of oil, with increased presence at eight weeks and near complete resorption by 12 weeks. In contrast, small and irregularly shaped adipocytes were evident at four weeks and increased in frequency at 8 weeks. The ratio of connective tissue (collagen indicated with blue) to whole area was calculated for each particle group [Figure 9]. Small particles had increased density of collagens at four and eight weeks ( $23\% \pm 4\%$  and  $20\% \pm 3\%$ ) but decreased density at 12 weeks ( $12\% \pm 1\%$ ,  $P < 0.05$ ) as it was replaced by adipocytes. In contrast, particles of 5-7 mm had significantly increased collagen matrix at 12 weeks compared to small particles ( $P < 0.05$ ), which may indicate that additional replacement by adipocytes would occur at a later timepoint.

#### *Gene expression analysis*

There were no statistical differences between measured gene expression between small and large particles for any gene at any timepoint, perhaps due to high inter-sample variability in each group [Figure 10]. In general, both particles had low *FABP4* expression at one week, suggesting low adipocyte function, and increased expression levels at each consecutive timepoint, suggesting adipocyte regeneration within the grafts [Figure 10A]. Glutathione peroxidase 1 is an enzyme which catalyzes the reduction of organic hydroperoxides and hydrogen peroxide ( $H_2O_2$ ) and thereby protects cells against oxidative damage. GPX1 is therefore upregulated by cells as a defense mechanism against reactive oxygen species. Small-sized particles had increased GPX1 expression at one week [Figure 10B] and simultaneously decreased expression of





**Figure 10.** Relative expression of adipocyte function (FABP4), protection from oxidative stress (GPX1), apoptosis (Caspase 3), and inflammation (TNF $\alpha$  and IL1) at one, four, and eight weeks post-grafted syngeneic fat particles. FABP4: fatty acid binding protein 4; GPX1: glutathione peroxidase; TNF $\alpha$ : tumor necrosis factor; IL1: interleukin 1

Caspase 3, which indicates active apoptotic pathways. Together, these results suggest reduced tissue survival in the larger particle group at early timepoints but equivalent and continuous tissue turnover throughout the regenerative process [Figure 10C]. We further speculate continuous infiltration of immune cells and tissue remodeling throughout fat graft regeneration due to consistently high levels of inflammatory genes that are typical of macrophages [Figure 10D and E]. Graft tissue replacement by circulating cells has been documented in other studies using fluorescently labeled bone-marrow cells; thus, these observations are in line with current observations in the literature<sup>[27,28]</sup>.

## DISCUSSION

Fat is a highly variable tissue that contains soft lipid-filled lobules and tough, fibrous connective tissue. Fat varies from patient to patient and across anatomical regions within a patient. To extract fat, a blunt end, hollow surgical cannula is passed many times through the tissue and, when suction is applied, small fat parcels are drawn into the cannula. Fat particles are defined as intact globules of adipocytes, microvasculature, and connective tissues. Particles do not occur in native adipose tissue, but rather are cut and formed during the harvesting step of fat grafting. Various cannula are commercially available with fixed aperture size and configuration to meet variable fat tissue needs. To obtain tissue for secondary grafting procedures, surgeons may attempt to use the smallest cannula possible, with the hypothesis that small fat particles will produce superior fat grafting results. However, our results suggest that the extra labor required to use small aperture cannula for the purposes of reducing particle size may not be necessary (or even helpful).

A common problem with current lipoplasty cannula is clogging due to parcels of fat becoming lodged in cannula apertures requiring surgeons to test multiple cannulas mid-surgery to find one that is appropriate. This cannula exchange process is tedious, time consuming, and requires additional operating room staff for

assistance. The primary driver of fat particle size manipulation is to avoid “large” fat particles in subsequent grafting procedures. These particles may clog injection cannula or have insufficient oxygen and nutrient diffusion to the interior particle core, resulting in large necrosis and oil cyst formation. These oil cysts are eventually resorbed through macrophage clearance resulting in graft tissue loss and inferior long-term fat grafting outcomes.

Clinically, it has been difficult to parse out the impact of fat particle size on graft retention from other confounding variables such as trauma associated with harvest or injection. Our study aimed to isolate the variable of fat particle size to determine the impact of fat particle size on *in vivo* survival and viability of fat grafts. To do this, an immunocompetent, inbred mouse model was selected to avoid donor variability with human adipose tissue. Adipose particles for grafting were prepared from whole subcutaneous fat pads by mincing tissue with surgical scissors. This allowed for consistent particle samples of known diameter while eliminating possible trauma from harvest cannulas. Study outcomes included graft retention, tissue histology, and measured genetic markers of inflammation, apoptosis, and tissue regeneration (qPCR).

Our data suggest that fat particles below 7 mm produce similar fat grafting outcomes despite increased hypoxia in 5-7-mm particles compared to 2-4-mm diameter particles. The histological findings from our study show that the small fat particles group led to milder necrosis and more neovascularization (especially in the early stages) while larger particles experienced increased necrosis and areas with irregular adipocyte morphology. Interestingly, while small fat particles showed superior architecture at the one-week study timepoint, this was reversed at the 12-week timepoint, with large fat particle grafts containing larger adipocytes and a better-organized histological structure. This may suggest that early adipocyte necrosis leading to extracellular matrix deposition through fibrosis results in a scaffold for tissue regeneration from circulating progenitor cells, as was also proposed by Del Vecchio *et al.*<sup>[29]</sup>, who found that smaller particles may lack connective tissue for structural support of adipocytes and proliferating stem cells. The regenerative role of fascia, connective tissue, and extracellular matrix components of the fat graft is often overlooked and yet to be fully understood. This study, along with our previous published work, aims to fill this knowledge gap.

Cumulatively, our studies suggest that, independent of adipose tissue quality, regenerative adipogenesis is highly dependent on the tissue scaffold, followed by angiogenesis, and lastly adipose induction or adipogenesis with all components playing important sequential and dynamic roles. It is hypothesized that finding the balance of these components will depend largely on the recipient site and the amount and nature of the host tissue in that region. As previous studies have demonstrated the angiogenic nature of proteins present in the fascia/connective tissue which angiogenesis occurs prior to adipogenesis, future studies on particle size could also include immunohistochemistry for CD31 to see if the larger particle group goes through this phase of angiogenesis at a different rate from small particles.

The results from this study have been corroborated elsewhere where particle size has shown no significant impact on long-term outcomes when the particle diameter is less than or equal to 7 mm<sup>[11,12]</sup>. Fisher *et al.*<sup>[30]</sup> transplanted lipoaspirate filtered with 500 and 800  $\mu$ m filters, whose average sizes were approximately 5.9 and 3.2 mm, and found there was no statistically significant difference between each other. Ozsoy *et al.*<sup>[31]</sup> compared lipoaspirate from human pannus harvested with 2-, 3-, and 4-mm diameter cannulas, and showed that the 4-mm cannulas had the highest level of adipocyte viability. Erdim *et al.*<sup>[15]</sup> evaluated viability of fat grafts, which was harvested from the abdomen of 10 consecutive patients using 2-, 4-, and 6-mm cannulas, by using colleganase digestion, supravital staining, and adipocyte counting using a haemocytometer. The results demonstrate a higher number of viable adipocytes from the aspirate occurred with the 6-mm cannula as opposed to the 2- and 4-mm cannulas. Therefore, the impact of harvesting cannula diameter may not be a critical factor on ultimate graft retention when fat particles are  $\leq 7$  mm in diameter, which we found to be the case in four commonly used lipoplasty harvesting cannula.

In conclusion, Fat particles with size below 7 mm in diameter do not alter graft survival in an immunocompetent mouse model. While larger fat particles experience early hypoxia and adipocyte loss, the remaining tissue serves as a scaffold for regeneration by circulating cells. This study provides important evidence on the fat grafting process, suggesting greater flexibility in lipoplasty cannula selection for fat grafting procedures than previously thought, allowing increased harvest yields and decreased effort and time.

## DECLARATIONS

### Authors' contributions

Performed partial examinations, analyzed and interpreted the research data: Yang X

Assisted with manuscript preparation: Egro FM, Nerone WV, Yousefpour M

Assisted with animal experiments, performed qRT-PCR experiments and assisted with data analysis: Jones T

Assisted with submission of the manuscript: Yousefpour M

Participated in overall study design and assisted with acquisition of human tissue specimens: Gusenoff JA, Rubin JP

Corresponding author is responsible for ensuring that all listed authors have approved the manuscript before submission and that all authors receive the submission and all substantive correspondence with editors: Kokai LE

All authors read and approved the final manuscript.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Financial support and sponsorship

This work was funded by a Pilot Funding Program for Early Stage Medical Technology Research and Development grant from the Center for Medical Innovation, Swanson School of Engineering, University of Pittsburgh (award no. F\_062-2013) and by National Institutes of Health for Dr. Rubin, Department of Plastic Surgery; University of Pittsburgh School of Medicine (award no. 5R01CA114246).

### Conflicts of interest

All authors declare that they are bound by confidentiality agreements that prevent them from disclosing their conflicts of interest in this work.

### Ethical approval and consent to participate

All animal experiments were performed under approved protocols by the University of Pittsburgh Institutional Animal Care and Use Committee (Protocol# 12080782).

### Consent for publication

Not applicable

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Cosmetic surgery national data bank statistics. *Aesthetic Surg* 2018;38:1-24.
2. Coleman SR. Structural fat grafting: more than a permanent filler. *Plast Reconstr Surg* 2006;118:108S-20S.
3. Gause TM, Kling RE, Sivak WN, Marra KG, Rubin JP, et al. Particle size in fat graft retention: a review on the impact of harvesting technique in lipofilling surgical outcomes. *Adipocyte* 2014;3:273-9.
4. Gir P, Brown SA, Oni G, Kashefi N, Mojallal A, et al. Fat grafting: evidence-based review on autologous fat harvesting, processing, reinjection, and storage. *Plast Reconstr Surg* 2012;130:249-58.

5. Smith P, Adams WP Jr, Lipschitz AH, Chau B, Sorokin E, et al. Autologous human fat grafting: effect of harvesting and preparation techniques on adipocyte graft survival. *Plast Reconstr Surg* 2006;117:1836-44.
6. Sommer B, Sattler G. Current concepts of fat graft survival: histology of aspirated adipose tissue and review of the literature. *Dermatol Surg* 2000;26:1159-66.
7. Crawford JL, Hubbard BA, Colbert SH, Puckett CL. Fine tuning lipoaspirate viability for fat grafting. *Plast Reconstr Surg* 2010;126:1342-8.
8. Rohrich RJ, Sorokin ES, Brown SA. In search of improved fat transfer viability: a quantitative analysis of the role of centrifugation and harvest site. *Plast Reconstr Surg* 2004;113:391-5.
9. Cheriyan T, Kao HK, Qiao X, Guo L. Low harvest pressure enhances autologous fat graft viability. *Plast Reconstr Surg* 2014;133:1365-8.
10. Yamaguchi M, Matsumoto F, Bujo H, Shibasaki M, Takahashi K, et al. Revascularization determines volume retention and gene expression by fat grafts in mice. *Exp Biol Med (Maywood)* 2005;230:742-8.
11. Carpaneda CA, Ribeiro MT. Percentage of graft viability versus injected volume in adipose autotransplants. *Aesthetic Plast Surg* 1994;18:17-9.
12. Khouri RK Jr, Khouri RE, Lujan-Hernandez JR, Khouri KR, Lancerotto L, et al. Diffusion and perfusion: the keys to fat grafting. *Plast Reconstr Surg Glob Open* 2014;2:e220.
13. Asken S. Autologous fat transplantation: micro and macro techniques. *Am J Cosmetic Surg* 1987;4:111-21.
14. Agris J. Autologous fat transplantation: a 3-year study. *Am J Cosmetic Surg* 1987;4:95-102.
15. Erdim M, Tezel E, Numanoglu A, Sav A. The effects of the size of liposuction cannula on adipocyte survival and the optimum temperature for fat graft storage: an experimental study. *J Plast Reconstr Aesthet Surg* 2009;62:1210-4.
16. Kirkham JC, Lee JH, Medina MA, McCormack MC, Randolph MA, et al. The impact of liposuction cannula size on adipocyte viability. *Ann Plast Surg* 2012;69:479-81.
17. Fagrell D, Eneström S, Berggren A, Kniola B. Fat cylinder transplantation: an experimental comparative study of three different kinds of fat transplants. *Plast Reconstr Surg* 1996;98:90-6.
18. Wree A, Mayer A, Westphal S, Beilfuss A, Canbay A, et al. Adipokine expression in brown and white adipocytes in response to hypoxia. *J Endocrinol Invest* 2012;35:522-7.
19. Van Pham P, Vu NB, Phan NK. Hypoxia promotes adipose-derived stem cell proliferation via VEGF. *Biomed Res Ther* 2016;3:1-7.
20. Wojciechowicz K, Gledhill K, Ambler CA, Manning CB, Jahoda CA. Development of the mouse dermal adipose layer occurs independently of subcutaneous adipose tissue and is marked by restricted early expression of FABP4. *PLoS One* 2013;8:e59811.
21. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006;444:860-7.
22. Mahmood DFD, Abderrazak A, El Hadri K, Simmet T, Rouis M. The thioredoxin system as a therapeutic target in human health and disease. *Antioxid Redox Signal* 2013;19:1266-303.
23. Lu J, Holmgren A. Thioredoxin system in cell death progression. *Antioxid Redox Signal* 2012;17:1738-47.
24. Linares M, Marin-García P, Martínez-Chacón G, Pérez-Benavente S, Puyet A, et al. Glutathione peroxidase contributes with heme oxygenase-1 to redox balance in mouse brain during the course of cerebral malaria. *Biochim Biophys Acta* 2013;1832:2009-18.
25. Wu Y, Wang D, Wang X, Wang Y, Ren F, et al. Caspase 3 is activated through caspase 8 instead of caspase 9 during H<sub>2</sub>O<sub>2</sub>-induced apoptosis in heLa cells. *Cell Physiol Biochem* 2011;27:539-46.
26. Park SA, Jeong S, Choe YH, Hyun YM. Sensing of vascular permeability in inflamed vessel of live animal. *J Anal Methods Chem* 2018;2018:5797152.
27. Zhao J, Yi C, Li L, Zheng Y, Wu K, et al. Observations on the survival and neovascularization of fat grafts interchanged between C57BL/6-gfp and C57BL/6 mice. *Plast Reconstr Surg* 2012;130:398e-406e.
28. Doi K, Ogata F, Eto H, Kato H, Kuno S, et al. Differential contributions of graft-derived and host-derived cells in tissue regeneration/remodeling after fat grafting. *Plast Reconstr Surg* 2015;135:1607-17.
29. Del Vecchio D, Rohrich RJ. A classification of clinical fat grafting: different problems, different solutions. *Plast Reconstr Surg* 2012;130:511-22.
30. Fisher C, Grahovac TL, Schafer ME, Shippert RD, Marra KG, et al. Comparison of harvest and processing techniques for fat grafting and adipose stem cell isolation. *Plast Reconstr Surg* 2013;132:351-61.
31. Ozsoy Z, Kul Z, Bilir A. The role of cannula diameter in improved adipocyte viability: a quantitative analysis. *Aesthet Surg J* 2006;26:287-9.

Review

Open Access



# The benefit of combined radiofrequency and ultrasound to enhance surgical and nonsurgical outcomes for the face and neck

Christine A. Catinis<sup>1</sup>, Suneel Chilukuri<sup>2</sup>

<sup>1</sup>Department of Medicine, Louisiana State University School of Medicine, New Orleans, LA 70112, USA.

<sup>2</sup>Refresh Dermatology, Department of Dermatology, Baylor College of Medicine, Houston, TX 77081, USA.

**Correspondence to:** Prof. Suneel Chilukuri, Refresh Dermatology, Department of Dermatology, Baylor College of Medicine, Houston, TX 77081, USA. E-mail: chilukuri@refreshdermatology.com

**How to cite this article:** Catinis CA, Chilukuri S. The benefit of combined radiofrequency and ultrasound to enhance surgical and nonsurgical outcomes for the face and neck. *Plast Aesthet Res* 2020;7:9. <http://dx.doi.org/10.20517/2347-9264.2019.68>

**Received:** 4 Dec 2019 **First Decision:** 27 Dec 2019 **Revised:** 6 Feb 2020 **Accepted:** 20 Feb 2020 **Published:** 28 Feb 2020

**Science Editors:** John Yousif Kai, O. Kaye **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

As people live longer and stay healthier, many want to look as youthful as they feel. The general population continues to explore nonsurgical options to augment and possibly delay traditional rhytidectomy. Herein, we present a unique nonsurgical option to enhance our current modalities of neuromodulators and fillers commonly used on the face and neck. In addition, we discuss the ideal application of this technology including treatment times and intervals between treatments.

**Keywords:** Facelift, neck lift, nonsurgical, radiofrequency, ultrasound, fillers, Botox, neurotoxin

## INTRODUCTION

Perhaps one of the most noticeable indicators of a person's age is the appearance of one's skin, particularly of the face and neck. Aging skin progressively becomes more wrinkled due to a loss of elasticity and volume over time. Other physical manifestations include uneven texture and discoloration due to sun damage. The increase in skin laxity is multifactorial and can be attributed to intrinsic factors, such as genetics, as well as extrinsic factors, such as UV exposure, gravity, and tobacco use<sup>[1]</sup>.

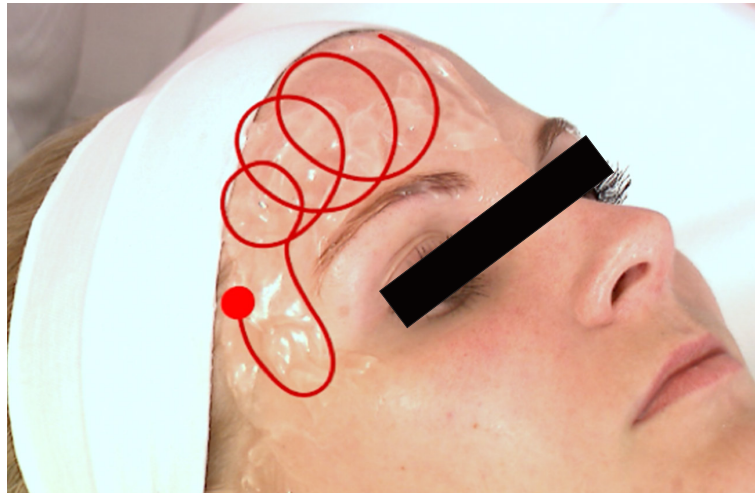
Physicians have traditionally relied on invasive procedures such as surgery and ablative skin resurfacing to combat the unwanted effects of skin aging. Over the last decade, however, noninvasive techniques have



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.







**Figure 1.** Treatment was started at the center part of the forehead and moved to the temporal region using slow, overlapping circular motions

greatly increased in popularity due to their effectiveness in improving skin laxity, while also minimizing recovery time, risk of adverse effects, and cost<sup>[2,3]</sup>. Such noninvasive methods include radiofrequency (RF), ultrasound (US), and a variety of other energy-based and mechanical devices.

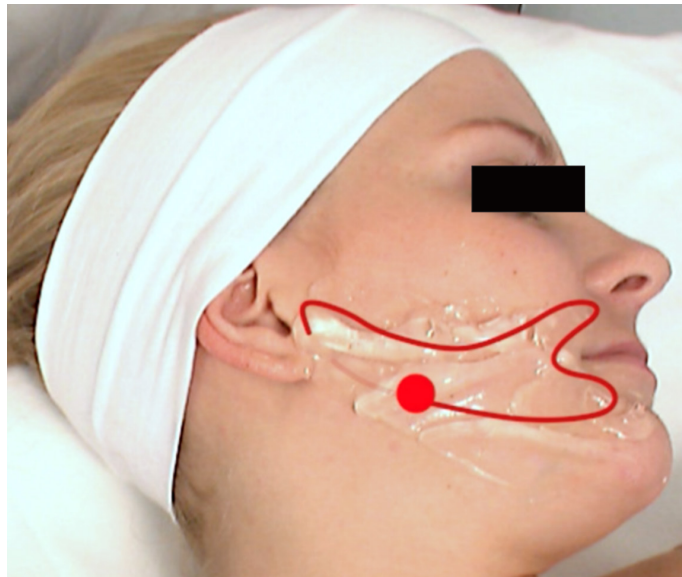
The application of RF to the skin generates heat at the level of the dermis by generating an oscillating current within the target tissue. The controlled heating of the dermis leads to the denaturation of the helical structure of collagen and an inflammatory response with subsequent activation of fibroblasts. As the collagen undergoes repair and remodeling, the dermal health improves with decreased skin laxity and increased elasticity<sup>[4-7]</sup>.

Ultrasound similarly induces dermal collagen synthesis and remodeling through the production of heat. The emission of sound waves through the tissue causes molecules to oscillate, which results in the production of thermal energy<sup>[4,8]</sup>.

Although the use of RF and US as individual modalities for the treatment of skin laxity has proven to be effective, the unique combination of the two in one handpiece allows shorter treatment times with more consistent results in our practice. The mechanical energy of ultrasound increases blood flow in the target tissue, which causes higher conductivity of the tissue and leads in homogenous heating of the area. In addition, ultrasound increases cell permeability, allowing the radiofrequency to have even more effect on fibroblast stimulation. The following details our modified treatment protocol that has improved patient outcomes.

## METHODS

After applying a ground pad opposite to the upper right back, the facial skin was cleaned with one pass with alcohol-soaked gauze and then one pass with acetone-soaked gauze. Ultrasonic gel was applied and a combined radiofrequency and ultrasound applicator (small handpiece of the Exilis Ultra by BTL Industries Inc., Boston, MA) was used to heat the facial skin to a therapeutic temperature of 41-43 °C within 1-1.5 min. With very slow sweeping motions with downward pressure, the right forehead, temple, medial and lateral cheek, and jawline (maximum of 10 cm × 10 cm area) were treated, as shown in [Figures 1 and 2]. Settings for the forehead and temple were power of 90 and duty factor of 80. Settings for the cheek and jawline were power of 90 and duty factor of 100. After reaching therapeutic temperature, these areas were treated for a



**Figure 2.** The applicator tip must be kept perpendicular to the treatment area while performing a very slow, large sweeping motion with some downward pressure. The soft tissue above the mandible was treated while avoiding direct treatment over the bone itself to ensure tolerability

total of 10 min. The contralateral forehead, temple, medial and lateral cheek, and jawline were then treated with the same protocol. The initial area was again heated to 41–43 °C and re-treated for 6 min at therapeutic temperature. The ultrasound gel was removed, and the patients were allowed to resume their routine skincare after 4 h. Included patients had no skin care regimen changes for a minimum of three months and were asked to refrain from altering their regimen until completion of the 12-month study. This protocol was repeated after two weeks. The results were examined at 3, 6, and 12 months post second treatment.

## RESULTS

In total, 30 patients (all women, age range of 40–55, mean age of 49) were treated over a 1-year period. Patient surveys showed 100% satisfaction at three months after the initial two treatments and 93% satisfaction at 6 months. Patients felt that their skin was “brighter” and “less saggy”. No complications were seen. In addition, all patients found the procedure tolerable. A pain scale of 0 (no discomfort) to 5 (worst pain imaginable) was utilized. The average rating was 1.8.

Figure 3 is an example of a patient three months after undergoing two treatments spaced two weeks apart.

## DISCUSSION

The best aesthetic outcomes in the treatment of age-related skin laxity of the face and neck often arise from a combination of modalities over time. These modalities include invasive approaches such as surgery and noninvasive methods including RF, US, and injectable neuromodulators and fillers. The surgical facelift is considered the gold standard in the treatment of age-related skin laxity due to its long-lasting results<sup>[9,10]</sup>. However, technological innovation is providing physicians and patients with novel noninvasive approaches. These new approaches have similar efficacy in combating skin laxity and have more favorable safety profiles. Regardless of which modality is chosen, the results of both invasive and noninvasive techniques can be enhanced and maintained by a combination of RF and US.

Radiofrequency exerts its effects by generating an oscillating electrical current within the target tissue. The current promotes collisions between ions and molecules in the dermal layer of the skin, leading to the



**Figure 3.** The right photo shows the patient three months after her second treatment with the protocol

formation of heat. In this way, RF produces thermal injury in the dermis, while preserving the integrity of the epidermis<sup>[4,5]</sup>. The controlled heating of the dermis induces immediate collagen denaturation and subsequent fibroblast activation, leading to synthesis of new collagen and elastin fibers<sup>[4,6,7]</sup>. The heat-induced contraction of the dermal layer tightens the skin while also increasing skin elasticity. The extent of tissue remodeling and contraction is dependent on a number of different variables. These include the depth of energy delivery, the time the desired temperature is maintained, the conductivity of the target tissue, and the frequency used<sup>[4]</sup>. It has also been shown that the heat generated by RF can induce apoptosis of subcutaneous adipocytes<sup>[4,8,11]</sup>. This finding has led to the extensive use of RF in body contouring and fat reduction, in addition to the treatment of skin laxity.

A novel two-treatment protocol using radiofrequency was proposed by McDaniel *et al.*<sup>[12]</sup> in 2014. Each treatment began with 6 min on the lower face (sub malar region to the mandible), followed by treatment of the submentum for 4 min. The lower face was then treated again for 6 min, with 3 min spent on the sub malar region and 3 min spent on the mandible. Next, the submentum was treated again for 4 min. These steps were then repeated on the contralateral face. The two treatments were spaced 10-14 days apart. This approach demonstrated a marked improvement in skin laxity in the majority of subjects. The treatments were well tolerated by patients, with no reported side effects. Ninety-two percent of subjects showed measurable improvement in the tightness of their skin after being evaluated three months post-treatment, demonstrating that maintaining a lower skin temperature over a longer period of time produces more favorable results. The data obtained in this study also confirmed an overall improvement in skin density (19% increase in three months) as well as improvements in dermal collagen and elastin deposition.

Ultrasound functions through the emission of high frequency sound waves within the tissue. The propagating sound waves excite charged molecules in the dermal layer and cause them to oscillate. By increasing the energy of the underlying molecules, US results in the generation of heat in a similar manner to RF, leading to collagen denaturation and remodeling<sup>[4,8]</sup>. When administered in combination with RF, US aids in efficiently and evenly dissipating the thermal energy to deeper skin layers by altering tissue resistance<sup>[4]</sup>.



**Figure 4.** This patient is 83 years old in the left photo and 88 years old in the right photo. She was treated with the protocol described in the Method Section and then continued with single quarterly treatments. During this five-year interval, she only received a total of 1.5 cc of hyaluronic acid filler. The only other treatment she received during that time is neuromodulators

Combined focused monopolar RF and US in a single applicator improves noninvasive treatment of skin laxity and fat reduction<sup>[5]</sup>. The mechanical energy of ultrasound increases blood flow in the target tissue, which causes higher conductivity of the tissue and leads in homogenous heating of the area. The other effect is to increase cell permeability - the mechanical energy or mechanical emission to the cells increases their responsivity to the treatment and the metabolic rate in the target area enables a faster and better response. The combined effect allows more rapid, uniform heating of the skin.

After reviewing the literature, we developed a modified treatment protocol using combined RF and US that has shown significant improvement in the maintenance and enhancement of both surgical and nonsurgical rejuvenation of the face and neck. We enhanced the double injury protocol first suggested by McDaniel *et al.*<sup>[12]</sup>. The advantage of a slightly longer protocol (10 min vs. 6 min) to uniformly heat the collagen to the ideal therapeutic temperature of 41-42 °C has proven to be comfortable and safe for the patient. By pausing for approximately 10 min before treating a second time for 6 min during the same visit, the chance of overheating and permanently denaturing collagen is reduced. After this initial injury, inflammatory cells including neutrophils and macrophages remove cellular debris before the second phase of repair begins. The tissue proliferation phase utilizes growth factors to increase fibroblast activity and subsequent collagen production. This second phase typically peaks at Day 6 or 7 with inflammatory cell response decreasing by Days 11-14. By repeating the RF/US protocol at two weeks, we suspect a sustained inflammatory response leads to greater tissue remodeling. In our practice, we performed a pilot split face study where one side of the face was treated with four sessions while the other side was treated according to the Method Section above. No visible difference was noted in 2D photography. When compared to other radiofrequency devices with protocols recommending three to five treatments, patients are pleased that fewer treatments are needed to obtain similar clinical results. In clinical practice, we routinely re-assess patients at three months after the second treatment and determine if he or she would benefit from another combined RF/US treatment or if other modalities can be utilized to enhance outcomes. We have found that our need for injectable fillers and collagen-stimulators has decreased in those patients who are receiving maintenance treatments 2-4 times per year. One such patient is the 88-year-old woman in [Figure 4](#).

There are several limitations to this unfunded pilot study. Only thirty subjects were treated, and no FDA-approved photometric scale was utilized. Biopsies were not performed to histologically quantify collagen



and elastin improvement. Future prospective studies with double-blinded investigators are indicated. In addition, there are numerous devices that may improve skin quality. Direct comparisons to these devices may be useful for physicians and health care providers.

We found this revised treatment protocol provides significant improvement in combating age-related changes in the face and neck, as a primary treatment, as post-surgical maintenance, and/or as enhancement to neuromodulators and fillers. The advantage of this device is that there is no down time, as seen with ablative lasers. However, in those patients with deep rhytids and severe actinic damage, laser resurfacing may be utilized first and the combined RF/US can be used to consistently enhance and maintain results. As with all aesthetic medicine, protocols should be customized to best benefit the patient.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Chilukuri S

Drafted the article and revised it critically for important intellectual content: Catinis CA

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

Both authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Consent was obtained for all patients.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Shah AR, Kennedy PM. The aging face. *Med Clin North Am* 2018;102:1041-54.
2. Chilukuri S, Lupton J. "Deep Heating" noninvasive skin tightening devices: review of effectiveness and patient satisfaction. *J Drugs Dermatol* 2017;16:1262-6.
3. Fritz M, Counters JT, Zelickson BD. Radiofrequency treatment for middle and lower face laxity. *Arch Facial Plast Surg* 2004;6:370-3.
4. Chilukuri S, Denjean D, Fouque L. Treating multiple body parts for skin laxity and fat deposits using a novel focused radiofrequency device with an ultrasound component: safety and efficacy study. *J Cosmet Dermatol* 2017;16:476-9.
5. Bonjorno AR, Gomes TB, Pereira MC, de Carvalho CM, Gabardo MCL, et al. Radiofrequency therapy in esthetic dermatology: a review of clinical evidences. *J Cosmet Dermatol* 2019;19:278-81.
6. Zelickson BD, Kist D, Bernstein E, Brown DB, Ksenzenko S, et al. Histological and ultrastructural evaluation of the effects of a radiofrequency-based nonablative dermal remodeling device: a pilot study. *Arch Dermatol* 2004;140:204-9.
7. Weiss RA. Noninvasive radio frequency for skin tightening and body contouring. *Semin Cutan Med Surg* 2013;32:9-17.
8. Fabi SG. Noninvasive skin tightening: focus on new ultrasound techniques. *Clin Cosmet Investig Dermatol* 2015;8:47-52.
9. Jones BM, Lo SJ. How long does a face lift last? Objective and subjective measurements over a 5-year period. *Plast Reconstr Surg* 2012;130:1317-27.
10. Hammoudeh ZS, Stevens WG. Nonsurgical adjuncts following facelift to achieve optimal aesthetic outcomes: "icing on the cake". *Clin Plast Surg* 2019;46:613-23.



11. McDaniel D, Fritz K, Machovcova A, Bernardy J. A focused monopolar radiofrequency causes apoptosis: a porcine model. *J Drugs Dermatol* 2014;13:1336-40.
12. McDaniel D, Weiss R, Weiss M, Mazur C, Griffen C. Two-treatment protocol for skin laxity using 90-Watt dynamic monopolar radiofrequency device with real-time impedance intelligence monitoring. *J Drugs Dermatol* 2014;13:1112-7.

Review

Open Access



# Differential diagnoses and treatment of lipedema

Maria Wiedner, Donia Aghajanzadeh, Dirk F. Richter

Department for Plastic and Aesthetic Surgery, Dreifaltigkeitskrankenhaus Wesseling, Wesseling 50389, Germany.

**Correspondence to:** Dr. Maria Wiedner, Department for Plastic and Aesthetic Surgery, Dreifaltigkeitskrankenhaus Wesseling, Bonner Strasse 84, Wesseling 50389, Germany. E-mail maria@wiedner-plastic.com

**How to cite this article:** Wiedner M, Aghajanzadeh D, Richter DF. Differential diagnoses and treatment of lipedema. *Plast Aesthet Res* 2020;7:10. <http://dx.doi.org/10.20517/2347-9264.2019.51>

**Received:** 3 Nov 2019 **First Decision:** 29 Nov 2019 **Revised:** 15 Jan 2020 **Accepted:** 3 Feb 2020 **Published:** 6 Mar 2020

**Science Editor:** Xiao Long **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

## Abstract

Lipedema is a frequently unrecognized and misdiagnosed disorder of the fatty tissue of extremities and hips, which affects almost purely women. The beginning of the disease usually occurs with hormonal changes, such as puberty, pregnancy, or menopause. Women suffer from pain, easy bruising, and disfigurement, which may lead to early immobility and social stress. Accurate diagnosis and treatment are essential. The differentiation between obesity and lipedema is difficult, as these two different entities often occur together. Other differential diagnoses are lymphedema, benign lipohypertrophy, and Dercum's disease. A therapy targeting the underlying cause of lipedema is not available because the exact etiology of the disorder is not clarified yet. Decongestive physical therapy is the basic conservative treatment, which is usually necessary lifelong. However, liposuction has led to a paradigm shift in the treatment of lipedema. The purposes of this article are to describe the symptoms and treatment options of the still fairly unknown disease Lipedema and to show the distinctions to its differential diagnoses.

**Keywords:** Lipedema, lymphedema, obesity, symmetrical limb enlargement, adipositas dolorosa

## INTRODUCTION

Lipedema is a painful disease of the subcutaneous tissue, which was first named in 1940 by Allen and Hines. They described a syndrome characterized by “large legs due to the subcutaneous deposition of fat in the buttocks and lower extremities and the accumulation of fluid in the legs”<sup>[1]</sup>. It is a painful, possibly chronically progressive disorder of adipose tissue that is characterized by symmetrical swelling of the lower and/or upper limbs. Patients typically complain about increased pressure sensitivity and easy bruising and may also experience ankle edema. In advanced stages, lymphedema may additionally occur, especially in those who are obese.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Lipedema is possibly a common but underdiagnosed disorder, which is almost exclusively found in females, but, as there is no standardized diagnostic test, the exact prevalence is unknown. According to German studies, 8%-18% of patients referred to a lymphedema clinic suffer from lipedema<sup>[2-4]</sup>.

The pathogenesis is not fully understood yet. Lipedema often affects several female members of the same family, suggesting a genetic disorder<sup>[5]</sup>. A positive family history is common and ranges between 16% and 64%<sup>[6]</sup>, but is likely higher due to under-diagnosis. Autosomal dominant inheritance with incomplete penetrance and sex limitation is the most likely mode of inheritance<sup>[5,7]</sup>. Since the disease usually manifests or is aggravated around hormonal changes (puberty, pregnancy, and menopause), lipedema is assumed to be an estrogen-regulated polygenetic disease. It is associated with vasculo- and lymphangiopathy<sup>[8]</sup>. There are numerous theories on pathogenesis. On the one hand, an altered estrogen-receptor pattern and responsiveness is assumed to exist centrally. On the other hand, pathologic estrogen-receptor patterns (alpha/beta) in the adipose tissue lead to increased lipogenesis and decreased lipolysis in the affected areas<sup>[8]</sup>.

Histologically, the fat deposition is a result of hyperplasia and hypertrophy of fat cells in the subcutaneous adipose tissue<sup>[8]</sup>. Additional mechanisms were demonstrated to play a role in the pathogenesis of lipedema including increased vascular permeability and damage (microangiopathy), excessive lipid peroxidation, and disturbances in adipocyte metabolism and cytokine production<sup>[9,10]</sup>. Inflammation of the peripheral nerves and sympathetic innervation abnormalities of the subcutaneous adipose tissue may be responsible for neuropathy<sup>[8]</sup>. One recent investigation on differences between adipose stem cells from lipedema and non-lipedema donors indicated that *in vitro* adipogenesis of lipedema adipose stem cells is severely hampered in comparison to non-lipedema adipose stem cells and that lipedema adipose stem cells not only differ in their lipid storage capacity but also in their adipokine expression pattern<sup>[11]</sup>. The findings indicate that this might serve as a valuable marker for diagnosis of lipedema, probably from an early stage.

Due to lack of knowledge, lipedema used to be a frequently unrecognized and misdiagnosed disorder. For a long time, the disease was equated with obesity, although lipedema fat is more resistant to reduction by diet and exercise than non-lipedema fat. The condition should be clearly distinguished from other dysfunctions of fat distribution, mixed forms of obesity and lymphedema. In addition to the progressive physical symptoms and consequences (lymphatic, dermatologic, and orthopedic problems), psychosocial distress with comfort eating and depression frequently arise in lipedema patients.

The diagnosis of lipedema is usually based on medical history and clinical features<sup>[6]</sup>. One criterion is the onset of the disease in parallel with hormonal changes and occurrence mainly in women<sup>[1,12]</sup>. Lipedema typically presents with a disproportionate enlargement of the limbs in relation to the upper part of the body<sup>[6,13]</sup> [Figure 1]. Increase of adipose tissue of the limbs is symmetrical, without involvement of feet or hands. Fat deposits begin abruptly above the malleoli, which creates the “cuff sign”<sup>[6]</sup> [Figure 2]. Other clinical criteria of lipedema comprise spontaneous or minimal trauma induced bruising, pain, and worsening during the day<sup>[5,14]</sup>.

The severity of lipedema can be classified into four clinical stages according to skin conditions and the sizes of the palpable and visible fat nodules<sup>[15,16]</sup> [Figure 3]:

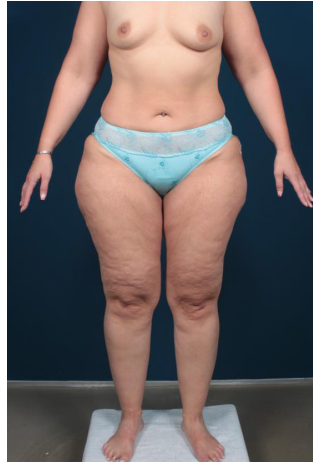
Stage 1: Flat skin with thickened subcutaneous tissue;

Stage 2: Increasing subcutaneous fat and walnut to apple-like indurations in the skin akin to a mattress;

Stage 3: Larger indurations and deforming skin-fat lobes, especially in the thighs and knee areas;

Stage 4: Development of additional lymphedema (lipolymphedema).

The development of lymphedema with lipedema (often known as lipolymphedema) can occur with any stage<sup>[10]</sup>. After a mean of about ten years suffering from lipedema, the lymphatics likely become insufficient.



**Figure 1.** Disproportionate enlargement of the lower limbs in relation to the upper part of the body



**Figure 2.** Typical cuff sign. The disproportionate fat accumulation in Stage 1-3 Lipedema patients stops proximal of the malleoli

In addition to the direct impairment of lymph vessels (fragility and compression by fat), a high volume insufficiency leads to increased edema.

In advanced stages, joint malformations are commonly seen due to the mass of soft tissue.

According to the pattern of fat distribution, one classification distinguishes five types of lipedema<sup>[17]</sup>:

Type I: Pelvis, buttocks, and hips (saddle bag phenomenon);

Type II: Buttocks to knees, with formation of folds of fat around the inner side of the knee;

Type III: Buttocks to ankles;

Type IV: Arms;

Type V: Lower leg.

There may be a mixture of lipedema types in one person. Only the arms may be affected in 3% of lipedema cases (Type IV)<sup>[18]</sup>.

## DIFFERENTIAL DIAGNOSIS

Lipedema is often misdiagnosed and differential diagnosis is sometimes challenging. The disease has to be clearly distinguished from other entities.



**Figure 3.** Different stages of lipedema according to the size of the fat nodules in the subcutaneous tissue: (left) Stage 1 shows a thickening of the subcutaneous tissue with small palpable nodules; (middle) Stage 2 already shows bigger fatty nodules up to walnut-size; and (right) Stage 3 is defined by overhanging skin-fat lobules, especially in the medial knee area or the development of severe column legs with cuff sign

One such is benign lipohypertrophy. Morphologically, lipohypertrophy may resemble lipedema. Women suffer from a constitutional disproportion of body shape with symmetrical hip- and thigh-obesity. The most common form of lipohypertrophy is the “riding breeches” obesity. The upper extremities are rarely affected. In contrast to lipedema, lipohypertrophy presents without pain, edema, or bruising<sup>[19]</sup>. It is thought that lipedema may develop from lipohypertrophy over time<sup>[20,21]</sup>. However, it is not entirely clear that these are truly separate conditions.

Another differential diagnosis of lipedema is primary lymphedema, which may also affect women around puberty. Concerning differences between lipedema and primary lymphedema, patients with lipedema present symmetrical swellings, whereas primary lymphedema is usually asymmetrical [Figure 4]. While lymphedema typically starts at the toes and subsequently reaches the thighs, swellings in lipedema patients usually affect the thighs first. One clinical differentiating factor is the Stemmer sign: lymphedema often presents a positive Stemmer sign, which describes the inability to pinch the skin over the proximal phalanx of the second toe, while Stemmer sign is negative in pure lipedema<sup>[22]</sup> [Figure 5]. Another significant difference between lipedema and lymphedema is the presence of pain and frequent bruising in lipedema. However, a visible lymphedema can occur with any stage of lipedema<sup>[10]</sup>.

Phlebolympheidema is the result of chronic venous insufficiency. It may occur in men and women, either uni- or bilaterally. Discolorations of the skin, varicose veins, or ulcer formations are typical symptoms. In contrast to lipedema, ultrasound examination in phlebolympheidema shows pathological findings. However, women with lipedema may also have varicose veins and may develop phlebolympheidema as a result of these<sup>[20]</sup>.

Dercum’s disease, also known as adipositas dolorosa, describes a condition characterized by generalized obesity and painful, fatty tumors (lipomata) in the adipose tissue, occurring almost exclusively in women. The tumors are found on the extremities, without involvement of the feet. It is said that it commonly develops around menopause. In contrast to lipedema, edema is not present. Muscular weakness and fatigue, emotional instability, depression, and alcohol abuse are potential features of the disease<sup>[23]</sup>. However, it is controversial whether Dercum’s disease represents a separate entity or it is only a variant of lipedema.





**Figure 4.** Asymmetrical limb swelling in a lymphedema patient with her left lower limb affected

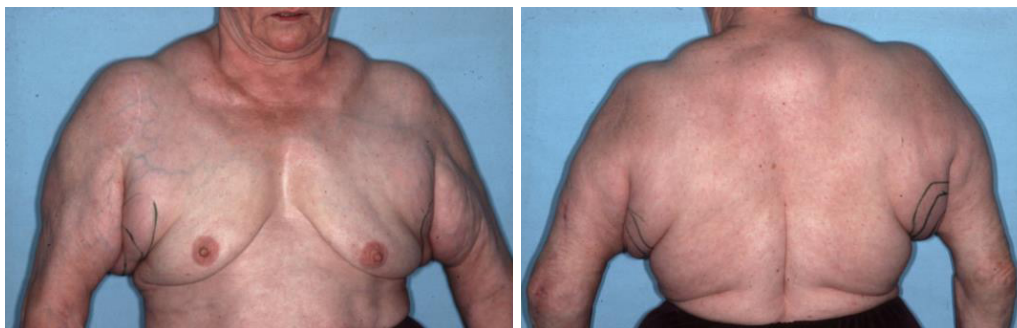


**Figure 5.** Stemmer sign. It is negative in Lipedema patients. Here you can lift the skin of the dorsum of the second toe, which is not possible in Lymphedema patients (positive Stemmer sign)

Madelung's Disease, also known as benign symmetrical lipomatosis and Launois-Bensaude disease, is a disorder of fat metabolism that results in an unusual accumulation of fat deposits around the neck (Type I), shoulder areas and upper arms (Type II), or pelvic areas (Type III). The condition is most common in men and almost always associated with alcohol abuse and liver damage<sup>[24]</sup> [Figure 6].

Lipedema is frequently misdiagnosed as obesity. While obesity affects the whole body, lipedema usually affects the upper and/or lower limbs and does not involve the feet and hands. Patients with lipedema hardly respond to restricted diet<sup>[25]</sup>. Even after extreme weight loss, for example after bariatric surgery or in cancer cachexia, patients typically lose less fat in the areas affected by lipedema than in the non-affected areas [Figure 7]. Lipedema and obesity share the hallmark of symmetrical fat increase<sup>[26]</sup>. However, differential diagnostic criteria include the different distribution of fat in obesity (which is typically more in the "central" pattern) and that the fat is not usually tender/painful. Weight loss by dieting and exercise in chronic lipedema patients can often be frustrating, because there is less fat reduction in the affected limbs than on the trunk. Furthermore, weight gain can result in excess fat deposition in the legs. Thus, in women with lipedema who cannot sustain weight loss, there is a risk of progression of the lipedema. This seems to be more likely in women who experience periods of weight loss followed by periods of weight gain. Management of any associated obesity is therefore crucial to the successful management of lipedema. Bariatric surgery may be a successful way of reducing weight and maintaining the achieved loss<sup>[27]</sup>.

A recent review highlights the utility of developing a genetic diagnostic test containing candidate genes for lipedema and causative genes of diseases that can be confused with lipedema, to help differentiate lipedema



**Figure 6.** Madelung's disease Type II with fat accumulations in the neck, shoulders, and arms



**Figure 7.** Patient after bariatric surgery and massive weight loss. She did not lose the lipedema fat in the affected lower legs

from other diagnoses<sup>[28]</sup>. The list of differential diagnoses criteria of lipedema modified from Schmeller in 2005 is shown in Table 1<sup>[29]</sup>.

## THERAPY

A targeted therapy aimed at the cause of lipedema is not known because the exact etiology is still unexplained. Therapy has essentially two objectives: (1) resolve or improve symptoms (edema, pain, and disproportion); and (2) prevent disease progression and the development of complications (lymphatic, dermatologic, and orthopedic problems). A distinction is made between conservative therapy to decongest the subcutaneous tissue and surgical therapy such as liposuction to reduce adipose tissue. As discussed above, weight management is a key component of both objectives.

Conservative approaches include compression garment therapy or wrapping to reduce edema and manual lymphatic drainage (MLD) as part of an outpatient context or as part of a complex decongestive physiotherapy (CDP). CDP is a very time-consuming therapy, being performed twice a day for 45-60 min over a period of 3-4 weeks, mainly in lymphedema clinics in an inpatient context. A component of CDP is manual lymphatic drainage. MLD is a type of gentle skin massage which stimulates contraction of the lymph collectors and enhances protein resorption. In addition to improving lymphatic circulation, MLD

**Table 1. List of differential diagnoses criteria**

	Sex	Onset	Localisation	Symmetry	Fat increase	Pressure sensitivity	Edema	Involvement	Diets successflu	Disproportion	Pain	Other
Lipedema	Women	Usually puberty	Legs, arms	+	+	+	+	-	-	+	+	Easy bruising
Lipohypertrophy	Women	Usually puberty	Legs, arms	+	+	-	-	-	-	+	-	Common condition
Primary Lymphedema	Women, men	Usually puberty	Legs	-	-	-	+	+	-	-	-	Positive Stemmer sign, pathologic lymphscintigraphy
Phlebedema	Women, men	Adolescence	Legs	-	-	-	+	+	-	-	-	Pathologic vein function test
Dercum's disease	Usually women	Usually menopause	Legs	-	+	+	-	+	-	+	+	Muscle weakness, alcohol abuse, depressions
Madelung's disease	Usually men	Adolescence	Neck, shoulder, pelvis	+	+	+	-	+	-	+	-	Alcohol abuse, liver damage
Obesity	Women, men	Each age	Whole body	+	+	-	-	+	+	-	-	BMI >25

Differential diagnoses criteria of lipedema listed regarding sex, onset of disease, affected localization, symmetry, pain, edema, and other symptoms

increases blood flow in deep and superficial veins<sup>[30]</sup>. Immediately after decongestive therapy, a compression bandage is applied in the form of garments or wrapping to reduce edema. CDP furthermore consists of decongestive exercises and meticulous skin care<sup>[31]</sup>.

CDP can also be combined with intermittent pneumatic compression (IPC). During this therapy, chambers in the sleeves, gloves, or boots of the device are inflated rhythmically from distal to proximal by an air pump to improve venous and lymphatic drainage of the limbs. Pressures between 30 and 60 mmHg are used. One cycle takes approximately 30 seconds and the treatment usually 30 min. IPC can also be used at home. Other components of conservative treatments are physical activity, healthy food plans to reduce any obesity component of lipedema, and psychosocial counseling. There is, however, a current debate about the value of the use of MLD in the routine management of lipedema<sup>[32]</sup>.

According to studies, decongestive treatments significantly reduce capillary fragility and the number of petechiae, as well as the mean limb volume in patients with lipedema<sup>[33]</sup>. However, conservative therapy usually presents only a short-term-success. For patients with minimal or no improvement with conservative treatment after at least 6-12 months, surgical treatment should be discussed.

Currently, there is growing interest in the use of liposuction as a surgical treatment for lipedema. Decades ago, surgical therapy of lipedema consisted of extensive lipectomies and conventional liposuctions with sharp needles without use of tumescent solution. This was associated with life-threatening



**Figure 8.** Lipedema Stage 3 patient before and after five sessions of liposuction in the lower legs

complications as well as persistent postoperative edema due to damage to the lymphatic vessels. As a result, conventional dry liposuction under general anesthesia was therefore contraindicated in lipedema patients<sup>[34]</sup>.

Nowadays, the “wet”, “super-wet”, and “tumescent” techniques are used, which are less likely to damage the lymphatic system compared to the conventional “dry” technique. The use of microcannulas as well as new liposuction techniques such as power-assisted liposuction with vibrating cannulas or waterjet-assisted liposuction have been shown to further minimize tissue trauma and complication rates.

When performing liposuction in lipedema patients, the crisscross technique, which is commonly used in aesthetic surgery, is contraindicated because of the higher risk of harming lymph vessels with consecutive development of lymphedema. Therefore, it is of great importance to take the lymph vessel anatomy into account and move the cannula parallel to the lymph vessels in order to save them. This is why more incisions are usually needed to remove the fat from the affected area.

Since 2005, guidelines of the German Society of Phlebology recommend liposuction as an integrated part of therapy<sup>[35]</sup>. Tumescent liposuction has been demonstrated to reduce disproportions and pain permanently<sup>[36]</sup>, stop progression of the disease<sup>[35,36]</sup>, and improve quality of life<sup>[37-40]</sup>. In addition, surgical therapy may reduce the amount of necessary conservative therapy<sup>[38,40]</sup>. Weight management is an important requirement for maintaining the benefits of liposuction. There are reports that fat can re-accumulate in those who put on weight after liposuction.

Most patients undergoing liposuction for lipedema require several treatments over several months. According to the guidelines, more than 4-6 liters of fat per session should not be removed because the risk of cardiopulmonary complications increases with increasing blood and fluid loss. Electrolyte imbalances are particularly dangerous. Usually, a minimal interval of at least three months between the sessions is recommended. [Figure 8](#) shows a 36-year-old patient after five sessions of liposuction.

In conclusion, Lipedema is a frequently unrecognized and misdiagnosed disorder. Clinicians should be aware of clinical signs and clearly distinguish the condition from other entities. Accurate diagnosis and treatment are essential because they determine the patient’s prognosis. A targeted therapy for lipedema is not known because the exact etiology of the disorder is not clarified yet. Decongestive physical therapy is the basic conservative treatment, which is usually necessary lifelong. However, liposuction has led

to a paradigm shift in the treatment of lipedema. While conservative therapy may reduce symptoms temporarily, liposuction is able to remove the pathologic adipose tissue, which may result in a sustainable symptom relief. Liposuction is an effective surgical method and should be individually considered for patients suffering from lipedema. Weight management is a key component of successful treatment whether conservative or surgical.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the article: Wiedner M, Aghajanzadeh D, Richter DF

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Written informed consent for publication of images was obtained from the patients shown on the images.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Allen EV, Hines EA. Lipedema of the legs: a syndrome characterised by fat legs and orthostatic edema. *Proc Staff Meet Mayo Clin* 1940;15:184-7.
2. Herpertz U. Krankheitsspektrum des lipödems an einer lymphologischen fachklinik- erscheinungsformen, mischbilder und behandlungsmöglichkeiten. *Vasomed* 1997;5:301-3.
3. Meier-Vollrath I, Schneider W, Schmeller W. Lipödem: Verbesserte Lebensqualität durch Therapiekombination. *Deutsches Ärzteblatt* 2005;15:A1061-7.
4. Lulay G. Lymphologische akutklinik- ein neues versorgungskonzept. *Lymphol Forsch Praxis* 2010;14:90-5.
5. Child AH, Gordon KD, Sharpe P, Brice G, Ostergaard P, et al. Lipedema: an inherited condition. *Am J Med Genet A* 2010;152A:970-6.
6. Langendoen SI, Habbema L, Nijsten TE, Neumann HAM. Lipoedema: from clinical presentation to therapy. A review of the literature. *Br J Dermatol* 2009;161:980-6.
7. Weissleder H, Schuchhardt C, et al. Lymphedema. Diagnosis and therapy. WVP. Wirtschafts- und Praxisverlag; 2007.
8. Szél E, Kemény L, Groma G, Szolnoky G. Pathophysiological dilemmas of lipedema. *Med Hypotheses* 2014;83:599-606.
9. Mayes JS, Watson GH. Direct effects of sex steroid hormones on adipose tissues and obesity. *Obes Rev* 2004;5:197-216.
10. Herbst K, Mirkovskaya L, Bharhagava A, Chava Y, Te CHT. Lipedema fat and signs and symptoms of illness, increase with advancing stage. *Arch Med* 2015;7:10.
11. Bauer AT, Lukowicz D, Lossagk K, Hopfner U, Kirsch M, et al. Adipose stem cells from lipedema and control adipose tissue respond differently to adipogenic stimulation in vitro. *Plast Reconstr Surg* 2019;144:623-32.
12. Brauer W, Cornely M, Faerber G, Lulay GR., Miller A, et al. Lipödem S1 Leitlinie. Available from: <https://www.phlebologie.de/aerzte/wissen/leitlinien/> [Last accessed on 16 Feb 2020] (in German)
13. Van Geest AJ, Esten SCAM, Cambier J-PRA, Gielen EGJ, Kessels A, et al. Lymphatic disturbances in lipoedema. *Phlebologie* 2003;32:138-42.
14. Wold LE, Hines Jr EA, Allen EV. Lipedema of the legs: a syndrome characterized by fat legs and edema. *Ann Intern Med* 1951;34:1243-



- 50.
15. Fife CE, Maus EA, Carter MJ. Lipedema: a frequently misdiagnosed and misunderstood fatty deposition syndrome. *Adv Skin Wound Care* 2010;23:81-92.
16. Meyer-Vollrath I, Schmeller W. Lipödem - aktueller stand, neue Perspektiven. *J Dtsch Dermatol Ges* 2004;2:181-6.
17. Schneider W, Meier-Vollrath I. Das lipödem: neue möglichkeiten der therapie. *Schweiz Med Forum* 2007;7:150-5.
18. Herpertz U. Lipedema. *Z Lymphol* 1995;19:1-11. (in German)
19. Müssig K, Gallwitz B. Lipohypertrophie. *Dtsch Med Wochenschr* 2006;131:1807-8. (in German)
20. Herpertz U. Ödeme und Lymphdrainage. *Diagnose und Therapie von Ödemkrankheiten*. 2nd ed. Stuttgart, New York: Schattauer; 2004. pp. 168-18122.
21. Marsch WC. Ist das Lipödem ein lympho-logisches krankheitsbild? *J Lymphologie* 2001;1:22-4.
22. Stemmer R. Ein klinisches zeichen für früh- und differentialdiagnostik des lymphödems. *Vasa* 1976;3:261-2.
23. DeFranzo AJ, Hall JH, Herring SM. Adiposis dolorosa (Dercum's disease): liposuction is an effective form of treatment. *Plast Reconstr Surg* 1990;85:289-92.
24. Ruzicka T, Vieluf D, Landthaler M, Braun-Falco O. Benign symmetric lipomatosis Lau-nois-Bensaude. Report of ten cases and review of the literature. *J Am Acad Dermatol* 1987;17:663-74.
25. Schmeller W, Meyer-Vollrath I. Erfolgreiche operative therapie des lipödems mittels liposuktion. *Phlebologie* 2004;33:23-9.
26. Bertsch T, Erbacher G. Lipoedema - myths and facts Part 3. *Phlebologie* 2018;47:188-97.
27. Bertsch T, Erbacher G, Torio-Padron N. Lipoedema - myths and facts Part 4. *Phlebologie* 2019;48:47-56.
28. Paolacci S, Precone V, Acquaviva F, Chiurazzi P, Fulcheri E, et al. Genetics of lipedema: new perspectives on genetic research and molecular diagnoses. *Eur Rev Med Pharmacol Sci* 2019;23:5581-94.
29. Schmeller W, Meyer-Vollrath I. Lipödem: ein Update. *LymphForsch* 2005;9:10-20.
30. Crisóstomo RSS, Candeias MS, Armada-da-Silva PAS. Venous flow during manual lymphatic drainage applied to different regions of the lower extremity in people with and without chronic venous insufficiency: a cross-sectional study. *Physiotherapy* 2017;103:81-9.
31. International Society of Lymphology. The diagnosis and treatment of peripheral lymphedema. Consensus document of the international society of lymphology. *Lymphology* 2003;36:84-91.
32. Bertsch T, Erbacher G. Lipoedema - myths and facts Part 2. *Phlebologie* 2018;47:120-6.
33. Szolnoky G, Nagy N, Kovács RK, Dósa-Rác E, Szabó A, et al. Complex decongestive physiotherapy decreases capillary fragility in lipedema. *Lymphology* 2008;41:161-6.
34. Stiefelhagen P. No lymphedema, no obesity. How can lipedema be treated? *MMW Fortschr Med* 2001;143:15.
35. Wienert V, Földi E, Schmeller W, Rabe E. Leitlinie: Lipödem der Beine. *Phlebologie* 2005;34:38-40.
36. Rapprich S, Loehnert M, Hagedorn M. Therapy of lipoedema syndrome by liposuction under tumescent local anaesthesia. *Ann Dermatol Venerol* 2002;129:1S-711.
37. Cornely ME. Fatter through lipids or water. Lipohyperplasia dolorosa versus lymphedema. *Hautarzt* 2010;61:873-9. (in German)
38. Rapprich S, Dingler A, Podda M. Liposuktion ist eine wirksame therapie beim lipödem - ergebnisse einer untersuchung mit 25 patientinnen. *J Deutsch Dermatol Ges* 2011;9:33-40.
39. Schmeller W, Tronnier M, Kaiserling E. Lymphgefäßschädigung durch liposuktion? Eine immunhistologische untersuchung. *LymphForsch* 2006;10:80-4.
40. Schmeller W, Huepp M, Meier-Vollrath I. Tumescent liposuction in lipoedema yields good long-term results. *Br J Dermatol* 2011;166:161-8.

Original Article

Open Access



# A minimally invasive midface suspension

Jorge I. de la Torre, John Lindsey Jr., Dean Cerio, Luis O. Vasconez

Division of Plastic Surgery, University of Alabama at Birmingham and Birmingham V.A. Medical Center Birmingham, Birmingham, AL 35294, USA.

**Correspondence to:** Dr. Jorge I. de la Torre, MD, MSHA, UAB Plastic Surgery, 500 22nd Street, South - JWB 103 Birmingham, Birmingham, AL 35294, USA. E-mail: jdlt@uab.edu

**How to cite this article:** de la Torre JI, Lindsey Jr. J, Cerio D, Vasconez LO. A minimally invasive midface suspension. *Plast Aesthet Res* 2020;7:11. <http://dx.doi.org/10.20517/2347-9264.2019.54>

**Received:** 8 Nov 2019 **First Decision:** 13 Jan 2020 **Revised:** 10 Feb 2020 **Accepted:** 20 Feb 2020 **Published:** 13 Mar 2020

**Science Editor:** John Yousif **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

**Aim:** With the normal aging process, the malar fat pad descends vertically, causing a number of characteristic changes to the face. Various techniques have been used to correct ptosis of the malar fat pad.

**Methods:** The authors describe a technique that is minimally invasive and can be used to correct malar fat pad ptosis. This technique uses a suspension suture to elevate the malar fat pad to a more youthful position. The technique has been successfully used in 71 patients.

**Results:** All of the cases were performed in the office setting under local anesthesia. There were no complications, and, by patient self-report and physician exam, results have been lasting and satisfactory.

**Conclusion:** The minimally invasive midface suspension is a safe and successful approach to midface rejuvenation in properly selected patients.

**Keywords:** Midface rejuvenation, midface suspension, malar fat pad, facial rejuvenation

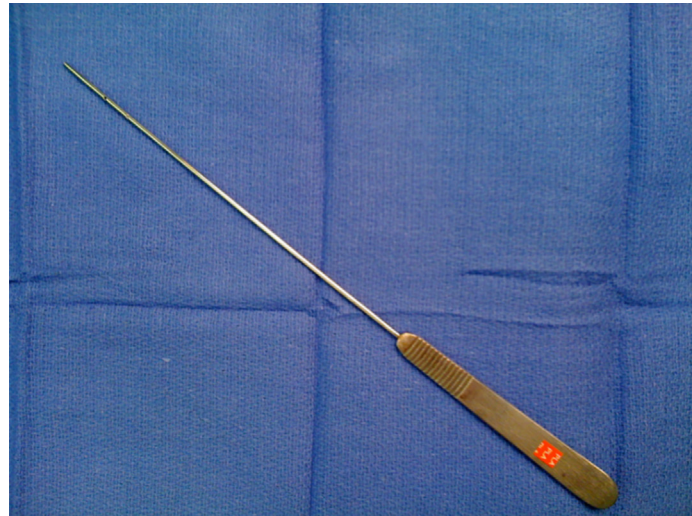
## INTRODUCTION

The malar fat pad is a critical component of the midface and its descent impacts several aspects of facial aging. It is a fibro-fatty triangular structure that is just below the dermis and subcutaneous tissue. It is superficial to the superficial muscular aponeurotic system (SMAS)<sup>[1]</sup>. This triangular structure has its apex located at the oral commissure; the lateral border is a line from the apex to the lateral canthus; the medial border is a line from the apex to the medial canthus; and the superior border is a horizontal line that runs along the inferior aspect of the lower eye<sup>[2]</sup>.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** Blunt passing instrument. Note the eye for the suture is 1 cm from the tip. There is a visible mark 2 cm away from the tip

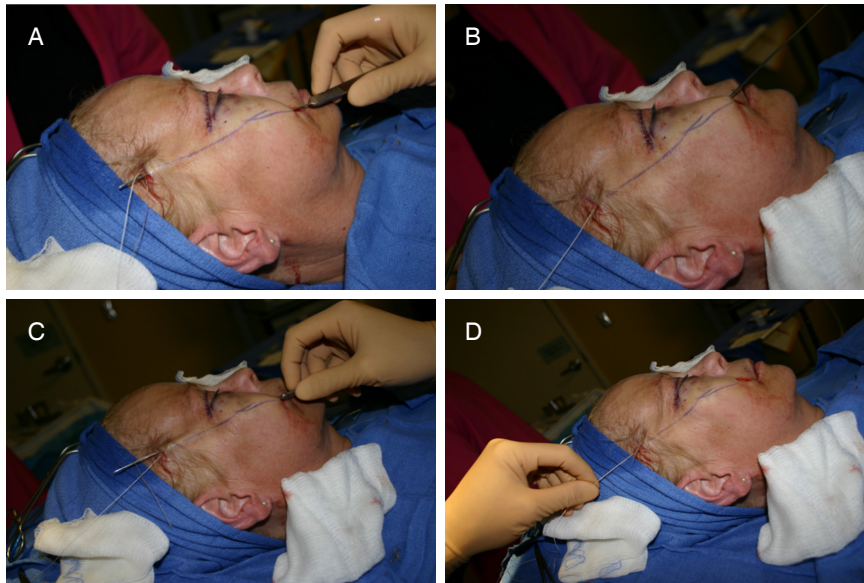
Numerous studies have described the etiology of ptosis of the malar fat pad<sup>[1-8]</sup>. These include gravitational pull, repetitive facial animation, laxity of the retaining ligaments, and loose attachment to the underlying SMAS. The effects of this descent have also been well described, such as hollowing of the infraorbital rim, deepening of the nasolabial folds, and the formation of jowls<sup>[1-8]</sup>. Myriad techniques have been used to rejuvenate the midface, ranging from open surgeries with extensive dissection to closed procedures with barbed sutures and zero dissection<sup>[2,3,8-11]</sup>. Furthermore, there is extensive variability in the location of incisions and the planes of dissection.

Despite the array of treatment options, there is an emerging interest in minimally invasive techniques. Reasons for this development may include shorter operative time, fewer complications, and decreased recovery time<sup>[12]</sup>. Additionally, these types of procedures can generally be performed in the office-based setting with local anesthesia. Patients are also more willing to accept less dramatic results for a less invasive procedure. There are several minimally invasive techniques, such as endoscopic approach with extensive dissection, U-suture suspension with minimal dissection, and unidirectional barbed suture suspension with zero dissection<sup>[9,10,13-17]</sup>. We describe here the Minimally Invasive Midface Suspension (MIMS), which is a technique with a short learning curve, reproducible results, high patient satisfaction, and low risk of complications.

## METHODS

### Surgical technique

The planned incisions are marked with the patient in the upright position. The anchor point incision is marked approximately 1-2 cm behind the hairline in the temporal region on a vertical line drawn from the superior lateral border of the malar fat pad. The inferior incision is marked along the nasolabial fold along the mid-pupillary line. Both incision sites are infiltrated with 0.5% lidocaine with 1:200,000 epinephrine. In addition, local anesthesia is administered as a block to the infraorbital and supraorbital nerves. After allowing the epinephrine to take effect, the temporal incision is made with a 15-blade approximately 2 cm in length beveling with the direction of the hair follicles. Gentle blunt dissection can be performed to dissect down to the level of the deep temporal fascia. A small stab incision is made near the nasolabial fold with an 11-blade. A specially designed long, blunt passing instrument [Figure 1] is then introduced through the naso-labial stab incision and directed through the malar fat pad toward the temporal incision in a subcutaneous plane. Care is taken to ensure that the passing instrument does not penetrate the



**Figure 2.** Technical details. A: The blunt passing instrument is introduced by stab incision up through the fixation incision. The Vicryl suture is passed through the eye of the passing instrument; B: The passing instrument is withdrawn, pulling the suture through the malar fat pad; C: The suture is engaged in the malar fat pad and then the tip of the passing instrument is passed up through the fixation incision; D: The suture is drawn out of the passing instrument. The passing instrument is removed through the naso-labial stab incision and the suture can then be secured to the temporal fascia

overlying skin or the underlying facial muscles. A 3-0 Vicryl, PDS absorbable suture, or 4-0 nylon clear permanent suture is threaded through the eye of the instrument and the suture needle is held in a needle diver. The instrument is then partially withdrawn through the stab incision [Figure 2A and B]. A mark on the instrument demarcates how close the tip of the instrument is to the puncture site so that the tip is not fully withdrawn. The passing instrument is re-advanced taking care to engage the malar fat pad tissue. The insertion path of the needle is in a slightly different path within the malar fat pad [Figure 2C and D]. The tip of the passing instrument exits the fixation incision and the suture is then withdrawn from the instrument leaving the two ends. The needle of the suture can then be used to attach the suture to the deep temporal fascia. Tension is placed on the suture to elevate the malar fat pad to the desired level. The suture is then secured to the deep temporal fascia. The temporal incision is closed using deep dermal sutures and subcutaneous sutures, and the stab incision is closed using 5-0 subcutaneous chromic suture.

## Patients

An institutional review board-approved retrospective review was performed to identify all patients who underwent a MIMS procedure between 2008 and 2018. Preoperative and postoperative photographs were reviewed as was the electronic medical record. There were a total of 71 patients, 59 females and 12 males with an average age of 59 years.

## RESULTS

A representative case is illustrated and described in Figure 3. There have been no major complications in any of the patients. No revision or re-elevation was necessary. Long-term results were assessed subjectively by patient report and surgeon examination. They were found to have been very satisfactory and lasting.

## DISCUSSION

Various techniques have been described to correct malar fat pad ptosis, including open surgical approaches and closed procedures<sup>[1-3,8,10,11,13-17]</sup>. In terms of minimal access procedures, endoscopic techniques with





**Figure 3.** Long-term follow-up of a 57-year-old patient undergoing minimally invasive midface suspension procedure: A: preoperative frontal view; B: postoperative frontal view (eight months); C: preoperative lateral view; D: postoperative lateral view (eight months). The patient maintained satisfactory results at the 18-month follow-up visit.

extensive subperiosteal dissection have been described<sup>[13]</sup>. These approaches may involve longer operative times, general anesthesia, lengthy recovery periods, and a higher risk of complications. Additionally, numerous studies describing thread lifts have been published. Thread lifts generally involve inserting a unidirectional suture with cones or barbs subcutaneously<sup>[9,10,16,17]</sup>. The tissue is then manually inset over the barbs or cones. The advantages of this technique are short procedure time and very limited dissection. Disadvantages of thread lifts include minimal fat pad elevation, reaction and extrusion of sutures, and temporary results.

The technique in this study involves making a small temporal incision and a small stab incision near the nasolabial fold. The malar fat pad is elevated by suspending it to the deep temporal fascia using a standard suture. Unlike thread lifts, which only elevate the skin, the technique in this study directly elevates the malar fat pad. This distinction is important because the ideal way to address midfacial aging is by elevating the malar fat pad to its original and youthful position over the malar eminence. In our series, we initially used permanent nylon suture; however, with the success of the absorbable barbed suture, we initiated the use of absorbable sutures. The monofilament sutures left a palpable area along the suture and a knot, which the Vicryl suture avoided. Although the suture material dissolves within a few weeks, the results last longer. Although nylon suture is frequently used for midface elevation, absorbable sutures including catgut, Vicryl, and PDS have been used to elevate the face with success<sup>[18-21]</sup>. This may be because the malar fat pad is anchored to the fixed tissue of the deep temporal fascia, unlike thread lifts, which typically involve floating sutures. The difference in fixation may account for results which last longer than those from the modified sutures since the malar fat pad is suspended to the deep temporal fascia. Although a stab incision is made in a visible area of the face, in all patients, the scar was extremely well-tolerated and imperceptible.

Although this technique has several advantages and satisfactory results, it does have limitations. MIMS is not ideal for patients with severe skin laxity; these patients are better served by traditional facelifts.





**Figure 4.** Short-term follow-up of a 47-year-old patient undergoing minimally invasive midface suspension procedure and resection of left temporal skin lesion: A: preoperative frontal view; B: immediate frontal view postoperative (6 days); C: postoperative frontal view (9 months); F: preoperative lateral view; E: immediate postoperative frontal view (6 days); and F: postoperative lateral view (9 months).

Therefore, the ideal candidates for minimally invasive midface suspension have mild-to-moderate skin laxity and are generally middle-aged seeking limited improvement. Periorbital pleating and wrinkling may occur if this procedure is performed in patients with significant skin laxity. However, in patients with some excess skin, a limited skin excision can be included. In addition, this procedure can be combined with another procedure, such as facial resurfacing, blepharoplasty, and fat grafting. Patients have self-reported satisfaction with outcomes that match or exceed those of other minimally invasive procedures; however, more studies are needed to better assess the longevity of the results.

## DECLARATIONS

### Authors' contributions

Review of data, supervision, editing: de la Torre JI

Writing, original draft: Lindsey Jr. J

Data curation: Cerio D

Conceptualization: Vasconez LO

### Availability of data and materials

Data and material are maintained at UAB Plastic Surgery.

### Financial support and sponsorship

There was no financial support. The project was supported by UAB Plastic Surgery.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Institutional Review Board approval was obtained.

### Consent for publication

The authors give consent for publication. Photographic consent was obtained for all patients.

### Copyright

© The Author(s) 2020.

### REFERENCES

1. de la Torre JI, Martin SA, Váscquez LO. Suture suspension of the malar fat pad. *Aesthet Surg J* 2002;22:446-50.
2. Engle RD, Pollei TR, Williams EF. Endoscopic midfacial rejuvenation. *Facial Plast Surg Clin North Am* 2015;23:201-8.
3. Tonnard P, Verpaele A, Monstrey S, Van Landuyt K, Blondeel P, et al. Minimal access cranial suspension lift: a modified S-lift. *Plast Reconstr Surg* 2002;109:2074-86.
4. Chalet SR, Williams EF. Understanding midfacial rejuvenation in the 21st century. *Facial Plast Surg* 2013;29:40-5.
5. Gamboa GM, de La Torre, Jorge I, Vasconez LO. Surgical anatomy of the midface as applied to facial rejuvenation. *Ann Plast Surg* 2004;52:240-5.
6. Collawn SS, Vasconez LO, Gamboa M, Guzman-Stein G, Carriquiry C. Subcutaneous approach for elevation of the malar fat pad through a prehairline incision. *Plast Reconstr Surg* 1996;97:836-41.
7. de la Torre, JI, Rosenberg LZ, De Cordier BC, Gardner PM, Fix RJ, et al. Clinical analysis of malar fat pad re-elevation. *Ann Plast Surg* 2003;50:244-8.
8. De Cordier BC, de la Torre JI, Al-Hakeem MS, Rosenberg LZ, Costa-Ferreira A, et al. Rejuvenation of the midface by elevating the malar fat pad: review of technique, cases, and complications. *Plast Reconstr Surg* 2002;110:1526-36.
9. Ugurbas SH, Goldberg RA, McCann JD, Shorr N, Murthy R, et al. Suture midface suspension. *Head Face Med* 2006;2:35.
10. Ogilvie MP, Few JW, Tomur SS, Teven CM, Semersky AJ, et al. Rejuvenating the face: an analysis of 100 absorbable suture suspension patients. *Aesthet Surg J* 2018;38:654-63.
11. Lindsey JT. Five-year retrospective review of the extended SMAS: critical landmarks and technical refinements. *Ann Plast Surg* 2009;62:492-6.
12. Chopan M, Buchanan PJ, Mast BA. The minimal access cranial suspension lift. *Clin Plast Surg* 2019;46:547-57.
13. Saltz R, Ohana B. Thirteen years of experience with the endoscopic midface lift. *Aesthet Surg J* 2012;32:927-36.
14. Abraham RF, DeFatta RJ, Williams EF. Thread-lift for facial rejuvenation: Assessment of long-term results. *Arch Facial Plast Surg* 2009;11:178-83.
15. Keller GS, Namazie A, Blackwell K, Rawnsley J, Khan S. Elevation of the malar fat pad with a percutaneous technique. *Arch Facial Plast Surg* 2002;4:20-5.
16. Laferriere KA, Castellano RD. Experience with percutaneous suspension of the malar fat pad for midface rejuvenation. *Facial Plast Surg Clin North Am* 2005;13:393-9.
17. Sasaki GH, Cohen AT. Meloplication of the malar fat pads by percutaneous cable-suture technique for midface rejuvenation: outcome study (392 cases, 6 years' experience). *Plast Reconstr Surg* 2002;110:635-54.
18. Owsley JQ, Zweifler M. Midface lift of the malar fat pad: technical advances. *Plast Reconstr Surg* 2002;110:674-85.
19. Fitzgerald R, Graivier MH, Kane M, Lorenc ZP, Vleggaar D, et al. Update on facial aging. *Aesthet Surg J* 2010;30 Suppl:11S-24S.
20. McCollough EG, Scurry WC, Shirazi MA. The "Midface-Lift" as a misnomer for correctly identifying procedures designed to lift and rejuvenate the cheeks and malar regions of the face. *Arch Facial Plast Surg* 2009;11:257-62.
21. Owsley JQ Jr, Zweifler M. Midface lift of the malar fat pad: technical advances. *Plast Reconstr Surg* 2002;110:674-85.

Original Article

Open Access



# The “central six” of ptosis repair: eliminating contour as a variable in external levator surgery

Benjamin C. Campbell<sup>1,2,3</sup>, Susuana T. Adjei<sup>1,3</sup>, William R. Nunery<sup>1,2,3</sup>, H. B. Harold Lee<sup>1,2,3</sup>

<sup>1</sup>Oculofacial Plastic and Orbital Surgery, Indianapolis, IN 46280, USA.

<sup>2</sup>Ascension St. Vincent Hospital, Indianapolis, IN 46260, USA.

<sup>3</sup>Department of Ophthalmology, Indiana University School of Medicine, Indianapolis, IN 46290, USA.

**Correspondence to:** Dr. H. B. Harold Lee, Department of Ophthalmology, Indiana University School of Medicine, Indianapolis, IN 46290, USA. E-mail: huiabae@gmail.com

**How to cite this article:** Campbell BC, Adjei ST, Nunery WR, Lee HBH. The “central six” of ptosis repair: eliminating contour as a variable in external levator surgery. *Plast Aesthet Res* 2020;7:12. <http://dx.doi.org/10.20517/2347-9264.2019.59>

**Received:** 26 Nov 2019 **First Decision:** 4 Feb 2020 **Revised:** 8 Feb 2020 **Accepted:** 26 Feb 2020 **Published:** 13 Mar 2020

**Science Editor:** Chau Pham **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

## Abstract

**Aim:** Eyelid contour is a key component to satisfactory lid position and appearance following ptosis repair, the components of which have been highly debated and remain difficult to objectively measure. We sought to minimize the number of intraoperative adjustments required and reduce reoperation rates by addressing only the central 6 mm of tarsus when reapproximating levator to the anterior surface of tarsus, thereby eliminating contour as an adjustable variable.

**Methods:** All patients who underwent external levator resection with blepharoplasty for correction of involutional ptosis between 2012 and 2019 by a single surgeon at one center were retrospectively reviewed. Patients who underwent concomitant brow lifting surgery were excluded. The same technique was used for each eyelid with uniform suture placement. One 6-0 silk horizontal mattress suture was placed partial thickness through the superior third of tarsus 3 mm lateral to the center of tarsus; another was passed 3 mm medial to the center of tarsus. No sutures were placed outside of this central 6-mm zone. Patient fixation was used to determine lid height and symmetry. Once satisfactory, the sutures were tied down in a permanent fashion and the eyelid position again verified. In total, 153 eyelids in 85 patients were evaluated. Data obtained included preoperative and postoperative margin-to-reflex distance (MRD<sub>1</sub>), intraoperative and postoperative complications, reoperation rates, and patient satisfaction with appearance of lid contour and symmetry.

**Results:** The mean follow up time was 3.41 months. The mean preoperative MRD<sub>1</sub> was 1.05 mm. The mean postoperative was 3.18 mm. All patients had recovery of an anatomically normal temporal peak height. Two of 153



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



eyelids (1.31%) required reoperation due to residual ptosis or overcorrection. No patients had postoperative lagophthalmos. Ninety-one percent of patients who underwent bilateral surgery had satisfactory symmetry defined as less than or equal to 1-mm difference between right and left MRD<sub>1</sub>. Eighty-two of the 85 patients were satisfied with their postoperative appearance.

**Conclusion:** This simple and standardized technique for suture placement gives reliable and effective results for external levator advancement for ptosis repair by eliminating contour as an adjustable variable. Addressing the central 6 mm of tarsus is not only paramount but also in and of itself satisfactory in achieving optimal contour during external levator resection, without regard to more medial or lateral lid anatomy.

**Keywords:** Ptosis, levator advancement, central 6

## INTRODUCTION

Acquired eyelid ptosis is most commonly due to involutional changes of the levator aponeurosis<sup>[1,2]</sup>. There are various surgical techniques to correct ptosis, and the majority of them focus on tightening or advancing the levator aponeurosis onto tarsus<sup>[3,4]</sup>. External levator advancement was first described in the 1880s<sup>[5]</sup>, and since then it has been repeatedly modified and improved. The traditional surgical technique is to place one or more sutures to reattach the levator aponeurosis to the anterior surface of tarsus once it has been carefully dissected and partially resected. The first suture is placed centrally to achieve appropriate eyelid height, and more sutures are then placed medially and laterally to achieve proper contour. This approach can be cumbersome and require multiple adjustments intraoperatively to achieve proper contour. There have been several studies suggesting modifications of the procedure in order to standardize and simplify the process<sup>[6-9]</sup>. It remains a challenging surgery in order to achieve adequate lift of the eyelid while retaining proper eyelid contour.

A recent study<sup>[10]</sup> describing a single stitch müller muscle conjunctival resection for ptosis repair has suggested that only the central portion of the eyelid needs to be addressed surgically. Excellent results were demonstrated with this technique, and it is similar to the concept we propose for the external levator advancement surgery. We propose a technique for external levator resection that standardizes suture placement on only the central 6 mm of tarsus, thereby eliminating contour as an adjustable variable and simplifying the surgery.

## METHODS

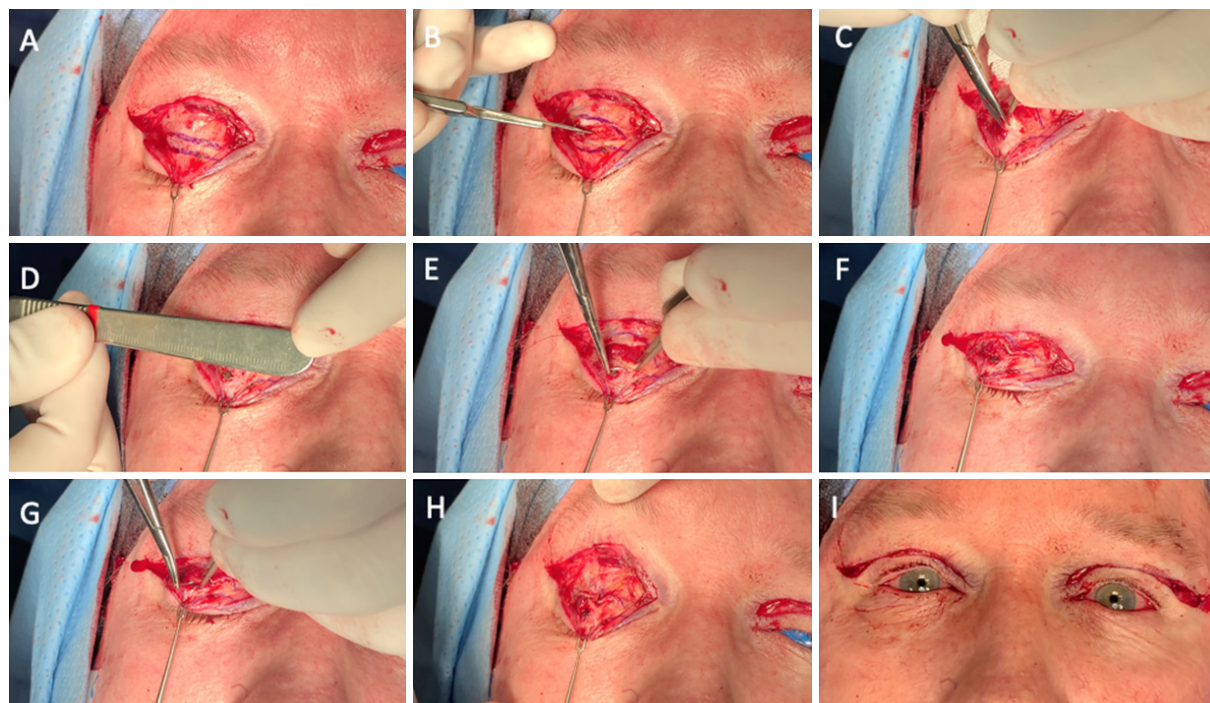
A retrospective chart review was performed at the practice of the main author. The institutional review board from Ascension St Vincent Hospital in Indianapolis granted exception status for the study. The research was Health Insurance Portability and Accountability Act compliant and adhered to the principles of the Declaration of Helsinki. All patients who underwent ptosis surgery by the main author from 2012 to 2019 using the central six technique were identified. The medical records were analyzed to record pertinent clinical examination measurements and outcomes, including measurements of margin-to-reflex distance (MRD<sub>1</sub>) and reoperation rates.

Patients with aponeurotic ptosis with levator function greater than 12 mm were included. Exclusion criteria included those who underwent concomitant brow lifting procedures, those with prior ptosis surgery, and those with inadequate follow up.

### Surgical technique

Cases were performed under monitored anesthesia care with approximately 4-5 mL of local anesthetic injected in each upper eyelid. Corneal protectors were placed to ensure no damage to the globe. A lid





**Figure 1.** Surgical steps. A: after a blepharoplasty has been performed, the amount of levator resection is marked; B: a 15 Bard-Parker blade is used to cut through superior marking down to Mueller's muscle; C, D: the upper border of tarsus is exposed and the central 6 mm of tarsus is marked; E, F: a 6-0 silk suture is placed at the medial mark in a horizontal mattress fashion; G: a second 6-0 silk suture is placed at the lateral mark; H: the levator is advanced with two paracentral sutures. No sutures are placed outside of the central 6 mm; I: adequate lid height and symmetry is checked

crease incision was made, and standard blepharoplasty performed, in which skin and orbicularis muscle were removed. The lid was stretched with gentle retraction, and the upper border of tarsus was marked. A parallel line was placed 3-4 mm above the upper border of tarsus to mark the amount of levator aponeurosis to be resected. A 15 Bard-Parker blade was used to cut through septum and levator aponeurosis, exposing Mueller's muscle underneath. The peripheral marginal arcade was preserved. Sharp dissection was used to expose the central upper border of tarsus. The center of the tarsal plate was determined as the widest portion of tarsus. This point may not coincide with the midpoint of the eyelid, as many patients have a temporal shift of the tarsus with age. Appropriate hemostasis was achieved. Two paracentral marks were placed 6 mm apart to indicate the placement of sutures. Each mark was placed approximately 3 mm on either side of the center of tarsus, thus marking the "central six". One 6-0 silk horizontal mattress suture was placed partial thickness through the superior third of tarsus 3 mm medial to the center of tarsus and another passed 3 mm lateral to the center of tarsus. No sutures were placed outside of this central 6 mm zone. Patient fixation was then used to ensure adequate lid height and symmetry with the patient supine. Any adjustments were made and sutures tied down permanently. The skin was closed in a standard fashion. [Figure 1](#) demonstrates the key steps of central six technique for suture placement.

## RESULTS

There were 85 patients identified who underwent surgery with the central six technique. The results are summarized in [Table 1](#). In total, 153 eyelids were included, with 68 patients undergoing bilateral surgery and 17 undergoing unilateral surgery. The average patient age was 68 years (range 38 to 73). The mean levator function was 14.45 mm (range 12 to 18 mm) and average follow-up was 3.4 months (range 1 to 17 months). The average preoperative MRD<sub>1</sub> was 1.05 mm (range -5 to 2 mm) and the average postoperative MRD<sub>1</sub> was 3.18 mm (range 1 to 5.5 mm), yielding a mean improvement in MRD<sub>1</sub> of 2.13 mm (standard deviation



**Table 1. Results**

Number of patients	85
Total eyelids	153
Bilateral surgery	68
Average age	72
Average preoperative MRD <sub>1</sub>	1.05 mm
Average postoperative MRD <sub>1</sub>	3.18 mm
Average improvement in MRD <sub>1</sub>	2.13 mm
Reoperation required	2
Post op symmetry (MRD <sub>1</sub> difference < 1 mm)	91.2%
Patients satisfaction	96.5%

MRD<sub>1</sub>: margin-to-reflex distance

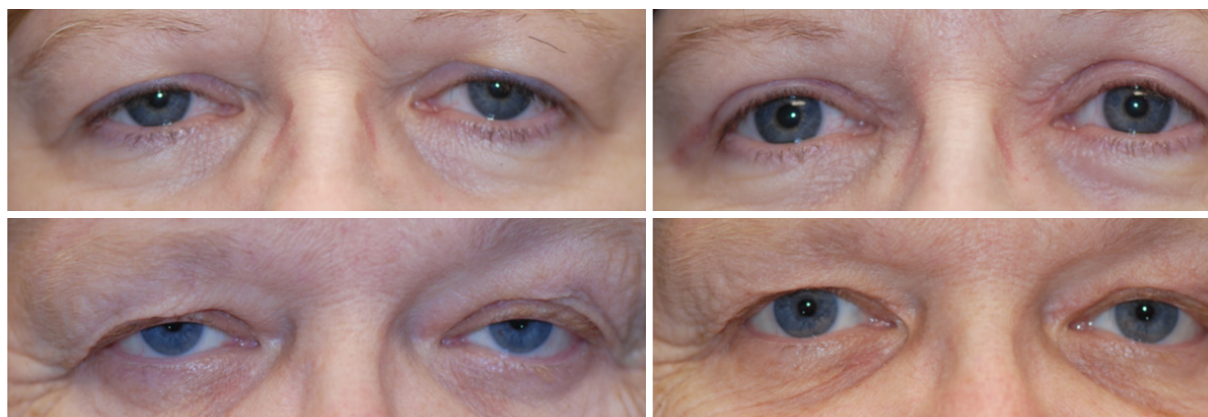
1.32 mm). Only two patients (1.31%) required reoperation: one for overcorrection and the second for residual ptosis. Both of these patients had undergone unilateral surgery. The patient with residual ptosis who underwent reoperation had to be converted to general anesthesia intraoperatively due to patient pain and discomfort, thus not allowing for intraoperative adjustment of eyelid height. Of those who underwent bilateral surgery, 62 patients (91.2%) had satisfactory postoperative symmetry of eyelid height defined as an MRD<sub>1</sub> difference less than 1 mm between the two eyes. Postoperatively, 82 of 85 patients (96.5%) were satisfied with the outcomes of surgery. The three patients who were not satisfied included the two who required reoperation, while the third patient elected not to have a reoperation performed for residual ptosis. The two who underwent a secondary surgery using the same technique had good results. No patients developed postoperative lagophthalmos. There were no immediate postoperative complications. [Figure 2](#) shows typical patients who underwent surgery with the central six technique.

## DISCUSSION

The two main goals of ptosis surgery are to restore eyelid height and contour, which can often be a challenging process requiring multiple intraoperative adjustments. The traditional approach is to place a central stitch to achieve the correct height, but this then leaves a peak centrally. Additional sutures are placed medially and laterally to restore a natural eyelid shape. Multiple techniques to simplify the procedure have been proposed since modern ptosis surgery was described in the 1970s by Jones *et al.*<sup>[4]</sup>. In the 1990s, there were several modifications. Liu *et al.*<sup>[6]</sup> proposed the concept of a single-suture ptosis repair, while Lucarelli and Lemke<sup>[7]</sup> later introduced small incision ptosis repair without concurrent blepharoplasty. Meltzer *et al.*<sup>[8]</sup> presented their experience using an adjustable suture. Later, Ahuero *et al.*<sup>[9]</sup> proposed a refinement to small-incision surgery with a standardized suture placement at the medial pupillary border and lateral limbus.

In a similar fashion, our proposed technique standardizes suture placement and thus eliminates contour as an adjustable variable. In our practice, those patients who have external ptosis surgery performed generally require concomitant blepharoplasty for dermatochalasis and are not good candidates for small-incision ptosis surgery. We have had excellent results with high patient satisfaction by only focusing on the central 6 mm of tarsus for suture placement. Operative time is reduced, thus leading to improved patient comfort and safety. Intraoperative adjustments for height can be made by tightening or loosening sutures, or on occasion a suture must be replaced to achieve more lift. Eyelid contour is typically excellent, without need to adjust suture placement horizontally.

Eyelid contour is a key component to satisfactory lid position and appearance following ptosis repair, the components of which have been highly debated and remain difficult to objectively measure<sup>[11-13]</sup>. External photos can be analyzed with geometrical models to quantify contour<sup>[11]</sup>. Another technique involves



**Figure 2.** Preoperative and postoperative photos of patients who underwent bilateral surgery with the “central six” technique

measuring distances from mid-pupil to different points on the upper eyelid<sup>[12]</sup>. Alternatively, blind graders can judge whether contour is adequate based on external photos. Ultimately, contour is important as it is a key component to postoperative patient satisfaction. A peaked eyelid or focal drooping results in abnormal appearance and displeased patients. In our study, we focused on patient satisfaction as an indirect measure of both good contour and adequate eyelid height. Eighty-two of the 85 patients (96.5%) in our study were satisfied with the outcomes of their surgery.

There are limitations to our study, including its retrospective nature and limited sample size. In addition, there was no control group to demonstrate statistically significant improvement over standard techniques. Our symmetry rate (91.2%) and satisfaction rate (96.5%) were among the high end of those published in the literature. We did not measure contour directly, but instead used patient satisfaction as an indirect measure of contour. The rates of intraoperative adjustment for eyelid height was not documented.

In conclusion, this simple and standardized technique for suture placement gives reliable and effective results for external levator resection for ptosis repair by eliminating contour as an adjustable variable. Addressing the central 6 mm of tarsus is not only paramount but also in and of itself satisfactory in achieving optimal contour during external levator resection, without regard to more medial or lateral lid anatomy.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Campbell BC, Nunery WR, Lee HBH

Performed data acquisition: Adjei ST

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

**Ethical approval and consent to participate**

The institutional review board from Ascension St Vincent Hospital in Indianapolis granted exception status for the study. The research was Health Insurance Portability and Accountability Act compliant and adhered to the principles of the Declaration of Helsinki.

**Consent for publication**

Written consent was obtained for publication for patient images.

**Copyright**

© The Author(s) 2020.

**REFERENCES**

1. Anderson RL, Beard C. The levator aponeurosis. Attachments and their clinical significance. *Arch Ophthalmol* 1977;95:1437-41.
2. Pearl RM. Acquired ptosis: a reexamination of etiology and treatment. *Plast Reconstr Surg* 1985;76:56-64.
3. Anderson RL, Dixon RS. Aponeurotic ptosis surgery. *Arch Ophthalmol* 1979;97:1123-8.
4. Jones LT, Quickert MH, Wobig JL. The cure of ptosis by aponeurotic repair. *Arch Ophthalmol* 1975;93:629-34.
5. Eversbusch O. Zur operation der congenitalen blepharoptosis. *Klin Monatsbl Augenheilkd* 1883;21:100.
6. Liu D. Ptosis repair by single suture aponeurotic tuck: surgical technique and long-term results. *Ophthalmology* 1993;100:251-9.
7. Lucarelli MJ, Lemke BN. Small-incision external levator repair: technique and early results. *Am J Ophthalmol* 1999;127:637-44.
8. Meltzer MA, Elahi E, Taupeka P, Flores E. A simplified technique of ptosis repair using a single adjustable suture. *Ophthalmology* 2001;108:1889-92.
9. Ahuero AE, Winn BJ, Sires BS. Standardized suture placement for mini-invasive ptosis surgery. *Arch Facial Plast Surg* 2012;14:408-12.
10. Ediriwickrema LS, Geng J, Nair AA, Prendes M, Gerber AL, et al. Single suture müeller muscle conjunctival resection (ssMMCR): a modified technique for ptosis repair. *Ophthalmic Plast Reconstr Surg* 2019;35:403-6.
11. Malbouisson JM, Baccega A, Cruz AA. The geometrical basis of the eyelid contour. *Ophthalmic Plast Reconstr Surg* 2000;16:427-31.
12. Golbert M, Pereira FJ, Garcia DM, Cruz AAV. Contour symmetry of the upper eyelid following bilateral conjunctival-Müller's muscle resection. *Aesthet Surg J* 2017;37:269-75.
13. Ahn S, Lee H, Lee J, Park J, Park M, et al. Analysis of surgical outcome after levator advancement by assessing changes in eyelid contour. *J Craniofac Surg* 2016;27:1147-50.

Systematic Review

Open Access



# Review of soft tissue coverage options in distraction osteogenesis of the extremity

Jacqueline Stoneburner<sup>1</sup>, Beina Azadgoli<sup>1</sup>, Anna C. Howell<sup>1</sup>, Douglass Tucker<sup>2</sup>, Geoffrey Marecek<sup>2</sup>, Joseph Carey<sup>1</sup>

<sup>1</sup>Plastic and Reconstructive Surgery, Keck School of Medicine of USC, Los Angeles, CA 90033, USA.

<sup>2</sup>Orthopaedic Surgery, Keck School of Medicine of USC, Los Angeles, CA 90033, USA.

**Correspondence to:** Dr. Joseph Carey, Division of Plastic and Reconstructive Surgery in the Department of Surgery, Keck School of Medicine of USC, 1510 San Pablo St, Los Angeles, CA 90033, USA. E-mail: joseph.carey@med.usc.edu

**How to cite this article:** Stoneburner J, Azadgoli B, Howell AC, Tucker D, Marecek G, Carey J. Review of soft tissue coverage options in distraction osteogenesis of the extremity. *Plast Aesthet Res* 2020;7:13. <http://dx.doi.org/10.20517/2347-9264.2019.028>

**Received:** 13 Sep 2019 **First Decision:** 15 Jan 2020 **Revised:** 12 Feb 2020 **Accepted:** 3 Mar 2020 **Published:** 20 Mar 2020

**Science Editor:** Matthew L. Iorio **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

**Aim:** To review the choices of soft tissue coverage in distraction osteogenesis of the extremity.

**Methods:** A PubMed literature search yielded 14 articles included for systematic review. Data were extracted from each article if available (sample size, patient age, surgical indications, type of flap, use of additional modalities, method of bone osteogenesis, postoperative events, follow-up, satisfaction, weight-bearing status, and success rate). Unpaired *t*-tests were performed to compare complication rates. A retrospective review of three cases was also conducted.

**Results:** Fourteen articles discussed 145 patients with a mean age of 33.4 years and 146 extremity injuries followed over 3.3 years on average. Indications included chronic osteomyelitis or nonunion (58.2%) and acute trauma (41.8%). Average time from injury was 1.1 years. Ilizarov frame was used in 12 articles. Free flaps (88.0%) or rotational flaps (12.0%) were used, with muscle flaps (96.7%) being most common. Most extremities received free latissimus dorsi or rectus abdominis flaps. Bone grafts and antibiotic beads were often used in conjunction. Although complications and reoperations were not uncommon (up to 30%), 98.8% of patients on average were ultimately weight bearing and all articles reported > 91% success rate. Additionally, the rates of any complication were not statistically different between "fix and flap" protocol and flap or frame first. Lastly, a three-patient case series is presented.

**Conclusion:** Bone transport with soft tissue reconstruction remains an excellent choice for patients with large bony defects or who are unable to undergo autologous bone grafting. Not one surgical approach to limb salvage is superior, and decision should be made on a case by case basis between the surgeon and the patient.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Keywords:** Soft tissue coverage, free flaps, rotational flaps, microsurgery, bone osteogenesis, bone transport, lower extremity reconstruction, limb salvage

## INTRODUCTION

High-energy lower extremity trauma with massive bone and soft tissue loss poses a challenge for orthopedic and reconstructive surgeons. Higher Gustilo-Anderson fracture type, Orthopaedic Trauma Association Open Fracture classification, and limb salvage index score are associated with a poor prognosis, including high risk of infection, nonunion, and amputation<sup>[1-3]</sup>. With improvements in surgical techniques and protocol-driven traumatic lower extremity management at tertiary care centers, limb salvage rates have significantly increased, reaching up to 90% in some reports<sup>[4-6]</sup>.

Early aggressive radical debridement, skeletal stabilization and soft tissue coverage is the critical first step in lower extremity salvage<sup>[7]</sup>. Depending on the extent of trauma and the size and nature of the defect, there are many reconstructive options available to the orthopedic and plastic surgeon. When the bony injury is “critical sized” - that is, too large to heal without additional intervention - additional steps must be taken to achieve bony union.

The choice of reconstructive technique is often determined by size. The induced membrane technique is a viable option for small and large defects, although results may differ based on anatomic location. Larger defects may require vascularized transfer from the fibula or iliac crest<sup>[7-9]</sup>. Some patients may be poor candidates for vascularized bone graft, the defect may exceed the size of the available graft or there may be concerns about size mismatch between vascularized bone and the defect location. In these cases, bone transport using distraction osteogenesis can be utilized. Bone transport in combination with free soft tissue transfer is an effective treatment for nonunions, segmental defects, and osteomyelitis.

This systematic review summarizes and discusses reconstructive options available for traumatic extremity injuries when bony transport and free tissue transfer are required for limb salvage. A series of case examples in combination with the senior authors’ surgical techniques at a single academic institution is described.

### Bone deficit treatment options

Critical bone loss is devastating for patients; orthopedic and plastic surgeons must be prepared to treat these patients with a multidisciplinary approach. It most commonly occurs in patients following trauma, infection, and oncologic resection. Critical bony defects, by definition, necessitate additional intervention, as the defect is too large to undergo the normal physiologic healing process. The inability for bone to spontaneously regenerate is often largely controlled by the presence or absence of periosteal blood supply, as well as a viable docking site for reduction<sup>[10]</sup>. Defects greater than 2.5 cm are usually considered critical, although there is poor evidence and thus some disagreement between experts with this definition<sup>[11-13]</sup>. In these situations, primary bone grafting is also insufficient to obtain union. More advanced treatment options include the induced membrane technique and bone transport through distraction osteogenesis.

#### *Induced membrane techniques*

The induced membrane technique, often eponymously referred to as the Masquelet technique, is a two-stage technique involving placement of a foreign body within the defect that results in a biologically-active membrane around the defect, which can then provide biology for subsequent bone formation using graft<sup>[14,15]</sup>. The first stage entails radical debridement and skeletal stabilization<sup>[15,16]</sup>. A spacer, typically made from polymethylmethacrylate cement, is placed. A pseudomembrane will form around the spacer. This spacer is biologically active and contains growth factors involved in fracture healing and angiogenesis. In



the second stage, the previous incision is reopened, the pseudomembrane is incised, bone graft is placed into the defect, and the membrane is closed around the graft.

#### *Autologous bone grafting*

The graft used in this technique is typically autogenous bone graft. Autologous graft is histocompatible, osteoconductive, osteoinductive, and osteogenic<sup>[17,18]</sup>. This provides a structure through which bone, host capillaries, and mesenchymal stem cells can have in-growth. The source of autogenous bone is determined by many factors including location of bone defect, desired function of bone graft, size of bone defect, associated complications, and expected graft site. Locations and methods for graft include the distal tibia, calcaneus, proximal tibia, iliac crest, and via the Reamer-Irrigator-Aspirator system. The optimal timing for placement for the bone graft is debated, with studies suggesting peak osteo- and angiogenic properties occur at 4 weeks, with a subsequent decline<sup>[19-21]</sup>. In situations in which the induced membrane technique is contraindicated, the orthopedic surgeon's best option for attaining bony union is by means of bone transport.

#### *Distraction osteogenesis*

Indications for bone transport via distraction osteogenesis include massive bone loss (greater than 5 cm), inadequate soft-tissue coverage, infection, and the need for prolonged stability. However, even with defects this large, other methods along with bone transport should be considered. Of paramount importance, the surgeon must be competent in managing such patients throughout the entirety of their care, as it has been suggested that the success of bone transport is highly dependent upon surgeon experience<sup>[22]</sup>.

Distraction osteogenesis (DO) was described by Ilizarov. The classic method utilizes thin-wire circular frames to provide stability and gradually distract at the osteotomy site. This method of DO was developed in the 1950s and has been a popular method for long bone expansion in Europe and the United States since the 1980s as a treatment modality for critical bone loss. All distraction osteogenesis is dependent on three things: a low-energy corticotomy, bony stability, and distraction with a specific rate and rhythm<sup>[23,24]</sup>. After the bone defect is defined, circular external fixation with thin K-wires are percutaneously fixated to healthy bone both proximal and distal to the site of bone loss. They are held under tension via fixation bolts to the rings surrounding the affected limb fragments. These rings are then joined using threaded rods or adjusters. The use of multiple fixation rings for each segment allows for increased control of the bone segments, allowing for an increased number of connecting rods and greater ability for bone gap repair<sup>[22]</sup>. Each case will progress through three phases: latency, in which the healing process begins; distraction, during which time the DO focus is distracted at a specific rate and rhythm; and consolidation, in which the regenerate ossifies<sup>[25]</sup>.

Ilizarov's method of DO can reliably achieve bony union in limbs that would otherwise not be salvageable. Time of distraction is variable and dependent on defect size, location, and host factors. The maximum rate of distraction is 1 mm/day, and consolidation typically requires twice as long as the distraction phase<sup>[26-29]</sup>. The published rates of successful limb reconstruction approach 100% in most publications<sup>[30-34]</sup>. Complication of DO include pin-site infections, stretching of neurovascular structures, soft-tissue scarring, poor regenerate, and non-union of the docking site<sup>[30,35]</sup>. Classic Ilizarov DO is still widely utilized, but alternative techniques such as cable transport and all-internal techniques using motorized medullary nails have proven successful. These techniques offer similar outcomes with decreased time with external fixation, allowing for improved patient comfort, decreased site infection risk, and sooner time to rehabilitation.

One potential alternative to classical Ilizarov DO is utilization of Taylor Spatial Frames. This unique frame has dual rungs with six struts allowing for telescoping<sup>[36,37]</sup>, thus providing many of the same benefits to Ilizarov DO but with the ability to provide correction in six planes (coronal angulation/translation, sagittal

angulation/translation, rotation, and shortening). In addition, this system has the ability to utilize computer accuracy<sup>[36-38]</sup>. Studies have shown this to be as effective as, but not superior to, classical Ilizarov DO in overall clinical outcomes<sup>[38-41]</sup>, while some advantages include use for residual deformity following Ilizarov DO, lower rate of return to the OR, and the ability to correct in all planes<sup>[36]</sup>.

Finally, the pull wire system is a newer technique that can induce bone transport with utilizing both internal and external fixation through medullary nail placement<sup>[42]</sup>. This dual distraction method provides similar rates of healing to classical Ilizarov DO, while limiting the risks associated with long-term external fixator use<sup>[42,43]</sup>. Rozbruch *et al.*<sup>[33]</sup> (2008) also suggested that there is expedited bone healing and a decreased risk of refracture of the site of bone transport.

### Soft tissue coverage in lower extremity trauma

In many cases of lower extremity trauma, free tissue transfer becomes necessary in an attempt at limb salvage. While successful flap reconstruction may be achieved, complication rates are relatively high in lower extremity reconstruction for a variety of reasons, which may include trauma-induced edema, pre-existing vascular conditions, other patient comorbidities, or poor patient compliance during postoperative recovery.

In a meta-analysis conducted by Xiong and colleagues on free flap reconstruction of lower extremity defects, total flap loss and the rate of thrombosis were both found to be about 6%<sup>[44]</sup>. Overall, 26.1% of the flap losses were due to venous thrombosis, whereas 10.1% were due to arterial thrombosis. Minor complications such as hematoma, partial necrosis, infection, and wound dehiscence occurred at rates between 4.0% and 8.0%<sup>[44]</sup>.

Given the heterogeneity of lower extremity trauma, several different reconstructive options can be utilized by the plastic surgeon, depending on the size of the defect, the structures involved, and the comfort level of the surgeon.

Historically, muscle flaps were believed to reduce infection rates in contaminated wounds. However, more recently, free fasciocutaneous flaps have proven to be comparable to muscle flaps in terms of success rates, infection rates, and bony union in lower extremity reconstruction<sup>[4,45-47]</sup>. Additionally, fasciocutaneous flaps are thought to be simpler to re-elevate for subsequent orthopedic procedures, require fewer secondary skin graft procedures, and result in lower donor site morbidity<sup>[46-49]</sup>.

In a meta-analysis conducted by Bekara *et al.*<sup>[50]</sup>, the most commonly used free flaps for distal third lower-limb reconstruction were found to be the latissimus dorsi muscle flap (25.5%), anterolateral thigh flap (19.7%), rectus abdominis muscle flap (8.5%), gracilis muscle flap (8.4%), and serratus anterior flap (6.4%)<sup>[50]</sup>. The majority of flaps used (56.5%) were muscular flaps, followed by fasciocutaneous (42%) and fascial (0.5%). The most common pedicled-propeller flaps were reportedly posterior tibial artery perforator (5.86%), peroneal artery perforator (30.1%), sural artery perforator (5.6%), metatarsal artery perforator (2.0%), anterior tibial artery perforator (1.6%), lateral retromalleolar artery perforator (1.6%), and dorsalis pedis artery perforator (0.3%)<sup>[50]</sup>.

Regarding free *versus* pedicled flaps in reconstruction of the distal third of the lower limb, the same review by Bekara *et al.*<sup>[50]</sup> concluded that, while partial flap necrosis is higher in pedicled-propeller flaps, wound dehiscence and infection rates are higher in free flaps. Failure and overall complication rates were similar in both groups<sup>[50]</sup>.

In addition to the flap that is used, the reconstructive surgeon must also select the appropriate recipient vessel and anastomotic technique. While studies have shown no difference in complication rates between

end-to-end and end-to-side arterial anastomoses, end-to-side anastomoses are generally the preferred choice for extremities at risk for vascular insufficiency in order to maintain perfusion to the distal leg and foot<sup>[51,52]</sup>.

While it was previously thought that only vessels proximal to the injury could be used as recipient vessels for free flap coverage, it has since been shown that there is no difference in outcomes or reoperation rates when flaps are anastomosed to vessels distal to the injury<sup>[53,54]</sup>.

### **Bone transport with simultaneous soft tissue reconstruction**

It is now well-established that early soft tissue coverage of complex lower extremity trauma leads to successful limb salvage with improved flap success rates and lower rates of infection<sup>[55-58]</sup>. Thus, the current practice at most institutions is simultaneous bony fixation and soft tissue coverage. This so-called “fix and flap” protocol, consisting of radical debridement and skeletal stabilization with immediate or very early free flap coverage, has resulted in faster union times with lower infection rates<sup>[59,60]</sup>.

To overcome segmental bone loss and manage later consequences of lower extremity trauma such as malunion or nonunion, bone transport is often used. By combining free tissue transfer with bone transport, large segmental and soft tissue defects can be simultaneously treated with great success. Flap coverage combined with bone transport allows for better limb length restoration by maintaining length from the start; improving vascularity, which is important for fracture healing; and facilitating bone grafting or other subsequent procedures at the docking site<sup>[61]</sup>.

While classically, the injured limb with critical bony defect was initially treated by debridement and resection and shortening of bone to allow for primary soft tissue closure, the more recent practice of combining free tissue transfer with bone transport allows for maintenance of the limb's original length and avoids the frequent complications associated with the traditional compression-distraction technique. The free flap provides healthy vascularized soft tissue, under which distraction osteogenesis can then take place<sup>[7]</sup>.

Few studies have described the effects of distraction on the transferred free tissue and its anastomotic pedicle. Jupiter *et al.*<sup>[62]</sup> reported that both the free tissue and the native tissue show equal magnitude of stretch and lengthening without any scar dehiscence after bone transport despite their different tensile strength and mechanical properties. Many studies have shown that, with major vessel repair in lower extremity trauma, the anastomotic site tolerates initial distraction process as early as 2-3 days after surgery<sup>[62-64]</sup>. However, the outcome of the microvascular anastomosis in these cases has not yet been reported. It has been demonstrated, however, that immediate distraction osteogenesis with a recent free flap has not been found to compromise the flap<sup>[61]</sup>.

Finally, while placement of pins through the free flap has also been noted to be safe, caution must be taken when placing the pins so that their anticipated path does not pierce the pedicle<sup>[62,65]</sup>. Careful planning and collaboration between the orthopedic surgeon and reconstructive surgeon are needed for these cases to ensure safe distraction against the pedicle and microvascular anastomosis.

Another potential option for patients needing both soft tissue coverage and assistance with bony growth is a medial femoral condyle vascularized graft. First described in the 1990s, this method utilizes the highly vascularized periosteum and either the medial superior genicular or more commonly the descending genicular artery, due to its length and ease of identification<sup>[66-68]</sup>. This method usually follows failure of conventional therapies and has been proven to be efficacious for osteomyelitis, avascular necrosis (AVN), and nonunions. Specifically, it has been used for the humerus and ulna<sup>[69-72]</sup>, as well as tibial and femoral

defects<sup>[70,73,74]</sup>. Notably, this methodology has been well studied in the treatment of scaphoid AVN<sup>[66,68,75]</sup>, with studies reporting up to 100% of patients achieving union<sup>[76,77]</sup> and level III evidence that it is superior to 1,2-intercompartmental supraretinacular artery graft<sup>[68,77,78]</sup>. Finally, these studies have reported limited to no complications at both donor and recipient sites<sup>[73,77,79,80]</sup>.

## METHODS

### Literature review

A systematic review of the literature was performed using PubMed database in adherence with PRISMA guidelines. Combinations of the following search terms were used: “‘bone transport’ AND ‘free tissue transfer’”, “‘Ilizarov’ AND ‘free tissue transfer’”, and “‘distraction osteogenesis’ AND ‘soft tissue coverage’”. No limits were placed on any of the search queries. All articles were independently reviewed by two authors. Following the primary search, titles and abstracts were either included or excluded based on predefined eligibility criteria. Inclusion criteria included bone transport as research theme, involvement of soft tissue coverage, relevance to limb salvage, and general population as the sample. Articles were limited to English language and review articles, letters, and comments. Subsequently, evaluation of the full text of selected articles was similarly performed and their bibliographies were assessed for additional articles to include.

Articles that described patient outcomes, details of bone transport, and type of soft tissue coverage were included. Studies were excluded if they had inadequate data, did not involve a combination of bone transport and soft tissue coverage, or were anecdotal in nature.

The following data from each article were extracted: sample size, mean patient age, indications for intervention, type of flap, use of additional modalities (antibiotic beads or bone substitute, vein or bone graft, and hardware), details of bone osteogenesis (average bone and soft tissue defect, time-to and rate of distraction, and total distraction), postoperative events (complications, total or partial flap loss, skin graft complication, hematoma, nonunion, malunion, infection, recurrent osteomyelitis, broken fixation wires, flap depression, bone exposure, fracture, limb-length discrepancy, second flap surgery or reoperation, and amputation), average follow-up, satisfaction, weight bearing status, and success rate.

Unpaired *t*-tests were utilized to assess if complication rates were statistically significant between the simultaneous placement of frame and flap and the placement of the flap before or after fixation. Fisher's exact test was used to compare differences in complication rates in patients with acute and chronic wounds. Statistical significance was determined at  $P < 0.05$ .

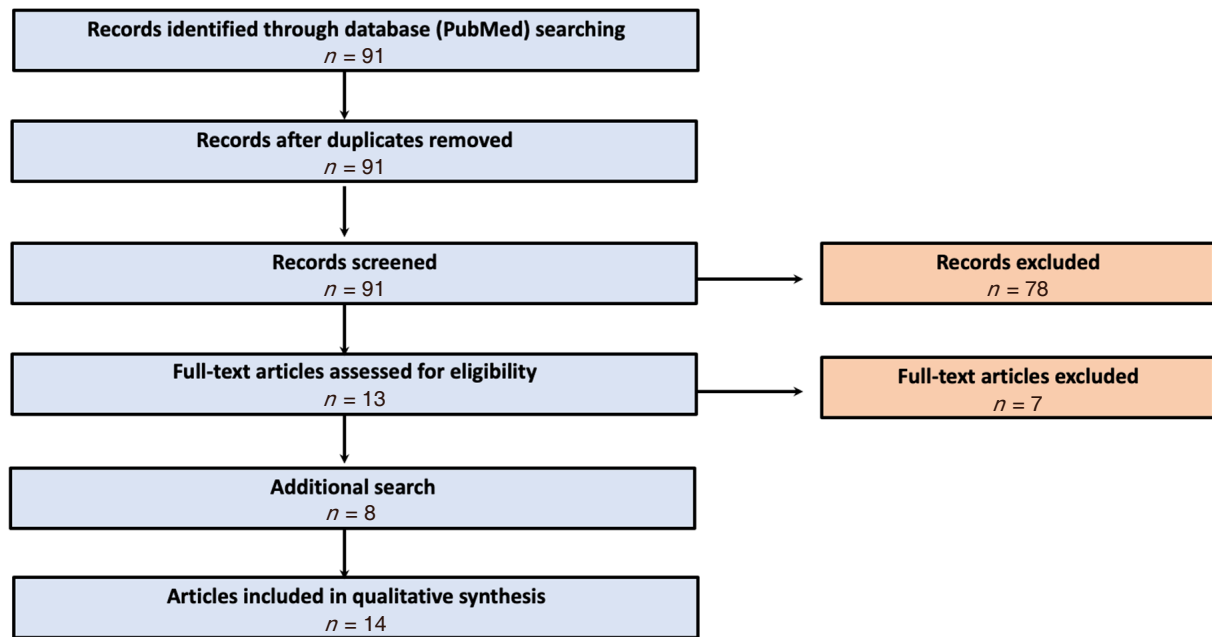
### Case series

A retrospective review was conducted of patients undergoing bone transport with simultaneous soft tissue coverage performed at the Keck Hospital of the University of Southern California between 2016 and 2019. Three non-consecutive cases performed by a single plastic and orthopedic surgeon are described below.

## RESULTS

Our initial literature search identified 91 unique articles, of which 13 full text-articles were assessed for inclusion. Six full-text articles from the search were ultimately included, added to eight articles selected from additional search. These 14 articles<sup>[7,9,61,65,81-90]</sup> were utilized for analysis [Figure 1].

The selected articles included bone transport with soft tissue reconstruction in 145 patients with a mean age of 33.4 years and a total of 146 extremity injuries (142 lower extremity and 4 upper extremity injuries). Ten of the 14 articles included average duration of follow up [Table 1]<sup>[7,9,61,65,81-90]</sup>. Patients were followed over 3.3 years

**Figure 1.** Flow diagram of the literature search**Table 1.** Studies included in systematic review

Ref.	No. of patients	Mean age (years)	No. of lower extremities	No. of upper extremities	Average time of repair from injury (months)	Frame	Avg follow up (years)
Lowenberg <i>et al.</i> <sup>[81]</sup>	34	40	34	0	NR	Ilizarov frame	11
Musharafieh <i>et al.</i> <sup>[82]</sup>	3	NR	3	0	0.71	Anterior double-stacking	1.5
Bibbo <sup>[83]</sup>	1	37	1	0	24	Ilizarov frame	2
Duman <i>et al.</i> <sup>[84]</sup>	9	22	9	0	NR	Ilizarov frame	NR
Fiebel <i>et al.</i> <sup>[65]</sup>	1	41	1	0	24	Ilizarov frame	3
Lowenberg <i>et al.</i> <sup>[61]</sup>							
Simultaneous group	13	31	12	1	NR	Ilizarov frame	2
Delayed group	23	44	20	3	NR	Ilizarov frame	2
Spiro <i>et al.</i> <sup>[9]</sup>	5	42	5	0	NR	Modified Ilizarov in 3 pts, Ilizarov frame in 2 pts	NR
Tukiainen and Asko-Seljavaara <sup>[7]</sup>	4	31	4	0	NR	Ilizarov frame	NR
Minehara <i>et al.</i> <sup>[85]</sup>	1	18	1	0	0.6	Ilizarov or hybrid frame	NR
Boopalan and Jepegnanam <sup>[86]</sup>	2	27.5	2	0	7.5	Ilizarov frame	1
Lowenberg and Van der Reis <sup>[87]</sup>	1	23	1	0	12	Ilizarov frame	1.25
Hutson <i>et al.</i> <sup>[88]</sup>	18	32	19	0	1.13	Ilizarov frame	4.83
Chim <i>et al.</i> <sup>[89]</sup>	28	45	28	0	36.1	Taylor spatial frame	4.1
Isik <i>et al.</i> <sup>[90]</sup>	2	NR	2	0	NR	Ilizarov frame	NR

NR: not reported

on average (range of 1-11 years). Indications for surgery included chronic osteomyelitis or nonunion (58.2% of patients) and acute trauma with or without infection (41.8% of patients) [Table 2]. No studies included patients with tumors or masses. The average time from injury was 1.1 years (range of 18 days to 3 years) in 8 of the 14 articles. An additional 2 of the 14 articles reported two separate times from injury: 1<sup>[7]</sup> or 3<sup>[81]</sup> weeks for patients with acute trauma and 10<sup>[81]</sup> or 18<sup>[7]</sup> months for patients with chronic osteomyelitis or nonunion.

All 14 articles described a surgical approach. In 11 of the 14 articles, the authors used a classic Ilizarov frame; in one, they used either an Ilizarov frame or segmental transport external fixator; and, in one, they used a hexapod frame. Only 1 of the 14 used anterior double-stacking external fixation. Some patients



**Table 2. Indications for surgery**

Indications for surgery	No. of articles	No. of extremities	Percentage of extremities (%)
Osteomyelitis	9	85	58.2
Nonunion	3		
Acute trauma (+/- infection)	9	61	41.8
Total articles	14	146	

**Table 3. Surgical approach**

Surgical approach	No. of articles	No. of extremities	Percentage of extremities (%)
Flap first	9	83	56.8
Simultaneous application of flap and frame	7	54	37.0
Frame first	4	9	6.2
Total articles	14	146	

**Table 4. Type of flap**

Type of flap	No. of articles	No. of extremities	Percentage of extremities (%)
Rectus abdominis	9	47	40.9
Free latissimus dorsi	12	42	36.5
Rotational gastrocnemius	4	11	9.6
Reverse sural	2	3	2.6
Soleus	1	3	2.6
Radial forearm flow-through	2	2	1.7
Rotational latissimus dorsi*	2	2	1.7
Cross leg	2	2	1.7
Serratus	1	1	0.9
Gracilis	1	1	0.9
Combined LD + serratus	1	1	0.9
Total articles	13	115	

\*Used for upper extremity reconstruction. LD: latissimus dorsi

underwent simultaneous application of frame and soft tissue flap (37.0%) while others had the application of a flap first (56.8%) or frame first (6.2%) [Table 3]. With respect to soft tissue reconstruction, four of the 14 articles quantified the soft tissue defect. The average soft tissue defect was 125 cm<sup>2</sup> (range of 88-219 cm<sup>2</sup>).

All 14 articles discussed the general type(s) of flaps used. Soft tissue reconstruction was most commonly performed with free flaps (88.0%) as opposed to rotational flaps (12.0%). Of these, most were muscle flaps (96.7%) with fewer being fasciocutaneous flaps (3.3%). However, only 13 of the 14 articles specified the type of flap used for each procedure. The most frequently used flaps were free latissimus dorsi and rectus abdominis, followed by rotational gastrocnemius [Table 4]. Seven of the 14 articles discussed type of anastomosis. End-to-side anastomosis (55.2%) was performed slightly more often than end-to-end anastomosis (44.8%), and, while a large range of recipient vessels was used, posterior tibial (53.2%) and anterior tibial (24.2%) were the most common [Table 5].

With regard to bone reconstruction and transport, bone defect was mentioned in nine articles, whereas total length of distraction was mentioned in four of the 14 articles. The average bone defect was 8.9 cm (range of 6.0-12.5 cm) and average total distraction was 6.1 cm (range of 4.3-10 cm). The time to distraction was discussed in five of the 14 articles, and rate of distraction was discussed in six of the 14 articles. The average time to distraction was 24.1 days (range of 7-73.5 days) and the average rate of distraction was 0.96 mm/day (range of 0.75-1 mm/day).

As discussed in 11 of the 14 articles, additional techniques were utilized for some extremities. These techniques included bone graft (62.3%), antibiotic beads (48.6%), antibiotic bone substitute (13.0%), vein

**Table 5. Recipient vessels**

Recipient vessels	No. of articles	No. of extremities	Percentage of extremities (%)
Posterior tibial	6	33	53.2
Anterior tibial	5	15	24.2
Peroneal	3	5	8.1
Sural	1	5	8.1
Popliteal	3	3	4.8
Femoral a./v. with saphenous graft	1	1	1.6
Total articles	7	62	

a.: artery; v.: vein

**Table 6. Complications in extremities based on timing of flap vs. frame placement**

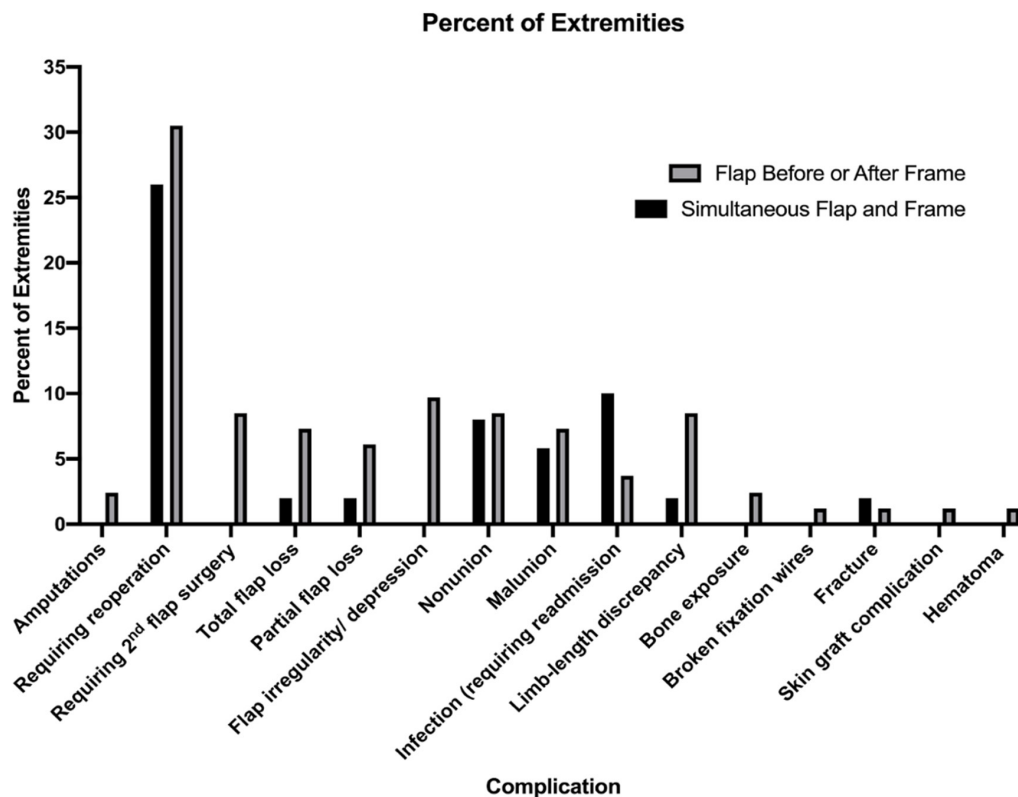
	Simultaneous flap and frame	Flap before or after frame	P-value
Amputations	0/50	2/82	0.45
Flap failure	1/50	6/82	0.32

*P* < 0.05 used for statistical significance

graft (2.7%), and hardware (0.7%). Eleven of the 14 articles documented complications in 141 patients, including nonunion (*n* = 11, 7.8%), malunion (*n* = 10, 7.1%), flap depression or irregularity (*n* = 10, 7.1%), total flap loss (*n* = 9, 4.9%), partial flap loss (*n* = 6, 4.3%), infection requiring readmission or i.v. antibiotics (*n* = 8, 5.7%), significant limb-length discrepancy (*n* = 8, 5.7%), bone exposure (*n* = 4, 2.8%), fracture (*n* = 2, 1.4%), hematoma (*n* = 2, 1.4%), skin graft complication (*n* = 1, 0.8%), and broken fixation wires (*n* = 1, 0.8%). Ten of the 14 articles also discussed 140 extremities requiring reoperation (*n* = 42, 30%), by far mostly for docking procedures, second flap surgery (*n* = 9, 6.4%), or amputations (*n* = 2, 1.4%).

Complications were further assessed based on timing of soft tissue coverage, which occurred simultaneously with bone transport frame as well as before or after bone transport. Starting with simultaneous placement of flap and frame, five of the 14 articles used this approach for 50 patients with a mean age of 34.4 years in 50 extremities [Table 6 and Figure 2]. Three extremities needed reoperation; however, none required amputation or a second flap surgery. These articles reported infections requiring readmission or i.v. antibiotics (*n* = 5, 10%), nonunions (*n* = 4, 8.0%), malunions (*n* = 3, 5.8%), partial flap loss (*n* = 1, 2%), total flap loss (*n* = 1, 2%), fracture (*n* = 1, 2%), and limb-length discrepancy (*n* = 1, 2%) in several extremities. In contrast, seven of the 14 articles documented complications after soft tissue reconstruction with flaps either before or after application of frame used for bone transport [Table 5 and Figure 2]. This method included 82 extremities of 81 patients with a mean age of 32.9 years. A higher number of extremities required amputations (*n* = 2, 2.4%), reoperation (*n* = 25, 30.5%), and second flap surgery (*n* = 7, 8.5%). Similar to the simultaneous group, these articles reported total flap losses (*n* = 6, 7.3%), partial flap losses (*n* = 5, 6.3%), nonunions (*n* = 7, 8.5%), malunions (*n* = 6, 7.3%), limb-length discrepancies (*n* = 7, 8.5%), infections requiring readmission or i.v. antibiotics (*n* = 3, 3.7%), and fracture (*n* = 1, 1.2%) in a number of extremities. Additionally, this group reported flap irregularities or depression (*n* = 8, 9.7%), bone exposures (*n* = 2, 2.4%), skin graft complication (*n* = 1, 1.2%), hematoma (*n* = 1, 1.2%), and broken fixation wires (*n* = 1, 1.2%). Of note, there was no statistical significance between the rates of any complication between the 2 groups.

Total flap loss, or flap failure, was identified in four different articles, one of which also reported amputation as subsequent management. Of the four articles, only one<sup>[81]</sup> employed the simultaneous “fix and flap” technique, in which one flap failed out of the 34 reconstructions performed (2.9%). However, this patient’s condition, flap choice, and postoperative management are not included. The remainder of the studies<sup>[82,88,89]</sup> utilized flap surgery before or after fixation. Musharafieh *et al.*<sup>[82]</sup> described 3 case reports,



**Figure 2.** Percentage of patients with complications after extremity soft tissue reconstruction, broken down by timing of soft tissue flap vs. bone transport frame placement

one of which is a 52-year-old male with a compound comminuted fracture of the distal leg, a resultant 14-cm tibial bony defect, and a large soft tissue defect. He received a free rectus abdominis muscle flap, which failed secondary to venous thrombosis about 55 days after injury. He then had a second and successful free rectus abdominis flap. Months later, he underwent bone transport, resulting in full weight bearing capacity 18 months after injury. Hutson *et al.*<sup>[88]</sup> denoted two flap failures out of 19 extremities undergoing reconstruction with flaps first. The methods utilized were two latissimus dorsi free flaps to recipient posterior tibial arteries anastomosed in either end-to-side or end-to-end fashion, both resulting in flap failure. The patients were reported to later undergo bone distraction, although further soft tissue rearrangement, if any, was not mentioned. Lastly, Chim *et al.*<sup>[89]</sup> reported on 28 extremities undergoing flap reconstruction before or after fixation, three of which failed. One patient had successful bone transport and was managed with distraction lengthening of soft tissue, whereas the other two had concurrent failure of distraction and were managed with amputations. One amputation occurred in a 34-year-old patient with a Gustilo II infected bony nonunion after a motor vehicle collision and an associated wound size of 50 cm<sup>2</sup>, who had a failed gracilis free flap to recipient posterior tibial artery with end-to-side anastomosis and venous commitantes in end-to-end fashion. The other amputation was on a 57-year-old patient with a Gustilo IIIB acute bone loss defect due to a crush injury and an associated wound size of 700 cm<sup>2</sup>, which was managed with a latissimus dorsi free flap through end-to-side anastomosis of posterior tibial artery and end-to-end anastomosis to anterior tibial venous commitantes.

Four studies with a total of 32 patients described the management of acute injuries. Regarding union, there was one nonunion and three malunions, while no patients suffered a refracture. Regarding the flap, there were 5 losses (three total and two partial) and 9 flap irregularities. Sixteen patients required a secondary reoperation, and seven of them were for the flap specifically. One patient suffered from a deep infection.

There were five studies that characterized their wounds as chronic. However, Lowenberg *et al.*<sup>[61]</sup> (1996) reported the lone study to clarify their complications and describe them. Of their 23 patients, six suffered from a complication. Ones of note include two with nonunion, one with malunion, and one with partial loss of a flap, in deep infection. Additionally, there was an increased rate of second surgery ( $P = 0.049$ ), as five patients needed a second surgery - one for a flap replacement specifically following initial failure.

Despite significant complications and reoperations, 98.8% (range of 92.9% to 100%) of patients averaged from 6 articles were ultimately weight bearing, and the success rate was 98.4% (range of 92.8% to 100%) averaged across 11 articles. One of the 14 articles reported a satisfaction rate of 100% from the 34 patients involved.

## Cases

### *Case 1: free anterolateral thigh flap with taylor spatial frame first*

A 69-year-old male with a history of obesity and hypertension was involved in a motor vehicle collision 40 years prior that resulted in an open tibia fracture that was treated with plate fixation at that time. He recovered from that surgery and did well without complication until 2 years prior to presentation when he was found to have purulent drainage around the plate, which was treated at an outside facility with serial debridement and skin grafting. He was ultimately referred to our center at Keck Hospital and was found to have chronic osteomyelitis of the tibia. After thorough debridement of bone and soft tissue, he was left with 17.5-cm bony defect of the tibia and a wound that was approximately 100 cm<sup>2</sup>. The bony defect was temporized with antibiotic spacer and culture-directed intravenous antibiotics until cultures were negative, at which point the patient was placed in a hexapod frame. Five days thereafter, he received free flap coverage of his large wound with an anterolateral thigh (ALT) fasciocutaneous flap anastomosed in end-to-side fashion into the posterior tibial vessels. Bony transport was initiated once he had completed 6 weeks of i.v. antibiotics and he could be scheduled for surgery for corticotomy, which was 98 days after his initial external fixator placement. He was distracted at a rate of approximately 0.5-1 mm per day. After two subsequent surgical revisions of his multiplanar external fixator, he underwent removal of the external fixator 505 days after his initial ex-fix placement, and he underwent a Masquelet procedure to complete bony union. He did suffer equinovarus deformity of the foot, for which he received tendon lengthening and ankle-spanning external fixation, as well as arthrofibrosis of his knee, for which he underwent two lysis of adhesions and quadricepsplasty. Currently, he is weightbearing as tolerated and proceeding to follow with physical therapy. At his most recent follow-up four years after presentation, he had achieved bony union but developed an infection for which he is currently being treated with oral antibiotics and is scheduled to undergo surgical irrigation and debridement.

### *Case 2: free latissimus dorsi and rotational gastrocnemius flap with delayed NuVasive precise frame*

A 45-year-old man sustained a left Gustilo-Anderson IIIB tibia/fibular shaft fractures with an associated distal fibular fracture, for which he underwent debridement and open reduction external fixation at an outside facility. He was transferred to our services when it was determined that he would need soft tissue coverage of his extremity. He was taken to the operating room for debridement of necrotic fibula stripped of periosteum and antibiotic spacer placement. His bony defect was approximately 8 cm and his soft tissue wounds were approximately 75 cm<sup>2</sup>. Five days after his initial debridement at our institution, the patient received both a free latissimus myocutaneous flap anastomosed in end-to-end fashion to the anterior tibial vessels as well as a rotational gastrocnemius myocutaneous flap for coverage of a more posterior wound. Split thickness skin grafting was employed to cover his remaining non-critical wounds. He proceeded with physical therapy and was ultimately weight bearing; however, it thereafter became apparent that he had nonunion of his tibia fracture, likely due to the extensive zone of injury. As such, 276 days after this initial treatment, he was taken to the operating room for placement of motorized magnetic transport nail (Nuvasive, Aliso Viejo, CA) for bone transport. He started distraction 11 days after placement of bone

**Table 7. Summary of cases series**

	Age (yrs)	Indication	Time from injury	Bone defect	Soft tissue defect	Frame	Surgical approach	Flap	Recipient vessel	Distraction rate	Management	Complications	Ultimate result
Case #1	69	Chronic OM	40 yrs	17.5 cm	100 cm <sup>2</sup>	Taylor spatial frame	Frame first	Free ALT	PT	0.5-1 mm/day	Masquelet for bony union	Tendon lengthening; ankle spanning external fixation; 2 lysis of adhesions; quadricepsplasty; infection	Weight bearing
Case #2	45	Acute trauma	NR	8 cm	75 cm <sup>2</sup>	NuVasive precise	Flap first	Free LD; rotational gastroc	AT	NR	Antibiotic spacer; STSG	Nonunion requiring reoperation for distraction and bone graft	Weight bearing
Case #3	31	Chronic OM and nonunion	6 mo	11.5 cm	500 cm <sup>2</sup>	Ilizarov frame	Frame first	Free LD	PT	NR	Skin grafting	Flap elevation and bone grafting	Weight bearing

OM: osteomyelitis; LD: latissimus dorsi; gastroc: gastrocnemius; SPSSG: split thickness skin graft; yrs: years; mo: months; PT: posterior tibial; AT: anterior tibial; NR: not reported; ALT: anterolateral thigh

transport nail. He went to the operating room 77 days later for preparation of the docking site as well as bone grafting from the iliac crest. He has completed transport, is fully weight bearing, and participating in physical therapy.

#### *Case 3: free latissimus dorsi with ilizarov frame first*

A 31-year-old man sustained a right distal tibia/fibula fracture from a motorcycle crash, treated at that time at an outside facility with debridement and stabilization with an external fixator, which two weeks later was converted to internal fixation. At the time of definitive fixation, the wounds were not able to be closed and ultimately a rotational flap was employed for closure. He thereafter developed infection and remained non-weightbearing. He was told that amputation was his only option, which he refused, and approximately 6 months after his initial fixation, he presented to our clinic to discuss limb salvage options. He was found at that time to have osteomyelitis and nonunion of his tibial fracture. His wounds had many sinus tracts, which were draining purulent effluent. He was taken to the operating room for debridement, hardware removal, and antibiotic-impregnated nail placement at the diaphyseal tibial fracture. He subsequently underwent two more debridements as well as removal of internal antibiotic coated nail and placement of multiplanar ringed external fixator. He was ultimately left with a bony defect of approximately 11.5 cm and soft tissue defect of approximately 500 cm<sup>2</sup>, at which point he was determined prepared for soft tissue coverage. One week after external fixation, he underwent free latissimus dorsi muscle flap with microvascular anastomosis in end-to-end fashion to the posterior tibial vessels as well as skin grafting for coverage. He underwent corticotomy in preparation for bony transport 42 days after his free flap, and subsequently initiated transport 55 days after his free flap. He received one additional surgery for elevation of the flap out of the docking site and simultaneous bone grafting to the docking site. Since that time, he has been distracting and has reached equal limb length. He has been weight bearing and working with physical therapy [Table 7].

## DISCUSSION

This systematic review evaluates the choices for soft tissue coverage in distraction osteogenesis for upper and lower extremities, and presents both indicators of success (weight bearing status and satisfaction) and complication rates to better inform the surgeon's decision-making process. The indications for surgical intervention include chronic nonunion, osteomyelitis, and, less commonly, acute trauma. Fewer articles detailed reconstruction after acute trauma, which was



reflected in the longer than expected average time from injury (1.1 years), which ranged from within weeks to up to three years. Two cases in the present study describe treatment within days to a few weeks, whereas the other indicates over 40 years since initial injury, which better aligns with what is seen in our review of the literature. Additionally, the present study includes a single article that reports flap and frame repair of upper extremity injuries<sup>[61]</sup>. Although there is a paucity of literature discussing the use of soft tissue coverage and distraction osteogenesis in upper extremity reconstruction, the study was included to bring light to the innovative, diverse applications of this technique.

The current gold standard for managing large soft tissue defects that cannot be closed directly is the “fix and flap” protocol, which has several recognized advantages<sup>[4,51,59,60]</sup>. However, with respect to the results of our literature review, only about one third of the extremities described underwent simultaneous flap and frame placement. Similarly, this simultaneous approach is taken in only 1 of the 3 cases presented. While this is partially due to the inclusion of older studies that were published prior to the acceptance of modern “fix and flap” approach<sup>[7,9,41,61,62,65,84-86,88,89]</sup>, it is also important to note that the gold standard typically applies to acute traumatic injuries, whereas many patients undergoing bone transport are patients with chronic extremity issues.

With respect to reconstruction of bone, the present review measured the average bone defect to be 8.9 cm, which required intervention to restore limb length. The mainstay of distraction osteogenesis is the application of the Ilizarov method for external fixation, although nowadays novel methods of complementary internal fixation have been innovated with improved patient comfort, decreased infection rates, and quicker recovery<sup>[43,91-95]</sup>. The present review identified that the majority of articles employed external fixation consistent with the Ilizarov method. The remaining articles used variations of the Ilizarov method, except one that used anterior double-stacking external fixation. However, for many reasons, an array of surgical techniques may be used to tailor reconstructive approach to the individual’s unique presentation.

While bone defects smaller than 8 cm can be successfully closed using bone grafting<sup>[7,9,96]</sup>, larger defects may require a vascularized bone graft<sup>[97]</sup>. Common vascularized bone grafts that have been described include contralateral fibula and iliac crest<sup>[8]</sup>. These operations, however, carry significant donor site morbidity and are also limited by the size of the donor site<sup>[7,77]</sup>. For patients with large defects, the anatomy of the iliac crest makes it unsuitable<sup>[27]</sup>, and harvesting a contralateral fibula in a patient with unilateral lower extremity trauma can be problematic due donor site complications<sup>[98]</sup> and insufficient pedicle length<sup>[99]</sup>. In certain circumstances, specifically in previously infected femoral shafts, efficacy has been shown for vascularized fibular grafts<sup>[100]</sup>. As seen in the present review, 62.3% of extremities were managed with both distraction osteogenesis and bone grafting. Two of the three cases discussed similarly received bone grafts.

When comparing bone grafting with soft tissue coverage against resection and bone transport, results have been shown to be similar, with significantly less limb length discrepancy in the bone transport group<sup>[101]</sup>. Although distraction osteogenesis in combination with free flap reconstruction has clearly been proven to be a useful treatment modality, the procedure is time consuming and can produce a multitude of challenging complications if not approached with great care.

Some of the common complications associated with this procedure are bone exposure<sup>[94]</sup>, nonunion<sup>[7]</sup>, flap necrosis<sup>[102]</sup>, and downward depression of the flap<sup>[90]</sup>. While some of the problems can be resolved with conservative care or minor revisions, there are others with more serious consequences. As found in the present review, flap failure may result from venous thrombosis or vasculature disruption due to acute or chronic pathologies<sup>[81,82,88,89]</sup>. The articles described these losses in patients with large bony and soft tissue defects undergoing a number of different free flaps (latissimus dorsi, rectus abdominis, and

gracilis) through both end-to-end and end-to-side anastomoses to either anterior or posterior tibial vessels, consistent with the most frequently used surgical techniques. While two patients eventually underwent amputations, this adverse event was seen in only 1.4% of all extremities. Likewise, there was no statistically significant difference in rates of flap failure or amputation between the extremities that were managed according to the “fix and flap protocol” *versus* the placement of a flap before or after the frame. While the difference in outcomes between the most commonly used free flaps, the rectus abdominis and latissimus dorsi flaps, would be beneficial to evaluate, this was not possible given the nature of the studies. Nearly all of the case series that were included in our review reported multiple different types of flaps that were used in their patients, however the results were not separated according to flap type.

Looking further into patient complications, the present study indicates that injuries managed in the acute setting were significantly more likely to undergo secondary surgical repair, compared to those who were chronic. While this may be due to more controlled surgical management of chronic wounds predating the beginning of the study time, the variability in injuries, patient morbidities, surgeon expertise, and postoperative care limits the value of this finding.

An additional issue that can arise during the distraction process is the necessity to revise pin position or flap configuration. In cases of flap necrosis, however, distraction is often delayed or stopped altogether, necessitating additional procedures such as bone grafting<sup>[102]</sup>. Thus, meticulous planning of the flap in addition to careful postoperative care is crucial for successful reconstruction.

Deciding the reconstructive method for soft tissue coverage can be challenging, as the options to choose from are vast. In the articles reviewed, free flaps and muscle flaps were more commonly utilized than rotational flaps and fasciocutaneous flaps, respectively. As for specific flaps, rectus abdominis flaps were used for the greatest number of extremities, whereas free latissimus dorsi flaps were cited in the greatest number of distinct articles. Microsurgical technique varied, with end-to-end anastomosis being used almost as often as end-to-side anastomosis. Recipient vessels were most commonly the anterior and posterior tibial arteries, although a diverse set of choices exist. Given the comparable success rates with all of these different techniques, flap choice is often left to the discretion of the surgeon.

Aside from infection and fracture, the present review found that simultaneous placement of flap and frame, or the “fix and flap” protocol, had fewer reported adverse events overall [Figure 1]. These results align with the well-documented faster union times but differ from reported lower infection rates in the current literature<sup>[59,60]</sup>, given that our review actually found a higher rate of infection in the simultaneous placement of flap and frame. Two of the cases presented required additional surgeries, one for knee arthrofibrosis and foot deformity and the other for flap elevation, bone grafting, and docking.

Other factors must be taken into consideration when attempting to mitigate the risk of flap loss, with one of which being rate of bone transport. While Jupiter *et al.*<sup>[62]</sup> concluded that the free tissue and the native tissue undergo equal amounts of stretch and lengthening, Horas *et al.*<sup>[103]</sup> noted a difference in speed between bone transport and soft-tissue movement, which could potentially jeopardize the vascular pedicle secondary. This risk increases with the amount of transport needed. Interestingly, all of the papers assessed in the review performed distraction at a rate between 0.75 and 1 mm per day, indicating that there is agreement that this range of rates produces optimal results.

Although limb salvage and reconstruction through soft tissue coverage and distraction osteogenesis comes with substantial risk of complications and reoperations, outcomes remain strong. Over 98% of patients were ultimately weight bearing, and article authors on average reported rates of success and patient satisfaction as greater than 98%. All three of the case examples outlined in the present study are fully weight bearing as tolerated and working with physical therapy.

There are several limitations to the present review. Due to the paucity of literature evaluating the outcomes after utilizing soft tissue coverage and bone transport after critical bone loss for limb salvage, this systematic review was limited to case reports and case series. Thus, the quality of studies included is a limitation. In addition, the studies included are very heterogeneous with the parameters discussed and outcomes evaluated, which led to an even smaller group of articles coinciding with any one parameter. Given the degree of inconsistency in the way results were reported in each article, many studies had to be excluded, leading to a lower power. Lastly, reoperation for cancellous bone grafting for nonunion was frequently performed across articles; however, it remained unclear in the literature if this was an expected complication that needs anticipated surgery vs. an actual complication. These events were included under the category of bone grafting.

In conclusion, the reconstruction of extremities with critical bone loss, whether due to acute trauma, nonunion, or chronic osteomyelitis, remains a significant challenge for surgeons. Our findings suggest that no one surgical approach is superior, and the treatment algorithm ultimately remains a decision to be made between the surgeon and the patient. In patients with bone defects too extensive for autologous bone grafting or in patients who are not candidates for this approach, a combination of bone transport with soft tissue reconstruction remains an excellent choice for satisfactory functional outcomes.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Stoneburner J, Azadgoli B, Carey J, Marecek G

Performed data acquisition, as well as provided administrative, technical, and material support: Stoneburner J, Azadgoli B, Howell A, Tucker D

### Availability of data and materials

All data is shared through the included tables and figures.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Hao J, Cuellar DO, Herbert B, Kim JW, Chadayammuri V, et al. Does the OTA open fracture classification predict the need for limb amputation? A retrospective observational cohort study on 512 patients. *J Orthop Trauma* 2016;30:194-98.
2. Tonnesen PA, Heerfordt J, Pers M. 150 open fractures of the tibial shaft--the relation between necrosis of the skin and delayed union. *Acta Orthop Scand* 1975;46:823-35.
3. Gustilo RB, Simpson L, Nixon R, Ruiz A, Indeck W. Analysis of 511 open fractures. *Clin Orthop Relat Res* 1969;66:148-54.
4. Yazar S, Lin CH, Lin YT, Ulusal AE, Wei FC. Outcome comparison between free muscle and free fasciocutaneous flaps for reconstruction

- of distal third and ankle traumatic open tibial fractures. *Plast Reconstr Surg* 2006;117:2468-75.
5. Fischer MD, Gustilo RB, Varecka TF. The timing of flap coverage, bone-grafting, and intramedullary nailing in patients who have a fracture of the tibial shaft with extensive soft-tissue injury. *J Bone Joint Surg Am* 1991;73:1316-22.
6. Rodriguez ED, Bluebond-Langner R, Copeland C, Grim TN, Singh NK, et al. Functional outcomes of posttraumatic lower limb salvage: a pilot study of anterolateral thigh perforator flaps versus muscle flaps. *J Trauma* 2009;66:1311-4.
7. Tukiainen E, Asko-Seljavaara S. Use of the Ilizarov technique after a free microvascular muscle flap transplantation in massive trauma of the lower leg. *Clin Orthop Relat Res* 1993;129-34.
8. Weiland AJ. Current concepts review: vascularized free bone transplants. *J Bone Joint Surg Am* 1981;63:166-9.
9. Spiro SA, Oppenheim W, Boss WK, Schneider AI, Hutter AM. Reconstruction of the lower extremity after grade III distal tibial injuries using combined microsurgical free tissue transfer and bone transport by distraction osteosynthesis. *Ann Plast Surg* 1993;30:97-104.
10. Watson TJ. Distraction osteogenesis. *JAAOS* 2006;14:S168-74.
11. Haines NM, Lack WD, Seymour RB, Bosse MJ. Defining the lower limit of a "critical bone defect" in open diaphyseal tibial fractures. *J Orthop Trauma* 2016;30:e158-63.
12. Nauth A, Schemitsch E, Norris B, Nollin Z, Watson JT. Critical-size bone defects: is there a consensus for diagnosis and treatment? *J Orthop Trauma* 2018;32:S7-11.
13. Schemitsch EH. Size matters: defining critical in bone defect size! *J Orthop Trauma* 2017;31:S20-22.
14. Masquelet AC, Fitoussi F, Begue T, Muller GP. Reconstruction of the long bones by the induced membrane and spongy autograft. *Ann Chir Plast Esthet* 2000;45:346-53. (in French)
15. Masquelet AC, Begue T. The concept of induced membrane for reconstruction of long bone defects. *Orthop Clin North Am* 2010;41:27-37.
16. Masquelet AC, Kishi T, Benko PE. Very long-term results of post-traumatic bone defect reconstruction by the induced membrane technique. *Orthop Traumatol Surg Res* 2019;105:159-66.
17. Khan SN, Cammisa FP Jr, Sandhu HS, Diwan AD, Girardi FP, et al. The biology of bone grafting. *J Am Acad Orthop Surg* 2005;13:77-86.
18. Christian EP, Bosse MJ, Robb G. Reconstruction of large diaphyseal defects, without free fibular transfer, in Grade-IIIB tibial fractures. *J Bone Joint Surg Am* 1989;71:994-1004.
19. Aho OM, Lehenkari P, Ristiniemi J, Lehtonen S, Risteli J, et al. The mechanism of action of induced membranes in bone repair. *J Bone Joint Surg Am* 2013;95:597-604.
20. Henrich D, Seebach C, Nau C, Basan S, Relja B, et al. Establishment and characterization of the Masquelet induced membrane technique in a rat femur critical-sized defect model. *J Tissue Eng Regen Med* 2016;10:E382-96.
21. Pelissier P, Masquelet AC, Bareille R, Pelissier SM, Amedee J. Induced membranes secrete growth factors including vascular and osteoinductive factors and could stimulate bone regeneration. *J Orthop Res* 2004;22:73-9.
22. Spiegelberg B, Parratt T, Dheerendra SK, Khan WS, Jennings R, et al. Ilizarov principles of deformity correction. *Ann R Coll Surg Engl* 2010;92:101-5.
23. Ilizarov GA. Clinical application of the tension-stress effect for limb lengthening. *Clin Orthop Relat Res* 1990;8-26.
24. Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: Part II. The influence of the rate and frequency of distraction. *Clin Orthop Relat Res* 1989;263-85.
25. Vauhkonen M, Peltonen J, Karaharju E, Aalto K, Alitalo I. Collagen synthesis and mineralization in the early phase of distraction bone healing. *Bone Miner* 1990;10:171-81.
26. Han CS, Wood MB, Bishop AT, Cooney WP 3rd. Vascularized bone transfer. *J Bone Joint Surg Am* 1992;74:1441-9.
27. Ikeda K, Yokoyama M, Okada K, Tomita K, Yoshimura M. Long-term follow-up of the vascularized iliac bone graft. *Microsurgery* 1998;18:419-23.
28. Dumont CE, Exner UG. Reconstruction of large diaphyseal defects of the femur and the tibia with autologous bone. *Eur J Trauma Emerg Surg* 2009;35:17.
29. Innocenti M, Abed YY, Beltrami G, Delcroix L, Manfrini M, et al. Biological reconstruction after resection of bone tumors of the proximal tibia using allograft shell and intramedullary free vascularized fibular graft: long-term results. *Microsurgery* 2009;29:361-72.
30. Bernstein M, Fragomen AT, Sabharwal S, Barclay J, Rozbruch SR. Does integrated fixation provide benefit in the reconstruction of posttraumatic tibial bone defects? *Clin Orthop Relat Res* 2015;473:3143-53.
31. Krappinger D, Irenberger A, Zegg M, Huber B. Treatment of large posttraumatic tibial bone defects using the Ilizarov method: a subjective outcome assessment. *Arch Orthop Trauma Surg* 2013;133:789-95.
32. Paley D, Maar DC. Ilizarov bone transport treatment for tibial defects. *J Orthop Trauma* 2000;14:76-85.
33. Rozbruch SR, Kleinman D, Fragomen AT, Ilizarov S. Limb lengthening and then insertion of an intramedullary nail: a case-matched comparison. *Clin Orthop Relat Res* 2008;466:2923-32.
34. Bernstein M, Fragomen A, Rozbruch SR. Tibial bone transport over an intramedullary nail using cable and pulleys. *JBJS Essent Surg Tech* 2018;8:e9.
35. Paley D. Problems, obstacles, and complications of limb lengthening by the Ilizarov technique. *Clin Orthop Relat Res* 1990;81-104.
36. Rozbruch SR, Fragomen AT, Ilizarov S. Correction of tibial deformity with use of the Ilizarov-Taylor spatial frame. *J Bone Joint Surg* 2006;88:156-74.
37. Feldman DS, Shin SS, Madan S, Koval KJ. Correction of tibial malunion and nonunion with six-axis analysis deformity correction using the Taylor Spatial Frame. *J Orthop Trauma* 2003;17:549-54.
38. Eidelman M, Katzman A. Treatment of complex tibial fractures in children with the Taylor spatial frame. *Orthopedics* 2008;31.
39. Eidelman M, Bialik V, Katzman A. Correction of deformities in children using the Taylor spatial frame. *J Pediatr Orthop B* 2006;15:387-95.
40. Fadel M, Hosny G. The Taylor spatial frame for deformity correction in the lower limbs. *Int Orthop* 2005;29:125-9.
41. Al-Sayyad J. Taylor spatial frame in the treatment of pediatric and adolescent tibial shaft fractures. *J Pediatr Orthop* 2006;26:164-70.
42. Bernstein R, Fragomen R, Rozbruch R. Tibial bone transport over an intramedullary nail using cable and pulleys. *JBJS Essent Surg Tech*

- 2018;8:e9.
43. Watanabe K, Tsuchiya H, Sakurakichi K, Yamamoto N, Kabata T, et al. Tibial lengthening over an intramedullary nail. *J Orthop Sci* 2005;10:480-5.
  44. Xiong L, Gazyakan E, Kremer T, Hernekamp FJ, Harhaus L, et al. Free flaps for reconstruction of soft tissue defects in lower extremity: a meta-analysis on microsurgical outcome and safety. *Microsurgery* 2016;36:511-24.
  45. Sofiadellis F, Liu DS, Webb A, Macgill K, Rozen WM, et al. Fasciocutaneous free flaps are more reliable than muscle free flaps in lower limb trauma reconstruction: experience in a single trauma center. *J Reconstr Microsurg* 2012;28:333-40.
  46. Hong JP, Shin HW, Kim JJ, Wei FC, Chung YK. The use of anterolateral thigh perforator flaps in chronic osteomyelitis of the lower extremity. *Plast Reconstr Surg* 2005;115:142-7.
  47. Cho EH, Shammass RL, Carney MJ, Weissler JM, Bauder AR, et al. Muscle versus fasciocutaneous free flaps in lower extremity traumatic reconstruction: a multicenter outcomes analysis. *Plast Reconstr Surg* 2018;141:191-9.
  48. Hanasono MM, Skoracki RJ, Yu P. A prospective study of donor-site morbidity after anterolateral thigh fasciocutaneous and myocutaneous free flap harvest in 220 patients. *Plast Reconstr Surg* 2010;125:209-14.
  49. Wei FC, Jain V, Celik N, Chen HC, Chuang DC, et al. Have we found an ideal soft-tissue flap? An experience with 672 anterolateral thigh flaps. *Plast Reconstr Surg* 2002;109:2219-26.
  50. Bekara F, Herlin C, Somda S, de Runz A, Grolleau JL, et al. Free versus perforator-pedicled propeller flaps in lower extremity reconstruction: what is the safest coverage? A meta-analysis. *Microsurgery* 2018;38:109-19.
  51. Samaha FJ, Oliva A, Buncke GM, Buncke HJ, Siko PP. A clinical study of end-to-end versus end-to-side techniques for microvascular anastomosis. *Plast Reconstr Surg* 1997;99:1109-11.
  52. Khouri RK, Shaw WW. Reconstruction of the lower extremity with microvascular free flaps: a 10-year experience with 304 consecutive cases. *J Trauma* 1989;29:1086-94.
  53. Kolker AR, Kasabian AK, Karp NS, Gottlieb JJ. Fate of free flap microanastomosis distal to the zone of injury in lower extremity trauma. *Plast Reconstr Surg* 1997;99:1068-73.
  54. Stranix JT, Borab ZM, Rifkin WJ, Jacoby A, Lee ZH, et al. Proximal versus distal recipient vessels in lower extremity reconstruction: a retrospective series and systematic review. *J Reconstr Microsurg* 2018;34:334-40.
  55. Byrd HS, Spicer TE, Cierney G 3rd. Management of open tibial fractures. *Plast Reconstr Surg* 1985;76:719-30.
  56. Fischer JP, Wink JD, Nelson JA, Cleveland E, Grover R, et al. A retrospective review of outcomes and flap selection in free tissue transfers for complex lower extremity reconstruction. *J Reconstr Microsurg* 2013;29:407-16.
  57. Pollak AN, McCarthy ML, Burgess AR. Short-term wound complications after application of flaps for coverage of traumatic soft-tissue defects about the tibia. The Lower Extremity Assessment Project (LEAP) Study Group. *J Bone Joint Surg Am* 2000;82:1681-91.
  58. Colen DL, Colen LB, Levin LS, Kovach SJ. Godina's principles in the twenty-first century and the evolution of lower extremity trauma reconstruction. *J Reconstr Microsurg* 2018;34:563-71.
  59. Hertel R, Lambert SM, Muller S, Ballmer FT, Ganz R. On the timing of soft-tissue reconstruction for open fractures of the lower leg. *Arch Orthop Trauma Surg* 1999;119:7-12.
  60. Gopal S, Majumder S, Batchelor AG, Knight SL, De Boer P, et al. Fix and flap: the radical orthopaedic and plastic treatment of severe open fractures of the tibia. *J Bone Joint Surg Br* 2000;82:959-66.
  61. Lowenberg DW, Feibel RJ, Louie KW, Eshima I. Combined muscle flap and Ilizarov reconstruction for bone and soft tissue defects. *Clin Orthop Relat Res* 1996;37:51.
  62. Jupiter JB, Kour AK, Palumbo MD, Yaremchuk MJ. Limb reconstruction by free-tissue transfer combined with the Ilizarov method. *Plast Reconstr Surg* 1991;88:943-51.
  63. Ilizarov GA, Zusmanovitch FN, Markhashov AM, Khelinskii AM, Levitina L, et al. Reconstruction of large defects of blood vessels on extremities by means of a gradual distraction. (An experimental study). *Acta Chir Plast* 1980;22:156-65.
  64. Ilizarov GA, Kuznetsova AB, Peschanskii VS, Shchudlo MM, Khanes GS. Blood vessels in different systems of limb traction (experimental study). *Arkh Anat Gistol Embriol* 1984;86:49-55. (in Russian)
  65. Fiebel RJ, Oliva A, Jackson RL, Louie K, Buncke HJ. Simultaneous free-tissue transfer and Ilizarov distraction osteosynthesis in lower extremity salvage: case report and review of the literature. *J Trauma* 1994;37:322-7.
  66. Friedrich JB, Pederson WC, Bishop AT, Galaviz P, Chang J. New workhorse flaps in hand reconstruction. *HAND* 2012;7:45-54.
  67. Larson N, Bishop T, Shin Y. Free medial femoral condyle bone grafting for scaphoid nonunions with humpback deformity and proximal pole avascular necrosis. *Tech Hand Up Extrem Surg* 2007;11:246-58.
  68. Vedung T, Vinnars B. Ectopic bone formation after medial femoral condyle graft to scaphoid nonunion. *J Wrist Surg* 2014;3:46-9.
  69. Penteado C, Masquelet A, Romana M, Chevrel J. Periosteal flaps: anatomical bases of sites of elevation. *Surg Radiol Anat* 1990;12:3-7.
  70. Doi K, Hattori Y. Vascularized bone graft from the supracondylar region of the femur. *Microsurgery* 2009;29:379-84.
  71. Sakai K, Doi K, Kawai S. Free vascularized thin corticoperiosteal graft. *Plast Reconstr Surg* 1991;87:290-8.
  72. Muramatsu K, Doi K, Ihara K, Shigetomi M, Kawai S. Recalcitrant posttraumatic nonunion of the humerus: 23 patients reconstructed with vascularized bone graft: 23 patients reconstructed with vascularized bone graft. *Acta Orthop Scand* 2003;74:95-7.
  73. Bakri K, Shin AY, Moran SL. The vascularized medial femoral corticoperiosteal flap for reconstruction of bony defects within the upper and lower extremities. *Semin Plast Surg* 2008;22:228-33.
  74. Jadhav C, Rawlins J, Walters AG. Descending genicular vessels as recipient pedicle for free flap cover of complex defects around upper and mid-lower third junction of tibia. *J Hand Microsurg* 2015;7:326-7.
  75. Jones DB Jr, Rhee PC, Shin AY. Vascularized Bone Grafts for Scaphoid Nonunions. *J Hand Surg Am* 2012;37:1090-4.
  76. Kollitz KM, Pulos N, Bishop AT, Shin AY. Primary medial femoral condyle vascularized bone graft for scaphoid nonunions with carpal collapse and proximal pole avascular necrosis. *J Hand Surg Eur Vol* 2019;44:600-6.
  77. Jones DB Jr, Bürger H, Bishop AT, Shin AY. Treatment of scaphoid waist nonunions with an avascular proximal pole and carpal collapse.



- a comparison of two vascularized bone grafts. *J Bone Joint Surg Am* 2008;90:2616-25.
78. Chang MA, Bishop AT, Moran SL, Shin AY. The outcomes and complications of 1,2-intercompartmental suprarreticular artery pedicled vascularized bone grafting of scaphoid nonunions. *J Hand Surg Am* 2006;31:387-96.
  79. Doi K, Oda T, Soo-Heong T, Nanda V. Free vascularized bone graft for nonunion of the scaphoid. *J Hand Surg Am* 2000;25:507-19.
  80. Jones B, Moran L, Bishop T, Shin Y. Free-vascularized medial femoral condyle bone transfer in the treatment of scaphoid nonunions. *Plast Reconstr Surg* 2010;125:1176-84.
  81. Lowenberg DW, Buntic RF, Buncke GM, Parrett BM. Long-term results and costs of muscle flap coverage with Ilizarov bone transport in lower limb salvage. *J Orthop Trauma* 2013;27:576-81.
  82. Musharafieh RS, Saghih SS, Nassar H, Hamdan AM, Hashim HA, et al. Microvascular soft-tissue coverage and distraction osteosynthesis for lower-extremity salvage. *Microsurgery* 1996;17:666-73.
  83. Bibbo C. Reverse sural flap with bifocal Ilizarov technique for tibial osteomyelitis with bone and soft tissue defects. *J Foot Ankle Surg* 2014;53:344-9.
  84. Duman H, Sengezer M, Celikoz B, Turegun M, Isik S. Lower extremity salvage using a free flap associated with the Ilizarov method in patients with massive combat injuries. *Ann Plast Surg* 2001;46:108-12.
  85. Minehara H, Yokoyama K, Sekiguchi M, Nakamura T, Shindo M, et al. Bone transport combined with free flap reconstruction and antibiotic bead spacers for a type IIIB open tibial fracture: case report. *J Trauma* 1998;44:1103-7.
  86. Boopalan PR, Jepeganiam TS. Reverse sural flap cover within a ring fixator. *Acta Orthop Belg* 2010;76:684-8.
  87. Lowenberg DW, Van der Reis W. One-stage muscle transfer and Ilizarov frame application. *Tech Orthop* 1996;11:144-9.
  88. Hutson JJ Jr, Dayicioglu D, Oeltjen JC, Panthaki ZJ, Armstrong MB. The treatment of Gustilo grade IIIB tibia fractures with application of antibiotic spacer, flap, and sequential distraction osteogenesis. *Ann Plast Surg* 2010;64:541-52.
  89. Chim H, Sontich JK, Kaufman BR. Free tissue transfer with distraction osteogenesis is effective for limb salvage of the infected traumatized lower extremity. *Plast Reconstr Surg* 2011;127:2364-72.
  90. Isik S, Guler MM, Selmanpakoglu N. Unexpected, late complication of combined free flap coverage and Ilizarov technique applied to legs. *Ann Plast Surg* 1997;39:437-8.
  91. Napora JK, Weinberg DS, Eagle BA, Kaufman BR, Sontich JK. Hexapod frame stacked transport for tibial infected nonunions with bone loss: analysis of use of adjunctive stability. *J Orthop Trauma* 2017;31:393-9.
  92. Napora JK, Weinberg DS, Eagle BA, Kaufman BR, Sontich JK. Hexapod stacked transport for tibial infected nonunions with bone loss: long-term functional outcomes. *J Orthop Trauma* 2018;32:e12-8.
  93. Quinnan SM, Lawrie C. Optimizing bone defect reconstruction-balanced cable transport with circular external fixation. *J Orthop Trauma* 2017;31:e347-55.
  94. Burghardt RD, Manzotti A, Bhave A, Paley D, Herzenberg JE. Tibial lengthening over intramedullary nails: a matched case comparison with Ilizarov tibial lengthening. *Bone Joint Res* 2016;5:1-10.
  95. Paley D, Herzenberg JE, Paremain G, Bhave A. Femoral lengthening over an intramedullary nail. A matched-case comparison with Ilizarov femoral lengthening. *J Bone Joint Surg Am* 1997;79:1464-80.
  96. Cierny G 3rd, Zorn KE, Nahai F. Bony reconstruction in the lower extremity. *Clin Plast Surg* 1992;19:905-16.
  97. Yakuboff KP, Stern PJ, Neale HW. Technical successes and functional failures after free tissue transfer to the tibia. *Microsurgery* 1990;11:59-62.
  98. Vail TP, Urbaniak JR. Donor-site morbidity with use of vascularized autogenous fibular grafts. *J Bone Joint Surg Am* 1996;78:204-11.
  99. McKee NH, Haw P, Vettese T. Anatomic study of the nutrient foramen in the shaft of the fibula. *Clin Orthop Relat Res* 1984;141:4.
  100. Wei FC, El-Gammal TA, Lin CH, Ueng WN, Wei FC. Free fibula osteoseptocutaneous graft for reconstruction of segmental femoral shaft defects. *J Trauma* 1997;43:784-92.
  101. Marsh JL, Prokuski L, Biermann JS. Chronic infected tibial nonunions with bone loss. Conventional techniques versus bone transport. *Clin Orthop Relat Res* 1994;139:46.
  102. Kim J, Park Y. Reconstruction of the lower leg using the Ilizarov devices and latissimus dorsi free flap. *J Korean Soc Plast Reconstr Surg* 1996;23:1707-802.
  103. Horas K, Schnettler R, Maier G, Schneider G, Horas U. The role of soft-tissue traction forces in bone segment transport for callus distraction. *Strategies Trauma Limb Reconstr* 2015;10:21-6.

Review

Open Access



# Role of the extracellular matrix in skin aging and dedicated treatment - State of the art

Adele Sparavigna

Derming, Clinical Research and Bioengineering Institute, Milan 20159, Italy.

**Correspondence to:** Dr. Adele Sparavigna, Derming, Clinical Research and Bioengineering Institute, Milan 20159, Italy.  
E-mail: adele.sparavigna@yahoo.it

**How to cite this article:** Sparavigna A. Role of the extracellular matrix in skin aging and dedicated treatment - State of the art. *Plast Aesthet Res* 2020;7:14. <http://dx.doi.org/10.20517/2347-9264.2019.73>

**Received:** 13 Dec 2019 **First Decision:** 25 Feb 2020 **Revised:** 3 Mar 2020 **Accepted:** 12 Mar 2020 **Published:** 20 Mar 2020

**Science Editors:** John Yousif, Kai O. Kaye **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

The extracellular matrix (ECM) occupies the space between cell and cell, and serves as a sort of intranet which connects the whole organism. Current research is focused on the ECM and, it is now possible to develop increasingly effective strategies for the prevention and treatment of degenerative diseases and even, cutaneous ageing. In fact, the most advanced anti-aging treatments are those that regenerate the ECM, which is now regarded as the main player in the physical support of, and exchange with and between cells of nutrients, cellular mediators and growth factors.

**Keywords:** Collagen, elastin, hyaluronic acid, extracellular matrix, matrisome

## INTRODUCTION

Skin aging is a complex and unavoidable biological phenomenon that starts in the third decade of life<sup>[1]</sup>. The aging processes is determined by a combination of intrinsic (chronological, hormonal and genetic) and extrinsic factors. The latter can be further divided into behavioural factors like sun exposure (responsible for photo-aging), cigarette smoking, dietary habits, alcohol intake, drug abuse and environmental factors such as pollution, weather, and humidity. Between individuals, genetics, as well as lifestyle habits, are all different. Therefore, aging at large, and skin aging in particular, are extremely variable<sup>[2]</sup>. Skin aging is a dynamic process that results in structural alterations of soft and bony tissues<sup>[3]</sup>. The silhouette of the face and body change as a result of atrophy of its various constituent layers: from skin to subcutaneous adipose tissue including loss of muscle mass and, to a lesser extent, resorption of bone, wrinkles, hypotonicity, lipotrophy, sarcopenia and volume displacement may result. Furthermore, to counteract atrophy, a



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



permanent muscle tone is established to neutralize incorrect relaxation of the skin through compensating mechanisms<sup>[4]</sup>.

## EXTRACELLULAR MATRIX AGING MECHANISMS

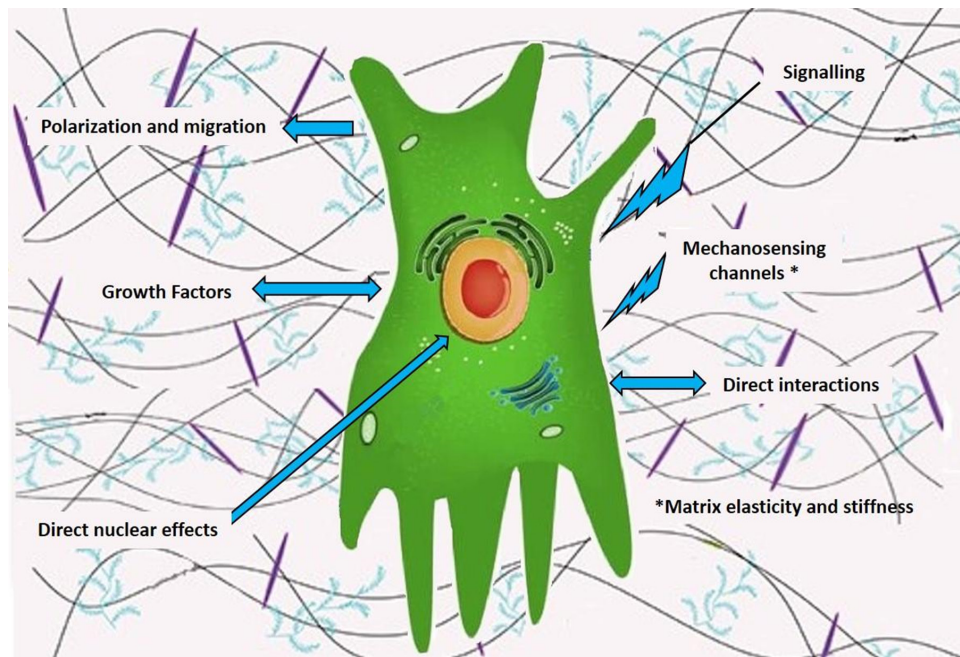
Over time, various body structures lose function in an unpredictable sequence. The aging process is thus not gradual nor uniform, and aging of the facies becomes even more complex due to the presence of mimetic muscles connected to the skin. Prevention and treatment strategies must therefore account for the above. The extracellular matrix (ECM) provides a commonality amongst these intricate processes and has emerged as an area of research focus for the development of more effective strategies against the cutaneous signs of aging<sup>[5]</sup>.

There are several molecular mechanisms underlying the aging process. Although still not completely undisclosed, such knowledge is very important clinically and is the focus of many current studies. The main extrinsic factor responsible is still photodamage, which causes the release of collagenases and neutrophil elastases that result in fragmentation of structural and tonic proteins. Additionally, the cellular responses of autophagy and Ubiquitin Proteasome systems are slowed. Current strategies of ECM remodeling are thus based on eliminating cellular debris and the stimulation of neocollagenesis, elastogenesis and glycosaminoglycans production through the use of various peptides and other active agents<sup>[6-8]</sup>. As far as we know, the main mechanism involved in preventing ECM changes is to keep free radical (i.e., ROS, reactive oxygen species) production under control. ROS are characterized by the presence of an unpaired electron, which is responsible for their instability and reactivity towards chemical structures to which they bind to capture another electron. In turn, this process generates unstable molecules and promotes chain reactions that ultimately, lead to functional and structural damage both within cells and extracellularly. ROS are produced in cellular structures such as membranes, lysosomes, and mitochondria. Their formation can be exacerbated by external agents including ultraviolet (UV) light, tobacco, infections and chemicals. The human body protects itself from ROS by utilizing antioxidant enzymes (SOD), such as catalases and glutathione peroxidases, which neutralize radicals and produce less harmful substances. However, as one's body declines with age, levels of these antioxidant enzymes also decrease correspondingly<sup>[9]</sup>.

Alteration of matrix metalloproteinase (MMP) levels can lead to dysregulation of skin homeostasis<sup>[10]</sup>. In the elderly, the levels of zinc-dependent proteases increase, causing degradation of ECM protein components. Physiologically, MMPs ensure protein turnover by stimulating the renewal of elastic and collagen fibers. MMP levels are regulated by tissue inhibitors, the expression of which can be disturbed by UV light, smoke and ROS. Protein-degrading MMPs in contrast, render connective tissue less elastic. Another critical point to be considered is protein glycation, namely the reaction of glucose and other sugars such as fructose. This post translational protein modification is associated with increased levels of glucose, affects protein structure and function through increased cross-linking, and results in the formation of "Amadori" and advanced glycation end products. Glycated proteins increase the formation of free radicals and release toxic products and pro-inflammatory components that cause protein damage, which ultimately is the fundamental cause of aging<sup>[11,12]</sup>. In women, menopause is associated with reduced ovarian endocrine activity leading to an increase in follicular stimulating hormone and decrease in estrogen and progesterone, with downstream metabolic disturbances. Alterations start from sexual organs and involve all tissues targeted by estrogen, in a multisystem framework that affects all connective tissues. Collagen atrophy is the main factor associated with menopause-related skin aging, which contributes to reduced skin elasticity<sup>[13]</sup>. In menopause, skin thickness and collagen content are initially reduced by 1.13% and 2.1% per year respectively; type I and III collagen decrease by 30%. The skin is not only a target for sex-hormones but also produces and releases estrogen from the enzymatic conversion of estrogen precursors. Estrogen exerts a number of functions on connective tissue such as counteracting the degradation of collagen by MMPs induced by UV light and ROS through the activation of the TGF- $\beta$ 1 pathway. Skin and bone thickness

**Table 1. Extracellular matrix components can be divided into 3 main groups**

Structural proteins	Adhesion proteins	Glycosaminoglycans	Proteoglycans
Collagen Elastin	Fibronectin Fibrillin Laminin Tenascin Vitronectine Osteonectine	Ialuronan Heparan-sulphate Chondroitin-sulphate	Biglycan Aggrecan Versican Neurocan



**Figure 1.** Interactions extracellular matrix cell

are also positively correlated with estrogen levels. Post-menopausal women with less estrogen, show a decreased expression of TGF- $\beta$ 1 in skin fibroblasts, which may hamper the ability of fibroblasts to produce collagen, elastin and proteoglycans.

### THE MATRISOME: SIGNIFICANCE AND FUNCTION

In recent years, both antiaging and medical research have focused on ECM biochemistry and pathophysiology as well as matrix dysregulation which can lead to disease progression<sup>[14,15]</sup>. The ECM fills the intercellular space and is present in all connective tissues [Table 1]. Dermis and adipose, muscle, bone, cartilage, and the surrounding parenchyma of organs, are all connective tissue, which highlights the relevance of the ECM in maintaining tissue homeostasis. Within the intercellular space, phenomena like cellular polarization and migration, regulation of growth factors, activation of signaling molecules, and processes translating mechanical stimulation into a chemical signal through the involvement of mechanosensitive channels are all essential for the maintenance of ECM elasticity and physiological tissue stiffness [Figure 1]. Mechano-transduction in particular, is an increasingly well-studied process. When the fibroblast is located in a stiff microenvironment, it will be stimulated continuously to produce new ECM. In addition, basement membranes play a fundamental role in maintaining tissue homeostasis and the transmission of mechano-transduction signals to the underlying dermis.

The molecular structure of the ECM is responsible for the above essential functions. The ECM is also comprised of a complex network of proteins defined as the “matrisome” [Table 1]. The matrisome is now

a matter of intense study, not only for the identification of all component proteins but also, for defining the molecular mechanisms regulated by the ECM<sup>[16]</sup>. To date, about 300 matrisome proteins have been identified including: 43 types of collagen subunits in fibrillar and non-fibrillar form (the most common is type 1 representing 80%-90%); 36 types of proteoglycans dispersed amongst collagen fibers to take up the interstitial space and maintain hydration; growth factors linked to glycosaminoglycans that are retained in the extracellular space to increase functionality; about 200 soluble proteins, which not only promote assembly of the ECM but are also involved in cell-ECM interactions, and act as ligands with receptors (integrins); and enzymes such as lysyl-oxidase, transglutaminase and hydroxylase<sup>[17]</sup>. To simplify, the matrisome is composed of 3 main molecular groups: structural proteins, adhesion proteins and proteoglycans. Among structural proteins, collagen is most abundant. Its peculiar amino acid sequence provides essential functions<sup>[18]</sup>. Aging reduces collagen synthesis and alters its structure (collagen fibers become fragmented and stiff). Amino acids are fundamental to the structure and thus, function of collagen - for example, the interaction of specific amino acids with potassium ions favors non-covalent binding and binding to proteoglycans. There is less elastin overall but it is still essential for maintaining the elastic properties of skin. Elastin fibers stretch under traction but with mechanical stress, can be restored to their original conformation. This process is due to the presence of hydrophobic amino acids such as valine and alanine, both of which are exposed to the hydrophilic components of ECM during mechanical stress. When the mechanical force is removed, elastin folds back on itself and its fibers become shortened. It goes without saying that the optimal functionality of elastin would also be dependent on adequate tissue hydration<sup>[19]</sup>.

Therefore, the aging process would undoubtedly influence the ECM and matrisome as a whole. All 300 matrisome proteins are affected and their turnover is related to their individual half-lives. To illustrate, the half-life of collagen is about 3 years and that of elastin is 70, which means that post-translational modifications like glycation have an essential role in skin elasticity and structure. The increase in MMPs is also part of aging and correspondingly, structural proteins such as collagen and elastin lose function and contribute towards a loss of skin tone. The imbalance of peptide content has to be taken into account as well, in terms of cellular cross-talk alterations.

## TREATMENT OF ECM ALTERATION IN SKIN AGING

Given the above discussion, the most advanced antiaging treatments would therefore be those that are able to rebuild the ECM, which is responsible for both physical support and the exchange of nutrients, cellular mediators, and growth factors<sup>[20,21]</sup>. The most effective treatment would target cell metabolism, autophagy, cell renewal and the production of new ECM. Such results can theoretically be obtained by providing the right metabolic and structural support to cells (i.e., hyaluronic acid, specific amino-acids and peptides, antioxidants) as well as mechanical stimulation which would, in turn, stimulate mechano-transduction (that is the conversion of mechanical forces into biochemical signals) through the creation of a scaffold in reduced volumes to enable stimulation of interstitial fluid circulation.

Low and/or high molecular weight, natural (not cross-linked) hyaluronic acid have been reported to be capable of hydrating the ECM, thereby stimulating cellular activity when administered as injectable formulas. Low molecular weight hyaluronic acid has the ability to stimulate cells thereby preventing aging, increasing skin brightness, hydration, tone, reducing wrinkles and restoring volume. On the other hand, high molecular weight forms exert powerful antioxidant activity by neutralizing free radicals and becoming a scaffold for the skin. Hyaluronic acid however, must be continuously produced as they have particularly short half-lives and are degraded by specific enzymes, hyaluronidases. To increase the consistency and longevity of injected hyaluronic acid in the dermis, it is necessary to render it less susceptible to hyaluronidases by chemical modification with cross-linking agents to bridge its filaments. The such chemically-modified hyaluronic acid has a greater filling effect but its biological properties are drastically reduced in favor of the filling capacity (filler) making fillers a different issue (they consist of cross-linked



hyaluronic acid, made denser and chemically modified by crosslinking agents) which actually fills the tissue with immediate results compared to natural hyaluronic acid, but with lower biological activity<sup>[22,23]</sup>.

It should be highlighted that the use of dermal fillers does not exclude treatment with hyaluronic acid and vice versa. Often, a combination of treatment strategies allows one to easily achieve the desired results<sup>[24]</sup>. Fibrous proteins of the dermis, such as collagen and elastin, are characterized by a specific aminoacid composition. With age, the ability of fibroblasts to synthesize collagen is reduced. Furthermore, alteration of collagen fibers imparts increasing stiffness and decreased elasticity to skin, with a consequent loss of tone and softness. An anti-aging treatment should therefore, always have, among others, the ability to increase the quantity and quality of collagen fibers. This can be achieved through specific cosmetic treatments capable of stimulating the production of collagen (peptides, retinol, vitamin C), food supplements containing specific amino acids or compounds capable of stimulating fibroblasts activity (i.e., collagen, amino acids and specific peptides), and even bio-revitalizing injections. In fact, in the absence of adequate amounts of amino acids, cells react by blocking catabolism. As a result, proteins age and become less efficient. "Old" proteins, whether enzymes, structural or contractile, become increasingly fragile<sup>[25]</sup>.

## CONCLUSION

Various factors associated with an individual's genetic background and physical environment can affect one's skin in different ways with regards to skin aging. Previously, skin aging was thought of as physiological or chrono-aging, and environmental or photo-aging. Other external factor have now been recognized to influence the skin aging process including gravity, environmental pollution, climate, and smoking. In order to provide a personalized treatment plan, a thorough analysis of an individual's aging processes will be required. For example, the shape of the face is of utmost importance - overtime, round faces tend to sag while thin and elongated faces have a tendency to become hollow. In the former, lifting would be required while in the latter, volumizing would be necessary. Treatment choice should be based on demonstrated safety and efficacy while injection technique is very important for delivery and stems from an in-depth knowledge of the anatomy and physiology of the treatment sites<sup>[26]</sup>. To illustrate, by specifically defining injection points or by combining injections with facial lymph node drainage, results will be immediately visible and continue to improve over the ensuing weeks and months<sup>[27]</sup>. Treatment protocols (number of sessions, frequency, maintenance, mix of introduced substances, combination with cosmetics, and nutraceuticals even) have to be individualized in order to suit the patient's needs. Aesthetic dermatology is perhaps the first medical discipline dedicated towards studying the properties of ECM and its treatment but has increasingly, gained the attention of other clinical fields<sup>[28]</sup> such as orthopedics, dentistry, pulmonology and gastroenterology. We believe that in the near future, the ECM will be recognized as a master element of many organs and body systems, overcome the barriers and concepts of current specialty fields, and improve our mechanistic understanding of the many internal and external dysfunctions and disease states for better treatment.

## DECLARATIONS

### Authors' contributions

The author contributed solely to the article.

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

The author declared that there are no conflicts of interest.

**Ethical approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Copyright**

© The Author(s) 2020.

**REFERENCES**

1. Tobin DJ. Introduction to skin aging. *J Tissue Viability* 2017;26:37-46.
2. Sparavigna A. How does face age? A retrospective observational study and meta-analysis. *J Plast Pathol Dermatol* 2019;15:1-8.
3. Michaud T, Gassia V, Belhaouari L. Facial dynamics and emotional expressions in facial aging treatments. *J Cosmet Dermatol* 2015;14:9-21.
4. Glogau RG. Aesthetic and anatomic analysis of the aging skin. *Semin Cutan Med Surg* 1996;15:134-8.
5. Hynes RO. The extracellular matrix: not just pretty fibrils. *Science* 2009;326:1216-9.
6. Widgerow AD, Fabi SG, Palestine RF, Rivkin A, Ortiz A, et al. Extracellular matrix modulation: optimizing skin care and rejuvenation procedures. *J Drugs Dermatol* 2016;15:s63-71.
7. Qa'aty N, Vincent M, Wang Y, Wang A, Mitts TF, et al. Synthetic ligands of the elastin receptor induce elastogenesis in human dermal fibroblasts via activation of their IGF-1 receptors. *J Dermatol Sci* 2015;80:175-85.
8. Widgerow AD, Jiang LI, Calame A. A single-center clinical trial to evaluate the efficacy of a tripeptide/hexapeptide antiaging regimen. *J Cosmet Dermatol* 2019;18:176-82.
9. Naylor EC, Watson REB, Sherratt MJ. Molecular aspects of skin ageing. *Maturitas* 2011;69:249-56.
10. Panwar P, Butler GS, Jamroz A, Azizi A, Overall CM, et al. Aging-associated modifications of collagen affect its degradation by matrix metalloproteinases. *Matrix Biol* 2018;65:30-44.
11. Langton AK, Sherratt MJ, Griffiths CEM, Watson REB. Review article: a new wrinkle on old skin: the role of elastic fibers in skin ageing. *Int J Cosmet Sci* 2010;32:330-9.
12. Zouboulis CC, Elewa R, Ottaviani M, Fluhr J, Picardo M, et al. Age influences the skin reaction pattern to mechanical stress and its repair level through skin care products. *Mech Ageing Dev* 2018;170:98-105.
13. Raine-Fenning NJ, Brincat MP, Muscat-Baron Y. Skin aging and menopause: implications for treatment. *Am J Clin Dermatol* 2003;4:371-8.
14. Theocharis AD, Skandalis SS, Gialeli C, Karamanos NK. Extracellular matrix structure. *Adv Drug Deliv Rev* 2016;97:4-27.
15. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol* 2014;15:786-801.
16. Hynes RO, Naba A. Overview of the matrisome - an inventory of extracellular matrix constituents and functions. *Cold Spring Harb Perspect Biol* 2012;4:a004903.
17. Raghunathan R, Sethi MK, Klein JA, Zaia J. Proteomics, glycomics, and glycoproteomics of matrisome molecules. *Mol Cell Proteomics* 2019;18:2138-48.
18. Shoulders MD, Raines RT. Collagen structure and stability. *Annu Rev Biochem* 2009;78:929-58.
19. Weihermann AC, Lorencini M, Brohem CA, de Carvalho CM. Elastin structure and its involvement in skin photoageing. *Int J Cosmet Sci* 2017;39:241-7.
20. Krutmann J, Boulou A, Sore G, Bernard BA, Passeron T. The skin aging exposome. *J Dermatol Sci* 2017;85:152-61.
21. Boulter E, Estrach S, Errante A, Pons C, Cailleteau L, et al. CD98hc (SLC3A2) regulation of skin homeostasis wanes with age. *J Exp Med* 2013;210:173-90.
22. Sparavigna A, Tenconi B, Giori AM, Bellia G, La Penna L. Evaluation of the efficacy of a new hyaluronic acid gel on dynamic and static wrinkles in volunteers with moderate aging/photoaging. *Clin Cosmet Investig Dermatol* 2019;12:81-90.
23. Sparavigna A, Orlandini A. Efficacy and tolerance of an injectable medical device containing hyaluronic acid and amino acids: a monocentric six-month open label evaluation. *J Clin Trials* 2017;7:4-12.
24. Sparavigna A, Tenconi B. Efficacy and tolerance of an injectable medical device containing stable hybrid cooperative complexes of high- and low-molecular-weight hyaluronic acid: a monocentric 16 weeks open-label evaluation. *Clin Cosmet Investig Dermatol* 2016;9:297-305.
25. Limbert G, Masen MA, Pond D, Graham HK, Sherratt MJ, et al. Biotribology of the ageing skin - why we should care. *Biotribology* 2019;17:75-90.
26. Hu S, Li Z, Cores J, Huang K, Su T, et al. Needle-free injection of exosomes derived from human dermal fibroblast spheroids ameliorates skin photoaging. *ACS Nano* 2019;13:11273-82.
27. Sparavigna A. The interstitial fluid technique and the extracellular matrix. *Prime Journal, Case Study, Injectables, Dermatology*. Available from <https://www.prime-journal.com/the-interstitial-fluid-technique-and-the-extracellular-matrix/> [Last accessed on 19 Mar 2020]
28. Bhattacharjee O, Ayyangar U, Kurbet AS, Ashok D, Raghavan S. Unraveling the ECM-immune cell crosstalk in skin diseases. *Front Cell Dev Biol* 2019;7:68.

Review

Open Access



# Breast cancer-related lymphedema: focus on surgical treatment

Maria Luisa Nardulli

Department of Plastic Surgery and Burns, Breast Reconstruction Unit, Perrino Hospital, Brindisi 72100, Italy.

**Correspondence to:** Dr. Maria Luisa Nardulli, Department of Plastic Surgery and Burns, Breast Reconstruction Unit, Perrino Hospital, Brindisi 72100, Italy. E-mail: marialuisanardulli@gmail.com

**How to cite this article:** Nardulli ML. Breast cancer-related lymphedema: focus on surgical treatment. *Plast Aesthet Res* 2020;7:15. <http://dx.doi.org/10.20517/2347-9264.2019.56>

**Received:** 20 Nov 2019 **First Decision:** 11 Feb 2020 **Revised:** 5 Mar 2020 **Accepted:** 10 Mar 2020 **Published:** 31 Mar 2020

**Science Editor:** Xiao Long **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

Breast cancer-related lymphedema (BCRL) can affect breast cancer patients, especially after axillary surgery and radiation treatment, for life. First line treatment is conservative and involves physical therapy and compression. It requires absolute, life-long compliance with treatment by the patient and, in some cases, it is ineffective. In recent years, surgery has emerged as a possible alternative or even, complementary therapy for BCRL. The most commonly reported techniques are reconstructive or debulking procedures. Reconstructive procedures are aimed at restoring the lymphatic pathways and can be effective early in the disease process, when increased arm volumes are mostly due to the accumulation of protein-rich fluid in the interstitial space. In more advanced stages, where fibrotic and hypertrophic adipose tissues are dominant, debulking techniques such as liposuction can be recommended. A standard of care for the treatment of BCRL has not been established. Currently, different techniques can be combined to optimize clinical outcomes, and the surgical approach must be individualized for each patient, based on sound clinical and imaging assessment. BCRL surgical treatment remains a challenging topic that requires further study before it can be standardized.

**Keywords:** Breast cancer-related lymphedema, vascularized lymph node transfer, lymphaticovenous anastomosis, liposuction, fat grafting

## INTRODUCTION

Breast cancer-related lymphedema (BCRL) is a well-known, potential sequela of breast cancer treatment, which can result in damage to and impairment of the lymphatic drainage system of the upper limb.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Consequently, protein-rich fluid accumulates in the interstitial space, leading to abnormal swelling and volume increase of the arm<sup>[1,2]</sup>. In more advanced stages, lymphedema is characterized by adipose deposition and irreversible fibrosis, together with residual lymphatic vessel disruption<sup>[3]</sup>. Despite reported incidence of BCRL being variable, DiSipio *et al.*<sup>[4]</sup> referred the overall estimated incidence of upper limb lymphedema after breast cancer to be 21.4%.

Symptoms of BCRL include arm heaviness, pain, impaired mobility, and recurrent skin and subcutaneous tissue infections. BCRL also affects the patient's body image with consequent psychological impairment<sup>[1,2,5]</sup>. For this reason, it is one of the most disabling sequelae of breast cancer treatment.

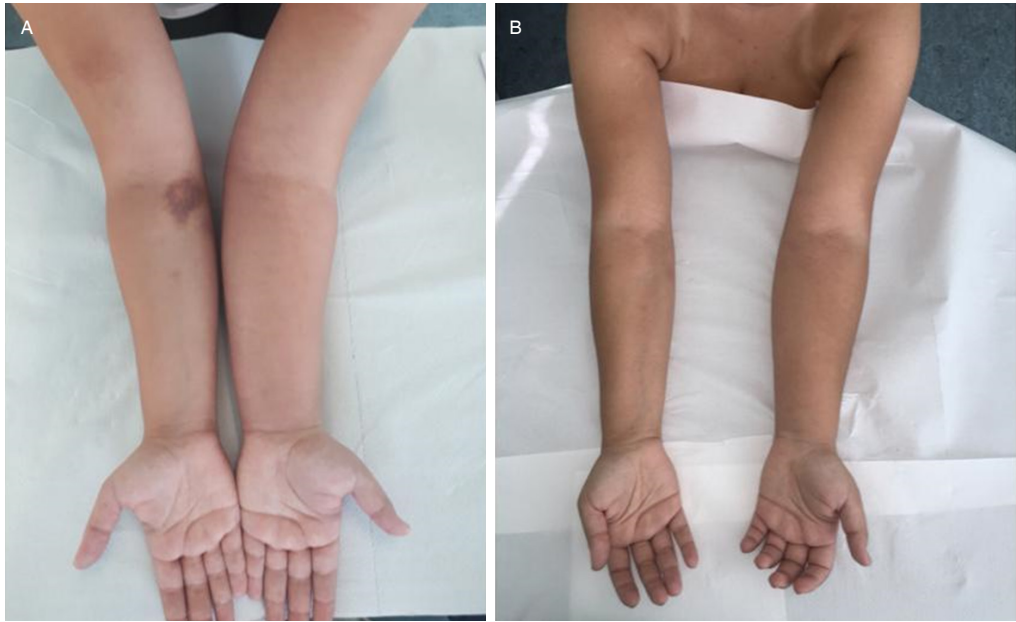
Risk factors for BCRL can be non-treatment or treatment related. Non-treatment related risk factors include BMI > 30 kg/m<sup>2</sup><sup>[6,7]</sup>, cellulitis<sup>[8]</sup> and genetic predisposition<sup>[9,10]</sup>. In fact, several studies have suggested that an underlying anomaly and/or dysregulation of the lymphatic system can lead to subclinical lymphatic dysfunction. In turn, this would increase the risk of developing lymphedema after disruption of the lymphatic network from surgery and/or radiation treatment. Treatment-related risk factors include axillary surgery, mastectomy and lack of breast reconstruction, regional lymph node radiation, and chemotherapy<sup>[1]</sup>. One of the main treatment-related risk factors for BCRL is axillary lymph node dissection (ALND)<sup>[1]</sup> as it involves axillary clearance and thus, iatrogenic lymphatic damage. In a meta-analysis, DiSipio *et al.*<sup>[4]</sup> reported the incidence of BCRL in patients with unilateral breast cancer at 19.9% after ALND. On the contrary, sentinel lymph node biopsy, an alternative procedure to ALND in clinically node negative breast cancer patients, is reported to be associated with a four times lower incidence of BCRL compared to ALND<sup>[4,11]</sup>. This is likely because sentinel lymph node biopsy is less invasive. On the other hand, several studies have shown an increasing incidence of BCRL with the number of axillary nodes removed<sup>[12,13]</sup>.

Interestingly, mastectomy itself has been reported to be a risk factor for BCRL<sup>[1,4,12]</sup>. Regarding breast reconstruction, some studies suggest that delayed autologous breast reconstruction can improve existing lymphedema<sup>[14]</sup>. Card *et al.*<sup>[11]</sup> concluded in a 6-year study that patients undergoing breast reconstruction after mastectomy had a lower risk of, and later onset of BCRL compared to patients who had undergone mastectomy alone. Card *et al.*<sup>[11]</sup> also found no difference in the risk of BCRL between the different types of breast reconstruction (tissue expander/implant, latissimus dorsi and implant, free autologous abdominal tissue only). The authors thus hypothesized that the transfer of vascularized tissue onto the chest reduces scarring, bridges damaged lymphatic vessels and promotes angiogenesis<sup>[11,14]</sup>. With regard to expander/implant based breast reconstruction, tissue expansion and capsule formation seem to increase the expression of vascular endothelial growth factor (VEGF), which has an important role in angiogenesis and lymphangiogenesis<sup>[10]</sup>, via chronic ischemia<sup>[15]</sup>.

Regional lymph node radiation (supraclavicular, with or without posterior axillary boost) is an independent risk factor for BCRL. It also conveys an increased risk of developing BCRL compared to breast/chest radiotherapy alone<sup>[16]</sup>.

In terms of the role of adjuvant and neoadjuvant chemotherapy in increasing BCRL risk, a literature review by Gillespie *et al.*<sup>[1]</sup> [Figure 1] suggested that the current evidence was not conclusive because several studies have chemotherapy as a potential risk factor for BCRL but data from others were non-confirmatory.

Several staging systems have been proposed for lymphedema. One of the most widely used is that of the International Society of Lymphology (ISL), as shown in Table 1<sup>[17]</sup>. Early or mild stages can be characterized by a positive "pitting" test, when pressure exerted to an edematous limb by the thumb for at least 60 seconds induces a depression or indentation on the skin. Such indentation is attributable to the displacement of interstitial fluid. In more advanced stages, when adipose hypertrophy is dominant in the affected limb, edema is typically "non-pitting"<sup>[18]</sup>.



**Figure 1.** A: A patient who developed left upper limb lymphedema during neoadjuvant therapy with Paclitaxel for breast cancer; B: After undergoing left nipple sparing mastectomy, sentinel lymph node biopsy and immediate breast reconstruction with a prepectoral implant, the lymphedema improved spontaneously, especially at the level of the forearm

**Table 1. Staging system for lymphedema**

Stage 0 (or Ia)	which refers to a latent or sub-clinical condition where swelling is not yet evident despite impaired lymph transport, subtle changes in tissue fluid/composition, and changes in subjective symptoms. It may exist months or years before overt edema occurs (Stages I-III)
Stage I	represents an early accumulation of fluid relatively high in protein content (e.g., in comparison with “venous” edema) which subsides with limb elevation. Pitting may occur. An increase in various proliferating cells may also be seen
Stage II	signifies that limb elevation alone rarely reduces tissue swelling and pitting is manifest. Late in Stage II, the limb may not pit as excess subcutaneous fat and fibrosis supervenes
Stage III	encompasses lymphostatic elephantiasis where pitting can be absent and trophic skin changes such as acanthosis, alterations in skin character and thickness, further deposition of fat and fibrosis, and warty overgrowths have developed

This table summarizes the lymphedema staging system according to ISL<sup>[17]</sup>, from the 2016 Consensus Document of the ISL<sup>[17]</sup> (adapted). These Stages only refer to the physical condition of the extremities. ISL: International Society of Lymphology

The first-line treatment for BCRL is a set of nonsurgical and conservative measures known as Complex Decongestive Therapy (CDT). CDT includes lymphatic manual drainage, bandaging, skin care, exercise, patient education and is coupled with the use of compression garments. The aim of CDT is to reduce limb volume first, followed by maintenance of the results achieved. Normally, patients suffering from lymphedema require lifelong adherence to such therapeutic measures<sup>[1,19,20]</sup>. Consequently, non-compliance to conservative therapy is frequent. Nevertheless, in recent years, interest in lymphedema surgery has increased all over the world and there have been many advances in both surgical techniques and imaging modalities. Therefore, surgery can be offered as a complementary or alternative therapeutic strategy for lymphedema, when conservative measures are inadequate<sup>[2,21]</sup>.

Below, we present an overview of the main surgical techniques currently adopted for the treatment of BCRL.

### SURGICAL TREATMENT FOR BCRL

Surgical techniques for BCRL can be classified as physiological or reconstructive, and ablative or debulking procedures<sup>[2,20]</sup>. Physiological or reconstructive techniques aim to restore lymphatic pathways in the axilla



and upper limb to drain excess fluid accumulating in the arm by creating bypasses of lymph flow, or are based on the concept of inducing lymphangiogenesis<sup>[21]</sup>. Such techniques can be utilized in the early stages of BCRL, when a residual and functional lymphatic system can still be identified. “Pitting” edema is often present because excess limb volume is mainly caused by the accumulation of interstitial fluid. When edema progresses, the affected limb is characterized by increased tissue fibrosis, hypertrophy of adipose tissue, and irreversible damage and obliteration of the lymphatic vessels, thereby becoming “non-pitting”. In such cases, physiological or reconstructive techniques are widely considered futile because the upper limb lymphatics are fibrotic and damaged. Moreover, the excess in limb volume is also attributed to fibrosis and fat hypertrophy instead. In these advanced, “non-pitting” stages, ablative or debulking procedures can reduce the excess volume of skin and/or subcutaneous tissue. In turn, this facilitates hygiene and improves limb functionality<sup>[2,19-21]</sup>.

### **Ablative or debulking surgical techniques**

Ablative techniques aim to remove excess limb bulk to reduce lymphedema-associated morbidity. The Charles<sup>[22]</sup> procedure was one of the earliest ablative techniques described. It involves the excision of excess skin and subcutaneous tissue up to the deep fascia, followed by skin grafting. However, this technique leads to extensive scarring, poor cosmetic results and the disruption of residual lymphatic vessels in the treated area. Therefore, it may even result in exacerbation of lymphedema. For these reasons, the Charles procedure is currently reserved for very advanced cases of lymphedema that are not susceptible to improvement through other measures<sup>[2,21]</sup>.

Excess fat and fibrotic tissue in the lymphadenomatous upper limb can now be removed through suction-assisted lipectomy. The technique was first applied to lymphedema treatment by O'Brien *et al.*<sup>[23]</sup>. Brorson *et al.*<sup>[24,25]</sup> then popularized a technique consisting of large volume lipoaspiration in a limb affected by lymphedema<sup>[24-26]</sup>. These procedures are typically indicated in advanced, “non-pitting” chronic edema that is non-responsive to conservative measures as explained above. Recently, Hoffner *et al.*<sup>[26]</sup> reported the 5-year results after liposuction and postoperative controlled compression therapy (CCT) in a series of 105 patients suffering from “non-pitting” or minimal “pitting” BCRL. The study protocol consists of the reduction of excess arm volume through liposuction or power-assisted liposuction, from the wrist to the shoulder, while the hand is spared. Volume reduction was performed according to previously assessed, contralateral limb volume measurements. In more recent cases, the authors used a tourniquet in addition to tumescence with adrenaline and lidocaine to reduce blood loss during surgery. A sterilized custom-made compression garment is also put on the arm in the operating theatre, as soon as surgery progresses, to reduce both blood loss and postoperative edema. With this approach, the authors obtained complete reduction of excess volume within 3-6 months and sometimes earlier. As expected, a key point of this approach is CCT, based on the constant use of made-to-measure compressive garments after surgery and indefinitely thence on. Despite the favorable long-term results achieved, CCT remains the main limitation of the treatment and requires absolute patient compliance. On the other hand, such liposuction-based techniques allow arm volume reduction and skin retraction without the need for skin excision or recurrence<sup>[26]</sup>, thereby improving patients' quality of life<sup>[25]</sup>. A reduced incidence of infections after liposuction has also been reported and is linked to the improvement of skin blood flow after the reduction of excess arm volume<sup>[25]</sup>. Moreover, if liposuction does not restore the lymphatic pathways in the affected limb, further impairment of lymphatic transport capacity after liposuction has not been proven<sup>[24-26]</sup>.

### **Reconstructive or physiological surgical techniques**

#### *Vascularized lymph node transfer*

In vascularized lymph node transfer (VLNT), the lymph nodes (LNs) are harvested as a vascularized free flap with a vascular pedicle. The flap contains donor LNs embedded within the surrounding fat, with or without a skin paddle<sup>[2,27]</sup>. The rationale for VLNT is based on the concept that axillary LNs, if surgically

removed or damaged by radiotherapy for breast cancer treatment, can be replaced by autologous healthy LNs harvested from an untreated donor site<sup>[27]</sup>. VLNT should therefore improve BCRL because new lymphatic connections are expected to form between new afferent and efferent lymphatics sprouting from the transferred LNs and residual lymphatics at the recipient site. VEGF-C secreted by transplanted LNs also seems to have an important role in this mechanism<sup>[28]</sup>. Additionally, VLNTs are believed to absorb lymph like a sponge, before redirecting it into the vascular network like a pump<sup>[19,29,30]</sup>.

This technique is especially appealing to patients who have undergone ALND (with or without radiation treatment) and with scarring in the axilla<sup>[21]</sup>. As donor LNs replace axillary LNs after ALND and/or radiotherapy, the most common recipient site is obviously the axilla<sup>[2,27]</sup>. Insetting of the flap in the axilla should be preceded by surgical release of scars as the scar itself may impede lymphatic flow<sup>[27]</sup>. Scar release would also provide a healthy environment for lymphangiogenesis. Other VLNT recipient sites have also been described, such as the wrist and elbow<sup>[30,31]</sup>, where distal inseting in the upper limb can take advantage of gravity for the flap to absorb excess fluid.

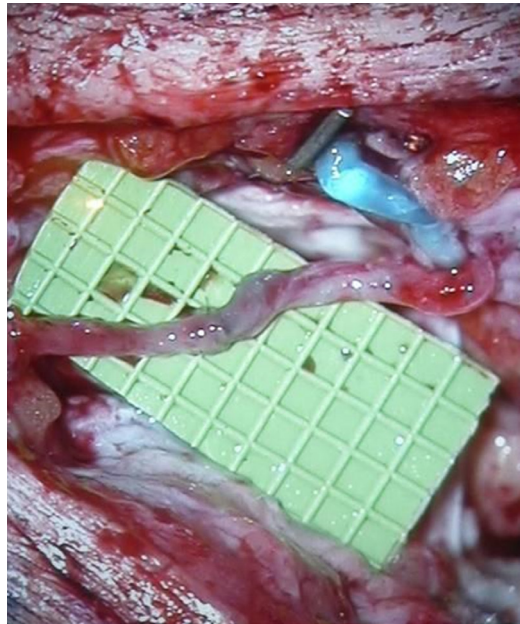
Donor LNs can be harvested from different sites with the groin being the most popular<sup>[2,27]</sup>. Other donor sites include the supraclavicular area, submental area, lateral thoracic area and intra-abdominal LNs<sup>[19]</sup>. In the groin, the LN-flap is supplied by the superficial circumflex iliac artery or superficial inferior epigastric artery. Scaglioni and Suami<sup>[32]</sup> found that in cadavers, the superficial inguinal LNs can be divided into 3 subgroups: the abdominal, medial thigh and lateral thigh groups. The abdominal group is utilized in VLNT. The LNs to be taken are located above the inguinal ligament, and superficial and lateral to the common femoral vessels. The harvesting of LNs below the inguinal ligament, medial to the femoral vessels or deep to the fascia of the thigh must be avoided because these LNs drain the lower limb and iatrogenic lower limb lymphedema may result<sup>[2,33]</sup>. Data now show that LN-flap harvesting is not a complication-free procedure and VLNT from the groin can lead to lower limb lymphedema<sup>[33]</sup>.

As such, reverse lymphatic mapping techniques are gaining popularity with VLNT procedures. These imaging techniques aim to identify the LNs that drain lymph from the donor limb, to avoid inadvertent harvest during VLNT. Indocyanine green (ICG)-lymphography has been shown to be useful for this purpose. ICG is injected intradermally into the foot web spaces, absorbed by lymphatic vessels and transported proximally to the LNs draining the lower limb. Lymphatic vessels and nodes can be visualized in real-time with a camera that captures the near-infrared fluorescence emitted from ICG so that the draining LNs can be spared<sup>[2]</sup>. Similarly, patent blue dye V can be injected intradermally at the level of the anterior superior iliac spine with the aim of identifying LNs draining the lower abdomen<sup>[2]</sup>.

#### *Lymphatico-venous anastomosis*

The lymphatico-venous anastomosis (LVA) [Figure 2] technique bypasses the lymphatic obstruction, creating a shunt between the lymphatic and blood circulation in the affected limb. The first LVA was performed several decades ago<sup>[34,35]</sup>, but the currently accepted technique was developed after the advent of supermicrosurgery<sup>[36]</sup>.

First, a small quantity of patent V blue dye is injected intradermally, a few centimeters distal to where the surgical incision will be performed. The dye is absorbed by lymphatic vessels which then turn blue and become easier to identify. Multiple anastomoses between superficial subcutaneous lymphatic vessels and venules are then performed, either end-to-end or end-to-side, in the affected upper limb. Lymph is thus diverted into the venous circulation<sup>[2]</sup>. This technique is widely accepted for the treatment of early BCRL, when lymphatic vessels with residual functionality can still be demonstrated, as lymph has to be pumped into venules through the LVA. ICG-lymphography and magnetic resonance lymphography can thus be useful for preoperative assessment for the presence of, and location of contractile lymphatics suitable for LVA<sup>[2]</sup>.



**Figure 2.** Magnified, intra-operative view of an lymphatico-venous anastomosis

### *Lymphaticolymphatic bypass*

This procedure has been popularized by Baumeister *et al.*<sup>[37,38]</sup>. Healthy lymphatic vessels are harvested from the lower extremity, used as a graft, and are inset subcutaneously under the skin of the anterior shoulder, between the upper arm and the neck. The ends of the grafted lymphatic vessels are anastomosed to recipient lymphatic vessels in the arm and supraclavicular region. Despite lymphoscintigraphy demonstrating the patency of lymphatic vessels in the graft and a significant reduction of arm volume, this technique leads to a long scar at the donor site and the risk of lymphedema in the donor lower limb cannot be excluded<sup>[1,20]</sup>. In turn, Campisi *et al.*<sup>[39]</sup> described the use of an autologous vein graft in a similar manner to bypass the lymphatic obstruction, thereby sparing the lymphatic vessels in the donor site.

### *Fat grafting*

Adipose-derived stem cells (ADSCs) are mesenchymal stem cells that can be collected through liposuction easily as fat tissue is an abundant and easily accessible source of ADSCs<sup>[40,41]</sup>. Recently, animal studies have demonstrated that the administration of ADSCs can increase the number of lymphatic vessels and improve secondary lymphedema<sup>[42]</sup>. The capacity of ADSCs to induce lymphangiogenesis seems to be mediated by VEGF-C and the release of other lymphangiogenic factors. Saijo *et al.*<sup>[40]</sup> studied the paracrine effects of ADSCs in promoting lymphangiogenesis in irradiated lymphatic endothelial cells *in vitro*. The results obtained suggest that ADSCs could have a role in the treatment of secondary-post irradiation limb lymphedema. Yet, few papers have described fat grafting in the axilla to improve BCRL clinically<sup>[41,43-45]</sup>. Maruccia *et al.*<sup>[45]</sup> retrospectively compared the efficacy of upper limb circumference reduction and the improvement in patients' quality of life between VLNT alone and VLNT plus scar revision through fat grafting with better outcomes reported in the latter. Toyserkani *et al.*<sup>[43]</sup> however, only reported a modest decrease in excess arm volume, that was not significant, after similar scar revision by means of fat grafting and ADSC injection in the axilla. Better results though were achieved in ISL stage I than in ISL stage II BCRL. The authors also observed an improvement in lymphedema symptoms and the decreased need for conservative treatment for the majority of patients. Recently, Toyserkani *et al.*<sup>[44]</sup> reported similar results after a 1-year period of follow-up. Quantitative lymphoscintigraphy was used to evaluate upper limb lymph drainage after ADSC injection in the axilla but no significant improvement was observed.



**Figure 3.** A: a patient with right breast cancer-related lymphedema, following axillary lymph node dissection and regional lymph node radiation. The patient had undergone right breast reconstruction with a TRAM-flap; B: in the same patient, fat grafting was carried out in the right breast for contour refinement and volume augmentation, and in the axilla for the release of fibrosis and filling of the residual defect from axillary lymph node dissection. TRAM: transverse rectus abdominis myocutaneous

The author of the present paper reports her preliminary experience in treating patients with BCRL by releasing fibrotic tissue in the axilla followed by fat grafting to the area [Figure 3A and B]. Five patients have been treated over 6 months. All patients underwent breast reconstruction and fat grafting. In addition to fat grafting of the breast, a small quantity (15 to 30 mL) of fat was also transferred to the axilla, with the aim of releasing fibrotic tissue and filling up the dead space following axillary surgery. Fat harvest and injection were performed according to the standard Coleman technique<sup>[46,47]</sup>. Before fat injection, scar tissue in the axilla was released percutaneously<sup>[48]</sup>. Four patients had improvement in upper limb circumferences at 1, 3 and 6 months follow-up. Furthermore, they reported subjective improvement in terms of reduction of arm heaviness and improved suppleness of the affected limb. No infections developed during the follow-up period. One patient however, reported a worsening in arm circumference volume and increasing heaviness after surgery.

This report does not claim to demonstrate the efficacy of fat grafting for BCRL. This is due to the small sample of patients treated, the short follow-up and the absence of significant results. These results though, show that the procedure is simple and quick, and appears to be safe, which is in line with previous studies. As fat grafting is often used in breast reconstruction, a small quantity of fat can easily be injected into the axilla for scar release. *In vitro* studies and animal models show that ADSCs induce lymphangiogenesis and could have a role in lymphedema treatment but there are few clinical studies on the same available. While the role of ADSCs in improving clinical BCRL requires further investigation, it is well accepted that axillary scar release can improve BCRL. This concept has also been highlighted in the context of VLNT<sup>[2,27]</sup>. Scar release through fat grafting should therefore be able to improve BCRL.

#### *Combined techniques and tailored treatment*

Currently, there is no gold standard for BCRL treatment but different surgical techniques can be combined to offer the patient a tailored approach based on staging and a global preoperative assessment. This is dependent on clinical examination and imaging modalities (e.g., ICG-lymphography, MR-lymphography, lymphoscintigraphy). As described earlier, non-pitting edema and the absence of residual functioning lymphatic vessels would necessitate an ablative procedure. Pitting edema in the advanced stages must be



managed with CDT first. Early stage BCRL with pitting edema can benefit from reconstructive procedures. If functional lymphatic channels are available still, LVA can be considered. In patients with a scarred axilla, they may benefit from VLNT instead. Some authors combine VLNT and LVA with satisfactory results reported<sup>[2,21]</sup>. VLNT from the groin can also be performed simultaneously with deep inferior epigastric perforator (DIEP)-flap breast reconstruction<sup>[49,50]</sup> to address BCRL concurrently<sup>[2,49]</sup>.

Finally, laser-liposuction in combination with VLNT has been described for treating II stage (ISL staging) BCRL<sup>[51]</sup>. Nicoli *et al.*<sup>[51]</sup> described the use of laser liposuction in combination with VLNT to treat ten patients with stage II (ISL staging) BCRL who had failed a 6-month-period of conservative treatment. The two-stage procedure involved VLNT from the supraclavicular or groin area to the wrist, and liposuction at 1 to 3 months later. Laser-assisted liposuction was carried out after tumescent solution infiltration, exsanguination and tourniquet positioning, using a high-power diode pulsed laser with 1470-nm wavelength. The laser light, conveyed through the microcannula, achieved both lipolysis and skin retraction in the affected arm. A traditional liposuction cannula was then used to aspirate the liquefied fat. Post-operatively, patients had to wear compressive garments at all times for the first 2 to 4 weeks and thereafter, only at night. Improvements in limb circumferences, skin tonicity and lymphoscintigraphic features in the treated arm have been reported by the authors. Histological changes including the reorganization of adipose cells and collagen in the reticular dermis have also been demonstrated in biopsies done post-procedure.

## CONCLUSION

BCRL is a disabling sequela of breast cancer and associated treatments. A conservative approach (i.e., CDT) is the first line treatment for newly diagnosed BCRL. This treatment is insufficient in some cases, however, and these patients can benefit from surgical intervention depending on clinical and imaging assessment. To date, there remains no gold standard in the surgical treatment of BCRL. However, it is generally agreed that early stage BCRL can benefit from reconstructive procedures. Advanced stages with no or minimal pitting edema can be improved through liposuction. Each patient should therefore be assessed thoroughly before surgery and have a tailored treatment plan to maximize benefits. Newer strategies such as fat grafting and ADSC injection have shown promising preliminary results but must be investigated further. BCRL remains a highly challenging surgical problem.

## DECLARATIONS

### Authors' contributions

The author contributed solely to the article.

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

There are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

A written informed consent for using photos for scientific purposes was obtained by each patient.



**Copyright**

© The Author(s) 2020.

**REFERENCES**

- Gillespie TC, Sayegh HE, Brunelle CL, Daniell KM, Taghian AG. Breast cancer-related lymphedema: risk factors, precautionary measures, and treatments. *Gland Surg* 2018;7:379-403.
- Masia J, Pons G, Nardulli ML. Combined surgical treatment in breast cancer-related lymphedema. *J Reconstr Microsurg* 2016;32:16-27.
- Hespe GE, Nores GG, Huang JJ, Mehrara BJ. Pathophysiology of lymphedema-is there a chance for medication treatment? *J Surg Oncol* 2017;115:96-8.
- DiSipio T, Rye S, Newman B, Hayes S. Incidence of unilateral arm lymphoedema after breast cancer: a systematic review and meta-analysis. *Lancet Oncol* 2013;14:500-15.
- De Brucker B, Zeltzer A, Seidenstuecker K, Hendrickx B, Adriaenssens N, et al. Breast cancer-related lymphedema: quality of life after lymph node transfer. *Plast Reconstr Surg* 2016;137:1673-80.
- Jammallo LS, Miller CL, Singer M, Horick NK, Skolny MN, et al. Impact of body mass index and weight fluctuation on lymphedema risk in patients treated for breast cancer. *Breast Cancer Res Treat* 2013;142:59-67.
- Ridner SH, Dietrich MS, Stewart BR, Armer JM. Body mass index and breast cancer treatment-related lymphedema. *Support Care Cancer* 2011;19:853-7.
- Ferguson CM, Swaroop MN, Horick N, Skolny MN, Miller CL, et al. Impact of ipsilateral blood draws, injections, blood pressure measurements, and air travel on the risk of lymphedema for patients treated for breast cancer. *J Clin Oncol* 2016;34:691-8.
- Hespe GE, Ly CL, Kataru RP, Mehrara BJ. Baseline lymphatic dysfunction amplifies the negative effects of lymphatic injury. *Plast Reconstr Surg* 2019;143:77e.
- Newman B, Lose F, Kedda MA, Francois M, Ferguson K, et al. Possible genetic predisposition to lymphedema after breast cancer. *Lymphat Res Biol* 2012;10:2-13.
- Card A, Crosby MA, Liu J, Lindstrom WA, Lucci A, et al. Reduced incidence of breast cancer-related lymphedema following mastectomy and breast reconstruction versus mastectomy alone. *Plast Reconstr Surg* 2012;130:1169-78.
- Kilbreath SL, Refshauge KM, Beith JM, Ward LC, Ung OA, et al. Risk factors for lymphoedema in women with breast cancer: a large prospective cohort. *Breast* 2016;28:29-36.
- Kim M, Kim SW, Lee SU, Lee NK, Jung SY, et al. A model to estimate the risk of breast cancer-related lymphedema: combinations of treatment-related factors of the number of dissected axillary nodes, adjuvant chemotherapy, and radiation therapy. *Int J Radiat Oncol Biol Phys* 2013;86:498-503.
- Chang DW, Kim S. Breast reconstruction and lymphedema. *Plast Reconstr Surg* 2010;125:19-23.
- Lantieri LA, Martin-Garcia N, Wechsler J, Mitrofanoff M, Raulo Y, et al. Vascular endothelial growth factor expression in expanded tissue: a possible mechanism of angiogenesis in tissue expansion. *Plast Reconstr Surg* 1998;101:392-8.
- Warren LE, Miller CL, Horick N, Skolny MN, Jammallo LS, et al. The impact of radiation therapy on the risk of lymphedema after treatment for breast cancer: a prospective cohort study. *Int J Radiat Oncol Biol Phys* 2014;88:565-71.
- Executive Committee. The diagnosis and treatment of peripheral lymphedema: 2016 consensus document of the international society of lymphology. *Lymphology* 2016;49:170-84.
- Hansson E, Brorson H. Liposuction of lymphedema of the extremities. In: Shiffman MA, Di Giuseppe A, editors. *Liposuction: principles and practice*. Berlin Heidelberg: Springer-Verlag Berlin Heidelberg; 2016. pp. 721-33.
- Chang DW, Masia J, Garza R 3rd, Skoracki R, Neligan PC. Lymphedema: surgical and medical therapy. *Plast Reconstr Surg* 2016;138:209S-18.
- Suami H, Chang DW. Overview of surgical treatments for breast cancer-related lymphedema. *Plast Reconstr Surg* 2010;126:1853-63.
- Kung TA, Champaneria MC, Maki JH, Neligan PC. Current concepts in the surgical management of lymphedema. *Plast Reconstr Surg* 2017;139:1003e-13e.
- Charles RH. Elephantiasis scroti. In: Latham A, English TC, editors. *A system of treatment*. Vol. 3. London: Churchill; 1912.
- O'Brien BM, Khazanchi RK, Kumar PA, Dvir E, Pederson WC. Liposuction in the treatment of lymphoedema; a preliminary report. *Br J Plast Surg* 1989;42:530-3.
- Brorson H, Svensson H, Norrgren K, Thorsson O. Liposuction reduces arm lymphedema without significantly altering the already impaired lymph transport. *Lymphology* 1998;31:156-72.
- Brorson H. From lymph to fat: liposuction as a treatment for complete reduction of lymphedema. *Int J Low Extrem Wounds* 2012;11:10-9.
- Hoffner M, Ohlin K, Svensson B, Manjer J, Hansson E, et al. Liposuction gives complete reduction of arm lymphedema following breast cancer treatment-a 5-year prospective study in 105 patients without recurrence. *Plast Reconstr Surg Glob Open* 2018;6:e1912.
- Becker C, Assouad J, Riquet M, Hidden G. Postmastectomy lymphedema: long-term results following microsurgical lymph node transplantation. *Ann Surg* 2006;243:313-5.
- Viitanen TP, Visuri MT, Hartiala P, Mäki MT, Seppänen MP, et al. Lymphatic vessel function and lymphatic growth factor secretion after microvascular lymph node transfer in lymphedema patients. *Plast Reconstr Surg Glob Open* 2013;1:1-9.
- Schaverien MV, Coroneos CJ. Surgical treatment of lymphedema. *Plast Reconstr Surg* 2019;144:738-58.
- Lin CH, Ali R, Chen SC, Wallace C, Chang YC, et al. Vascularized groin lymph node transfer using the wrist as a recipient site for management of postmastectomy upper extremity lymphedema. *Plast Reconstr Surg* 2009;123:1265-75.
- Cheng MH, Chen SC, Henry SL, Tan BK, Lin MC, et al. Vascularized groin lymph node flap transfer for postmastectomy upper limb lymphedema: flap anatomy, recipient sites, and outcomes. *Plast Reconstr Surg* 2013;131:1286-98.
- Scaglioni MF, Suami H. Lymphatic anatomy of the inguinal region in aid of vascularized lymph node flap harvesting. *J Plast Reconstr*

- Aesthet Surg 2015;68:419-27
33. Pons G, Masia J, Loschi P, Nardulli ML, Duch J. A case of donor-site lymphoedema after lymph node-superficial circumflex iliac artery perforator flap transfer. *J Plast Reconstr Aesthet Surg* 2014;67:119-23.
  34. Yamada Y. The studies on lymphatic venous anastomosis in lymphedema. *Nagoya J Med Sci* 1969;32:1-21
  35. O'Brien BM, Shafiroff BB. Microlymphaticovenous and resectional surgery in obstructive lymphedema. *World J Surg* 1979;3:3-15,121-3.
  36. Koshima I, Inagawa K, Urushibara K, Moriguchi T. Supermicrosurgical lymphaticovenular anastomosis for the treatment of lymphedema in the upper extremities. *J Reconstr Microsurg* 2000;16:437-42.
  37. Baumeister RG, Siuda S. Treatment of lymphedemas by microsurgical lymphatic grafting: what is proved? *Plast Reconstr Surg* 1990;85:64-74.
  38. Baumeister RG, Siuda S, Bohmert H, Moser E. A microsurgical method for reconstruction of interrupted lymphatic pathways: autologous lymph-vessel transplantation for treatment of lymphedemas. *Scand J Plast Reconstr Surg* 1986;20:141-6.
  39. Campisi C. Use of autologous interposition vein graft in management of lymphedema: preliminary experimental and clinical observations. *Lymphology* 1991;24:71-6.
  40. Saijo H, Suzuki K, Yoshimoto H, Imamura Y, Yamashita S, et al. Paracrine effects of adipose-derived stem cells promote lymphangiogenesis in irradiated lymphatic endothelial cells. *Plast Reconstr Surg* 2019;143:1189e-200e.
  41. Toyserkani NM, Jensen CH, Sheikh SP, Sørensen JA. Cell-assisted lipotransfer using autologous adipose-derived stromal cells for alleviation of breast cancer-related lymphedema. *Stem Cells Transl Med* 2016;5:857-9
  42. Hayashida K, Yoshida S, Yoshimoto H, Fujioka M, Saijo H, et al. Adipose-derived stem cells and vascularized lymph node transfers successfully treat mouse hindlimb secondary lymphedema by early reconnection of the lymphatic system and lymphangiogenesis. *Plast Reconstr Surg* 2017;139:639-51.
  43. Toyserkani NM, Jensen CH, Andersen DC, Sheikh SP, Sørensen JA. Treatment of breast cancer-related lymphedema with adipose-derived regenerative cells and fat grafts: a feasibility and safety study. *Stem Cells Transl Med* 2017;6:1666-72.
  44. Toyserkani NM, Jensen CH, Tabatabaeifar S, Jørgensen MG, Hvidsten S, et al. Adipose-derived regenerative cells and fat grafting for treating breast cancer-related lymphedema: lymphoscintigraphic evaluation with 1 year of follow-up. *J Plast Reconstr Aesthet Surg* 2019;72:71-7.
  45. Maruccia M, Elia R, Ciudad P, Nacchiero E, Nicoli F, et al. Postmastectomy upper limb lymphedema: combined vascularized lymph node transfer and scar release with fat graft expedites surgical and patients' related outcomes. A retrospective comparative study. *J Plast Reconstr Aesthet Surg* 2019;72:892-901.
  46. Coleman SR. Long-term survival of fat transplants: controlled demonstrations. *Aesthetic Plast Surg* 1995;19:421-5.
  47. Coleman SR, Saboeiro AP. Fat grafting to the breast revisited: safety and efficacy. *Plast Reconstr Surg* 2007;119:775-85.
  48. Khouri RK, Smit JM, Cardoso E, Pallua N, Lantieri L, et al. Percutaneous aponeurotomy and lipofilling: a regenerative alternative to flap reconstruction? *Plast Reconstr Surg* 2013;132:1280-90.
  49. Saaristo AM, Niemi TS, Viitanen TP, Tervala TV, Hartiala P, et al. Microvascular breast reconstruction and lymph node transfer for postmastectomy lymphedema patients. *Ann Surg* 2012;255:468-73.
  50. Chang EI, Masia J, Smith ML. Combining autologous breast reconstruction and vascularized lymph node transfer. *Semin Plast Surg* 2018;32:36-41.
  51. Nicoli F, Constantinides J, Ciudad P, Sapountzis S, Kiranantawat K, et al. Free lymph node flap transfer and laser-assisted liposuction: a combined technique for the treatment of moderate upper limb lymphedema. *Lasers Med Sci* 2015;30:1377-85.

Review

Open Access



# The use of botulinum toxin A in chemical component separation: a review of techniques and outcomes

Sharbel A. Elhage, Eva B. Deerenberg, Jenny M. Shao, Vedra A. Augenstein, B. Todd Heniford

Department of Gastrointestinal and Minimally Invasive Surgery, Carolinas Medical Center, Charlotte, NC 28204, USA.

**Correspondence to:** Dr. B. Todd Heniford, Department of Gastrointestinal and Minimally Invasive Surgery, Carolinas Medical Center, 1025 Morehead Medical Drive, Suite 300, Charlotte, NC 28204, USA. E-mail: todd.heniford@gmail.com

**How to cite this article:** Elhage SA, Deerenberg EB, Shao JM, Augenstein VA, Heniford BT. The use of botulinum toxin A in chemical component separation: a review of techniques and outcomes. *Plast Aesthet Res* 2020;7:16.  
<http://dx.doi.org/10.20517/2347-9264.2020.03>

**Received:** 3 Jan 2020 **First Decision:** 12 Mar 2020 **Revised:** 18 Mar 2020 **Accepted:** 26 Mar 2020 **Published:** 10 Apr 2020

**Science Editor:** Sahil Kuldip Kapur **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

Fascial closure is crucial for abdominal wall reconstruction (AWR) but can be especially difficult in patients with massive ventral hernias or loss domain. Recently, botulinum toxin A (BTA) has been increasingly utilized as an adjunct in AWR to aid in fascial closure. This review aims to evaluate the current literature on the use of BTA in AWR to assess current treatment regimens, side effects, outcomes and complications. A literature search was performed, yielding 10 studies that met the inclusion criteria. There was a significant amount of heterogeneity in treatment regimens, with studies differing in BTA injection timing, dosage, concentration, and location. The majority of studies showed that injection of BTA preoperatively was able to augment abdominal wall musculature, with many showing a decrease in mean transverse defect size and high rates of successful fascial closure. No major complications were reported from BTA administration, with only mild side effects reported by some studies. The most common side effects include a weak cough or sneeze, bloating, and back pain, which generally all resolved prior to surgery. While BTA appears to be a promising adjunct for AWR, further investigation is needed to determine optimal patient selection and treatment regimens.

**Keywords:** Hernia, abdominal wall reconstruction, botulinum toxin, botox, chemical component separation

## INTRODUCTION

Ventral hernias are a frequent complication after open abdominal surgery and up to 30% develop incisional hernias<sup>[1-3]</sup>. Repair of incisional hernias is difficult due to scarring and the distortion of tissue planes innate to a reoperative field. In complex cases, such as massive hernias, those with loss of domain, recurrent hernias, or hernias with infection or contamination, patient morbidity can be greatly increased.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Despite advances in mesh technology, refinement of surgical techniques, and the advent of myofascial reconstruction techniques, abdominal wall reconstruction (AWR) in these patients is still associated with high rates of recurrence<sup>[4-6]</sup>. In addition, each failed attempt at hernia repair results in higher morbidity, costs, and risk of recurrence for each subsequent repair<sup>[7,8]</sup>.

An especially challenging patient population for hernia specialists is those with loss of domain. This occurs when an abdominal wall defect progresses to a size at which it may no longer accommodate the abdominal viscera, leading to irreducible protrusion into the hernia sac. Chronic muscle retraction reduces the volume of the peritoneal cavity and precludes tension-free fascial closure during AWR, with potential problems such as abdominal compartment syndrome, ventilatory restriction, and an elevated risk of hernia recurrence. In hernia defects over 8.3 cm in width, component separation is often needed to achieve primary, tension-free fascial closure<sup>[9]</sup>. Other techniques that have been utilized to increase the abdominal domain in large hernias with loss of domain include progressive pneumoperitoneum and soft tissue expanders. These are not without risk of morbidity however<sup>[10,11]</sup>.

More recently, preoperative injection of botulinum toxin A (BTA) has been added to the surgeon's armamentarium for AWR as an off-label use. BTA is a neurotoxin produced by the bacterium *Clostridium botulinum*, which has been shown to have a variety of therapeutic uses through its inhibitory effect on presynaptic cholinergic nerve terminals<sup>[12]</sup>. Treatment of a muscle with BTA results in functional denervation within 2 days and with peak effect after 4-6 weeks, leading to flaccid paralysis. This has been successfully used for several ocular and facial nerve disorders, as well as a range of other neuromuscular disorders including laryngeal, cervical and hand dystonia<sup>[12]</sup>. The first experimental study reporting the benefits of BT and its application in abdominal wall repair was published by Cakmak *et al.*<sup>[13]</sup> In a rat study, they demonstrated increased abdominal wall laxity after BTA injection in the lateral abdominal wall. The amount of intraperitoneal saline required to reach a fixed intraabdominal pressure after BTA administration was significantly higher than before treatment with BTA. The neurotoxin BTA results in a form of chemical component separation, causing relaxation of the lateral muscles of the abdominal wall. The first report of preoperative injection of BTA for AWR was by Ibarra-Hurtado *et al.*<sup>[14]</sup> in 2009, where BTA was used to facilitate fascial closure in 12 patients. The lateral muscle paralysis achieved, and transverse hernia defect reduction in size, allowed for closure with minimal tension. BTA might also decrease pain after AWR and the need for narcotic analgesia. Besides blocking the release of acetylcholine, BTA also prevents release of the pain-modulating molecules calcitonin gene-related peptide and substance P from the presynaptic motor nerve terminal<sup>[15]</sup>.

The following review aims to summarize the literature regarding the clinical use of BTA in AWR, and assess the varying regimens, published effects, outcomes, and complications reported.

## METHODS

A literature review was performed using the PubMed Medline database. The following search and keyword techniques were used: [(“botulinum”) AND (“hernia”) NOT (“paraesophageal” OR “esophageal” OR “hiatal” OR “diaphragmatic” OR “disk”)]. We limited our review to studies written in English.

Our search yielded 47 articles, and 10 met our inclusion criteria for review<sup>[14,16-24]</sup>. Two independent reviewers assessed the articles for inclusion. Studies that did not report outcomes of preoperative BTA use in AWR for ventral hernia were excluded. We also excluded review articles, case reports, cadaveric studies, and studies on groin hernias.

Two BTA products were utilized in the 10 studies included for analysis - onabotulinum toxin A (Botox®, Allergan, Dublin, Ireland) and abobotulinum toxin A (Dysport®, Ipsen, Boulogne-Billancourt, France).

**Table 1. Summary of botulinum toxin A treatment regimens**

Ref.	Year	n	Total BTA units	Injection sites	Timing of injections
Ibarra-Hurtado <i>et al.</i> <sup>[14]</sup>	2009	12	166 units*	10 total, 5 sites bilaterally, 16.6 units per site*	At least 4 weeks prior to surgery
Ibarra-Hurtado <i>et al.</i> <sup>[16]</sup>	2014	17	166 units*	10 total, 5 sites bilaterally between EO and IO, 16.6 U/5 mL per site*	At least 4 weeks prior to surgery
Zendejas <i>et al.</i> <sup>[17]</sup>	2013	22	300 units	6 total, 3 sites bilaterally at EO, IO, and TA, 50 U/25 mL per site (16.6 units/8.3 mL per muscle)	0-9 days prior to surgery (median 6 days)
Farooque <i>et al.</i> <sup>[18]</sup>	2016	8	300 units	6 total, 3 sites bilaterally at EO, IO, and TA, 50 U/25 mL per site (8 mL per muscle)	2 weeks prior to surgery
Rodriguez-Acevedo <i>et al.</i> <sup>[19]</sup>	2018	56	200-300 units	6 total, 3 sites bilaterally at EO, IO, and TA	1-2 weeks prior to surgery
Bueno-Lledó <i>et al.</i> <sup>[20]</sup>	2018	70	166 units*	10 total, 5 sites bilaterally, 16.6 U/5 mL per site, divided between EO, IO, and TA*	4 weeks prior to surgery
Elstner <i>et al.</i> <sup>[22]</sup>	2020	46	200 units	6 total, 3 sites bilaterally at EO, IO, and TA, or 6 total, 3 sites bilaterally at EO and IO	2-4 weeks prior to surgery
Bueno-Lledó <i>et al.</i> <sup>[21]</sup>	2019	80	166 units*	10 total, 5 sites bilaterally, 16.6 U/5 mL per site, divided between EO, IO, and TA*	4 weeks prior to surgery
Nielsen <i>et al.</i> <sup>[23]</sup>	2020	37	300 units	6-10 total, 3-5 sites bilaterally, 5 U/1 mL or 2 U/1 mL evenly distributed to all sites between EO, IO, and TA	3-4 weeks prior to surgery
Chan <i>et al.</i> <sup>[24]</sup>	2020	12	200-300 units	6 total, 3 sites bilaterally at EO and IO	4-6 weeks prior to surgery

EO: external oblique muscle; IO: internal oblique muscle; TA: transversus abdominis muscle. BTA: botulinum toxin A. All botulinum units in this table correspond to onabotulinum toxin A (Botox®). \*166 units of onabotulinum toxin A (Botox®) corresponds to the 500 units of abobotulinum toxin A (Dysport®) used in these studies

These two BTA products have varying potency and conversion to onabotulinum toxin A (Botox®, Allergan) dosing was performed based on the most commonly published conversion ratio of 1:3 onabotulinum toxin A:abobotulinum toxin A to report the total BTA units used in each study <sup>[25]</sup>.

## RESULTS

### Botulinum toxin A regimens

The treatment regimens for BTA administration are outlined in Table 1. Studies ranged from 500 units of abobotulinum toxin A or 200-300 units of onabotulinum toxin A in total. All studies diluted BTA in 0.9% normal saline to varying concentrations. Eight studies injected BTA at least 2 weeks prior to surgery, with five waiting at least 4 weeks before <sup>[14,16,18,20-24]</sup>. Rodriguez-Acevedo *et al.* <sup>[19]</sup> injected BTA 1-2 weeks prior to surgery, while Zendejas *et al.* <sup>[17]</sup> performed injections on the day of surgery in 13 patients, and a median of 6 days prior in the remaining nine patients. Either ultrasound guidance or electromyography was used to confirm injection location. Injection sites ranged from 3-5 points on each side of the abdomen. Six studies injected BTA into all three muscle layers of the abdominal wall for each injection site <sup>[17-21,23]</sup>. Elstner *et al.* <sup>[22]</sup> compared injection at all three of the external oblique (EO), internal oblique (IO), and transversus abdominis (TA) muscles, to injection at only the EO and IO. Chan *et al.* <sup>[24]</sup> injected only at the level of the EO and IO while Ibarra-Hurtado *et al.* <sup>[14,16]</sup> injected between the EO and IO in their 2013 study and did not specify the location of BTA injection in their 2009 study.

### Botulinum toxin A efficacy

Ibarra-Hurtado *et al.* <sup>[14]</sup> published the first description of AWR with preoperative BTA in a prospective case series of 12 patients between 2007-2009. The authors reported that 4 weeks after BTA injection, patients had a decrease in mean transverse defect by 5.25 cm and achieved fascial closure in 100% of patients. Six (50%) patients required concomitant external oblique release to achieve fascial closure. At mean follow-up of 9 months, there were zero recurrences.



The same group then conducted another prospective study of a series of 17 patients between 2009-2011. These patients were all males with midline ventral hernias who were at least 12 months out from laparotomy and open abdomen without formal abdominal closure. On CT scans performed 4 weeks after BTA injection, statistically significant decreases in bilateral muscular thickness and length, and transverse hernia defect sizes (all  $P < 0.0001$ ) were found. As with their prior study, the authors were able to achieve fascial closure in 100% of patients, with nine patients (53%) requiring additional EO release. Similarly, no recurrences developed at a mean follow-up of 49 months<sup>[16]</sup>.

Reporting on a series of 22 patients, Zendejas *et al.*<sup>[17]</sup> evaluated BTA use in AWR and its effect on post-operative pain control in 2010. These 22 patients received preoperative BTA injections and were matched to 66 controls based on age, sex, BMI, number of hernia recurrences and type of hernia repair. Thirteen (59%) of the patients in the BTA group received injections on the day of surgery, while the remaining nine had injections on an average of 6 days preoperatively. They found that the BTA group used 44% less morphine equivalents on hospital day two and 64% less on hospital day five, while reporting significantly lower pain scores at both time points (all  $P < 0.05$ ). Hernia size was only reported in 73% of patients with an average of 59.7 cm<sup>2</sup> in the BTA group vs. 117.5 cm<sup>2</sup> in the control. While there was no statistically significant difference between the two groups, this clinical difference may have played a role in the results. In the BTA group, 54.6% of cases were completed laparoscopically and fascia was only reapproximated in 40.9% of patients. No difference in the rates of component separation, length of stay, or recurrence between the two groups was found at a mean follow-up of 18 months.

Farooque *et al.*<sup>[18]</sup> described a prospectively collected series of eight patients from 2012-2013 who underwent AWR with preoperative BTA injections. All patients were considered complex, having had at least two prior hernia repairs, with defect sizes ranging from 5 cm × 9 cm to 24 cm × 24 cm, and an 11 cm mean transverse defect size. On post-BTA imaging, a decrease in the thickness of the abdominal wall musculature by an average of 6.3 mm, and a decrease in abdominal wall muscle length by an average of 2.8 cm per side was noted. All hernia repairs were performed laparoscopically with intraperitoneal onlay mesh (IPOM) placement. Fascial closure rates and long term outcomes were not reported however.

In a prospective case series of 56 patients from 2012-2017, Rodriguez-Acevedo *et al.*<sup>[19]</sup> evaluated patients with complex ventral hernias who received BTA 1-2 weeks prior to AWR. They defined complex hernias as recurrent or traumatic hernias having a minimum defect width of 6 cm, and/or loss of domain greater than 20%. They also reported a mean transverse defect size of 11.6 cm prior to BTA administration. Defects ranged from 5 cm × 9 cm to 28 cm × 19 cm. In addition to BTA, patients with a transverse defect size greater than 15 cm, and infraumbilical defect greater than 9 cm, or loss of domain greater than 20% (18/56 patients) received preoperative progressive pneumoperitoneum (PPP) of up to 1000 cc for 3-7 days before surgery. Patients averaged an increase in abdominal wall muscle length of 4.0 cm per side, with no statistical difference between those receiving 200 and 300 units of BTA. No statistical difference in muscle length gain between the patients receiving BTA alone vs. those receiving BTA plus PPP was reported. Fascial closure was achieved in 100% of patients, with eight (14%) requiring component separation. Fifty-three patients had a laparoscopic or laparoscopic assisted repair with intraperitoneal mesh placement, and the remaining three patients had open repairs with retro-rectus mesh placement. At 26 months post-repair, there was one case of recurrence but the overall mean follow-up period was not reported.

Publishing the largest series thus far with 70 patients, Bueno-Lledó *et al.*<sup>[21]</sup> reported a retrospective case series of prospectively collected data on patients who received BTA 4 weeks prior to AWR from 2010-2016. All patients also received 500-1000 cc of PPP for 1-2 weeks prior to surgery. Using CT imaging before and after administration of BTA and PPP, they calculated volume of the hernia (VIH) and volume of the abdominal cavity (VAC). The VIH:VAC ratio decreased by an average of 16.6% after administration of BTA

and PPP ( $P = 0.02$ ) but there was no significant difference in median transverse or longitudinal hernia defect size. Fascial closure was achieved in 95.7% of patients using a variety of techniques: 54 (77.1%) external oblique release, 14 (20%) transversus abdominis release, and 2 (2.9%) retro-rectus repairs. Mesh was used in all patients. At a mean follow-up of 34.5 month, 4 recurrences (5.7%) were reported<sup>[20]</sup>. In 2019, the authors updated their series with 10 more patients with overall data showing a similar reduction in VIH:VAC ratio (16.3%), fascial closure (96.3%) and recurrence rates (6.2%) with a mean follow-up of 38.5 months.

In the only study comparing BTA injection techniques, Elstner *et al.*<sup>[22]</sup> evaluated a total of 46 patients in a prospective, observational fashion from 2015-2018. They compared two consecutive cohorts: 23 patients received BTA at three sites bilaterally, targeting each muscle of the abdominal wall (EO, IO, TA) at each injection site, and 23 patients who also received the same, but only targeting the EO and IO. Age, BMI, transverse defect size, and number of failed hernia repairs were similar in both groups. Using serial CT imaging, there was no difference in abdominal wall length gain between the groups ( $P = 0.37$ ). All patients underwent laparoscopic or laparoscopic-open-laparoscopic hernia repair with IPOM. Fascial closure rate was 100%, and there were no recurrences at mean follow-up of 24 months. They concluded that BTA injection of the TA can be omitted without compromising fascial closure in complex ventral hernias.

Nielsen *et al.*<sup>[23]</sup> retrospectively evaluated the short-term safety of BTA for the treatment of large hernias in 37 patients from two centers. The mean defect width was 12.1 cm, and 33 (89.2%) patients had no prior hernia operations. All patients underwent open repair with 95% being retro-muscular. Component separation was used in 15 (40.5%) patients and fascial closure was achieved in 100% of cases. Six patients required readmission within 30 days however, three for wound complications. In total, nine patients developed wound related complications. Outcomes beyond 30 days and recurrence rates were not reported.

In the most recent study by Chan *et al.*<sup>[24]</sup>, 12 patients underwent preoperative BTA injections prior to hernia repair. On CT imaging after BTA, there was a statistically significant increase in both left ( $P = 0.004$ ) and right ( $P = 0.014$ ) sided mean abdominal wall lengths, with an increase in mean abdominal wall length of  $4.0 \pm 2.2$  cm per side. Nine patients underwent laparoscopic repair with IPOM, and one patient had a robotic IPOM repair. The remaining two patients underwent laparoscopic-open-laparoscopic repair, one with IPOM and the other with a retro-rectus mesh placement. Based on the description of the “Venetian Blinds” surgical technique used, it appears that fascia was reapproximated in all patients although this was not directly reported. No hernia recurrences at a median follow-up of 18.3 months was observed.

### Complications and side effects of botulinum toxin A

Nine of ten studies in our review reported on specific BTA related complications or side effects<sup>[16-24]</sup>. Five did not have any complications or side effects related to BTA administration<sup>[16,17,20,21,24]</sup> while the remaining four reported side effects without major complications<sup>[18,19,22,23]</sup>.

Nielsen *et al.*<sup>[23]</sup> reported one patient (2.7%) who had pain related to BTA injections. This was managed without narcotic pain medications and resolved prior to surgery.

The remaining three studies shared a common theme of patients reporting a weak cough or sneeze following BTA administration<sup>[18,19,22]</sup>. Farooque *et al.*<sup>[18]</sup> found that in patients experiencing these symptoms, this improved with application of an abdominal binder, and all resolved prior to surgery.

Rodriguez-Acevedo *et al.*<sup>[19]</sup> found that several patients developed superficial bruising at the BTA injection sites. In addition to the weak cough, patients also reported a sensation of bloating that resolved only after the hernia repair. Four patients in their study also reported back pain after BTA injections but with unclear duration. For these complaints, an abdominal binder again proved to manage symptoms adequately.

This study included 18 patients who also received PPP, but because data on which patients developed these complications was not reported, it is not certain these symptoms can be attributed solely to BTA administration.

Elstner *et al.*<sup>[22]</sup> did not report any complications related to BTA administration but do describe similar side effects of weak cough and sneeze, and a sensation of bloating. Patients also described back pain, and one experienced dyspnea. Similarly, abdominal binders were found to aid in symptom resolution. The authors theorized that sparing the TA from BTA injection may allow for increased core stability and reduction of these side effects. However, they no statistical analysis was performed to compare the three muscle layer group and the EO and IO only group regarding these side effects.

## DISCUSSION

Improving outcomes of AWR in large hernias starts with optimization of modifiable patient factors, such as weight loss, smoking cessation and controlling diabetes. Furthermore, to reduce complication and recurrence rates, the goal of AWR should be achievement of primary fascial closure with mesh reinforcement, instead of bridging the hernia defect with mesh<sup>[26]</sup>. Both increased intrabdominal pressure, as well as morbid obesity which is associated with increased intrabdominal pressure, may play a role in the development of hernias and hernia recurrences<sup>[27]</sup>. Different component separation techniques can be used to increase abdominal cavity volume and decrease intrabdominal pressure, but this necessitates destruction of the anatomical tissue planes of the abdominal wall<sup>[28,29]</sup>. The use of BTA as a chemical rather than surgical technique for components separation achieves abdominal wall compliance by elongation and thinning of the musculature, although such application is currently off-label<sup>[16]</sup>.

While there are relatively few studies evaluating the efficacy of BTA in AWR, and no randomized controlled trials, the existing evidence remains promising. Studies evaluating abdominal wall muscle thickness and length were able to show significant differences after administration of BTA<sup>[16,18-22,24]</sup>. As would be expected with these effects on the musculature, many studies then showed a decrease in the mean transverse defect size. In studies reporting fascial closure, all but one achieved very high success rates after pre-operative BTA. Zendejas *et al.*<sup>[17]</sup> reported a low fascial closure rate in their BTA patients (40.9%), but this was not dissimilar from their propensity matched control group (36.4%). This raises the question of whether this lower fascial closure rate reflects surgeon preference and surgical technique as opposed to BTA treatment effect. Three studies, including the largest review to date by Bueno-Lledó *et al.*<sup>[20,21]</sup>, used PPP as an adjunct to BTA in AWR, which makes extrapolation of results to patients receiving BTA only difficult<sup>[19]</sup>. Further limitations of this review include the significant amount of heterogeneity in regard to hernia characteristics, surgical techniques, and the type of mesh used. While BTA appears to be an effective adjunct therapy in AWR, none of the studies in this review detailed the selection criteria and hernia characteristics that may define patients who would benefit most from BTA injection.

There is significant variation in technique, number of sites and timing of injection in the reviewed articles. Functional denervation starts after 2 days and the effect peaks at approximately 2 weeks after BTA injection and lasts beyond 30 days<sup>[12,30]</sup>. Injection at least 2 weeks prior to surgery seems most beneficial for AWR. Ultrasound guidance appears sufficient for targeted BTA injection and was used in all but one study. Guidance techniques should be based on the comfort of the provider however<sup>[16-24]</sup>. All studies injected at least the EO and IO, with most injecting the TA as well. Theoretically, paralysis of all 3 layers of the lateral abdominal wall could interfere with the stabilizing function of the core abdominal muscles, potentially contributing to back pain and predisposing to injury, as hypothesized by Elstner *et al.*<sup>[22]</sup> However, in large ventral hernias, this stabilizing function is likely already impaired due to disruption of the midline. If this is a concern in patients with pre-existing conditions, application of an abdominal binder or selective injection

of the EO and IO can be performed, sparing the TA and its truncal stabilizing function. Elstner *et al.*<sup>[22]</sup> found comparable results of two and three layer BTA injection in laparoscopic hernia repair. A total of at least 200 BTA units at a concentration of 2 U/mL or more was used in all studies. Since there is great heterogeneity in timing, sites and total BTA units injected in the studies, no conclusion on the optimal dose, concentration, sites, or target muscle layer(s) can be formulated. Furthermore, one study which assessed abdominal wall effects of BTA with both functional CT and EMG at 1-4 weeks after injection showed non-uniform distribution and duration of the induced paralysis<sup>[31]</sup>.

BTA has also been shown to prevent release of the pain-modulating molecules calcitonin gene-related peptide and substance P from the presynaptic motor nerve terminal and could have a positive effect on postoperative pain control<sup>[15]</sup>. One study reported on post-operative pain and found promising results with decreased morphine use and pain reported in the short-term postoperative period. Nevertheless, the analgesic effects of BTA would benefit from further research<sup>[17]</sup>.

With the introduction of new medications for clinical use, patient safety is of utmost importance. The majority of studies did not report complications with the use of BTA and of those that did, they were minor in nature. Complaints after BTA injection included a mild cough or sneeze, bloating, and back pain, and nearly all resolved after application of an abdominal binder. However, serious adverse effects of BTA injection can occur and have been described after administration at other sites of the body. These adverse effects are often related to the specific injection site, non-sterile injection technique, or injection into infected tissue<sup>[32]</sup>. Four cases of life-threatening botulism have occurred after injection of highly concentrated, unlicensed preparation of BTA for cosmetic purposes<sup>[33]</sup>. Although the precise human lethal dose of crystalline BTA is not known, extrapolation from primate studies suggest an approximate intravenous or intramuscular lethal dose of 40 U/kg of BTA in humans<sup>[34,35]</sup>. Therefore, for an average 70 kg human, the lethal dose would be 2800 units in total; in AWR, the maximum reported total dose used is 300 units of BTA, which is drastically lower than the described lethal dose. Proper storage of the product, selection of the correct dose, and proper administration techniques are necessary to prevent these adverse events when injecting BTA into the abdominal wall. Patients with neuromuscular junction disorders such as myasthenia gravis, Lambert-Eaton syndrome, and anterior horn disorders are particularly susceptible to the adverse events of botulinum toxin and should not be injected with BTA<sup>[32]</sup>.

## CONCLUSION

The initial results of BTA use in AWR are promising and safe, with beneficial alterations to the abdominal wall and high rates of fascial closure, and only minor side effects reported. There remains much variance in BTA treatment regimens and at this time, a consensus on the dosage, technique, and timing of injection has yet to be reached. There remains a lack of high-level evidence and the existing literature is limited by studies with small sample sizes, unclear patient selection criteria, and treatment regimen heterogeneity.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Elhage SA, Deerenberg EB, Shao JM, Augenstein VA, Heniford BT

Performed data acquisition, as well as provided administrative, technical, and material support: Elhage SA, Deerenberg EB, Shao JM

Drafting of manuscript and critically important revisions: Elhage SA, Deerenberg EB, Shao JM, Augenstein VA, Heniford BT

### Availability of data and materials

Not applicable.

## Financial support and sponsorship

None.

## Conflicts of interest

Dr. Heniford has received honoraria, speaker's fees, and research support from Allergan, W. L. Gore, and Stryker. Dr. Augenstein has received honoraria and speaker's fees for Allergan, Intuitive, Acelity, W. L. Gore, and Medtronic. The remaining authors have no other relevant financial or personal relationships that could inappropriately influence this work or its conclusions.

## Ethical approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Copyright

© The Author(s) 2020.

## REFERENCES

1. Kingsnorth A, LeBlanc K. Hernias: inguinal and incisional. *Lancet* 2003;362:1561-71.
2. Deerenberg EB, Harlaar JJ, Steyerberg EW, Lont HE, van Doorn HC, et al. Small bites versus large bites for closure of abdominal midline incisions (STITCH): a double-blind, multicentre, randomised controlled trial. *Lancet* 2015;386:1254-60.
3. Jairam AP, Timmermans L, Eker HH, Pierik REGJM, van Klaveren D, et al. Prevention of incisional hernia with prophylactic onlay and sublay mesh reinforcement versus primary suture only in midline laparotomies (PRIMA): 2-year follow-up of a multicentre, double-blind, randomised controlled trial. *Lancet* 2017;390:567-76.
4. Weissler JM, Lanni MA, Tecce MG, Carney MJ, Shubinets V, et al. Chemical component separation: a systematic review and meta-analysis of botulinum toxin for management of ventral hernia. *J Plast Surg Hand Surg* 2017;51:366-74.
5. Deerenberg EB, Timmermans L, Hogerzeil DP, Sliker JC, Eilers PH, et al. A systematic review of the surgical treatment of large incisional hernia. *Hernia* 2015;19:89-101.
6. Luijendijk RW, Hop WC, van den Tol MP, de Lange DC, Braaksma MM, et al. A comparison of suture repair with mesh repair for incisional hernia. *N Engl J Med* 2000;343:392-8.
7. Flum DR, Horvath K, Koepsell T. Have outcomes of incisional hernia repair improved with time? A population-based analysis. *Ann Surg* 2003;237:129-35.
8. Heniford BT, Park A, Ramshaw BJ, Voeller G. Laparoscopic repair of ventral hernias: nine years' experience with 850 consecutive hernias. *Ann Surg* 2003;238:391-9.
9. Blair LJ, Ross SW, Huntington CR, Watkins JD, Prasad T, et al. Computed tomographic measurements predict component separation in ventral hernia repair. *J Surg Res* 2015;199:420-7.
10. Mayagoitia JC, Suárez D, Arenas JC, Díaz de León V. Preoperative progressive pneumoperitoneum in patients with abdominal-wall hernias. *Hernia* 2006;10:213-7.
11. Wooten KE, Ozturk CN, Ozturk C, Laub P, Aronoff N, et al. Role of tissue expansion in abdominal wall reconstruction: a systematic evidence-based review. *J Plast Reconstr Aesthet Surg* 2017;70:741-51.
12. Jankovic J, Brin MF. Therapeutic uses of botulinum toxin. *N Engl J Med* 1991;324:1186-94.
13. Cakmak M, Caglayan F, Somuncu S, Leventoglu A, Ulusoy S, et al. Effect of paralysis of the abdominal wall muscles by botulinum A toxin to intraabdominal pressure: an experimental study. *J Pediatr Surg* 2006;41:821-5.
14. Ibarra-Hurtado TR, Nuño-Guzmán CM, Echeagaray-Herrera JE, Robles-Vélez E, De Jesús González-Jaime J. Use of botulinum toxin type A before abdominal wall hernia reconstruction. *World J Surg* 2009;33:2553-6.
15. Jankovic J, Albanese A, Atassi MZ, Dolly JO, Hallet M, Mayer NH. *Botulinum Toxin: Therapeutic Clinical Practice and Science*. Philadelphia: Saunders Elsevier; 2009. p. 512.
16. Ibarra-Hurtado TR, Nuño-Guzmán CM, Miranda-Díaz AG, Troyo-Sanromán R, Navarro-Ibarra R, et al. Effect of botulinum toxin type A in lateral abdominal wall muscles thickness and length of patients with midline incisional hernia secondary to open abdomen management. *Hernia* 2014;18:647-52.
17. Zendejas B, Khasawneh MA, Srivastyan B, Jenkins DH, Schiller HJ, et al. Outcomes of chemical component paralysis using botulinum toxin for incisional hernia repairs. *World J Surg* 2013;37:28307.
18. Farooque F, Jacombs AS, Roussos E, Read JW, Dardano AN, et al. Preoperative abdominal muscle elongation with botulinum toxin A for complex incisional ventral hernia repair. *ANZ J Surg* 2016;86:79-83.
19. Rodríguez-Acevedo O, Elstner KE, Jacombs ASW, Read JW, Martins RT, et al. Preoperative botulinum toxin A enabling defect closure and



- laparoscopic repair of complex ventral hernia. *Surg Endosc* 2018;32:831-9.
20. Bueno-Lledó J, Torregrosa A, Jiménez R, Pastor PG. Preoperative combination of progressive pneumoperitoneum and botulinum toxin type A in patients with loss of domain hernia. *Surg Endosc* 2018;32:3599-608.
  21. Bueno-Lledó J, Torregrosa-Gallud A. Preoperative botulinum toxin and progressive pneumoperitoneum are useful in the treatment of large incisional hernias. *Am Surg* 2019;85:e189-92.
  22. Elstner KE, Read JW, Saunders J, Cosman PH, Rodriguez-Acevedo O, et al. Selective muscle botulinum toxin A component paralysis in complex ventral hernia repair. *Hernia* 2020;24:287-93.
  23. Nielsen M, Bjerg J, Dorfelt A, Jørgensen LN, Jensen KK. Short-term safety of preoperative administration of botulinum toxin A for the treatment of large ventral hernia with loss of domain. *Hernia* 2020;24:295-9.
  24. Chan DL, Ravindran P, Fan HS, Elstner KE, Jacombs ASW, et al. Minimally invasive Venetian blinds ventral hernia repair with botulinum toxin chemical component separation. *ANZ J Surg* 2020;90:67-71.
  25. Scaglione F. Conversion ratio between botox®, dysport®, and xeomin® in clinical practice. *Toxins (Basel)* 2016;8.
  26. Booth JH, Garvey PB, Baumann DP, Selber JC, Nguyen AT, et al. Primary fascial closure with mesh reinforcement is superior to bridged mesh repair for abdominal wall reconstruction. *J Am Coll Surg* 2013;217:999-1009.
  27. Cobb WS, Burns JM, Kercher KW, Matthews BD, James Norton H, et al. Normal intraabdominal pressure in healthy adults. *J Surg Res* 2005;129:231-5.
  28. Ramirez OM, Ruas E, Dellon AL. "Components separation" method for closure of abdominal-wall defects: an anatomic and clinical study. *Plast Reconstr Surg* 1990;86:519-26.
  29. Novitsky YW, Elliott HL, Orenstein SB, Rosen MJ. Transversus abdominis muscle release: a novel approach to posterior component separation during complex abdominal wall reconstruction. *Am J Surg* 2012;204:709-16.
  30. Eleopra R, Tugnoli V, De Grandis D. The variability in the clinical effect induced by botulinum toxin type A: the role of muscle activity in humans. *Mov Disord* 1997;12:89-94.
  31. Tomazini Martins R, Elstner KE, Skulina C, Rodriguez-Acevedo O, Read JW, et al. Limitations of electromyography in the assessment of abdominal wall muscle contractility following botulinum toxin a injection. *Front Surg* 2019;6:16.
  32. Yiannakopoulou E. Serious and long-term adverse events associated with the therapeutic and cosmetic use of botulinum toxin. *Pharmacology* 2015;95:65-9.
  33. Chertow DS, Tan ET, Maslanka SE, Schulte J, Bresnitz EA, et al. Botulism in 4 adults following cosmetic injections with an unlicensed, highly concentrated botulinum preparation. *JAMA* 2006;296:2476-9.
  34. Scott AB, Suzuki D. Systemic toxicity of botulinum toxin by intramuscular injection in the monkey. *Mov Disord* 1988;3:333-5.
  35. Herrero BA, Ecklund AE, Spencer Streett C, Ford DF, King JK. Experimental botulism in monkeys-A clinical pathological study. *Exp Mol Pathol* 1967;6:84-95.

Original Article

Open Access



# Safety and efficacy of surgical treatments for axillary osmidrosis: a retrospective cohort study comparing conventional open excision with cartilage-shaver closed curettage

Ryutaro Tanaka, Daichi Morioka, Syuryo Akamine, Takafumi Shimizu, Koichi Kadomatsu

Department of Plastic Surgery, Showa University, Tokyo 142-8666, Japan.

**Correspondence to:** Prof. Daichi Morioka, Department of Plastic Surgery, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan. E-mail: dmorioka@gmail.com

**How to cite this article:** Tanaka R, Morioka D, Akamine S, Shimizu T, Kadomatsu K. Safety and efficacy of surgical treatments for axillary osmidrosis: a retrospective cohort study comparing conventional open excision with cartilage-shaver closed curettage. *Plast Aesthet Res* 2020;7:17. <http://dx.doi.org/10.20517/2347-9264.2020.12>

**Received:** 21 Jan 2020 **First Decision:** 6 Mar 2020 **Revised:** 14 Mar 2020 **Accepted:** 2 Apr 2020 **Published:** 10 Apr 2020

**Science Editor:** Raúl González-García **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

## Abstract

**Aim:** The aim of this study was to evaluate the usefulness of suction-assisted cartilage shaver (SACS) system closed curettage by comparing it with open excision regarding safety and efficacy.

**Methods:** A retrospective chart review was conducted for patients with axillary osmidrosis (AO) who underwent either open excision or SACS closed curettage between 2006 and 2018. We investigated the demographic data of patients and compared the postoperative complications and outcomes of the patients undergoing the two procedures.

**Results:** A total of 91 patients underwent SACS closed curettage and 188 patients underwent open excision. The complication rate in the SACS group (10.4%) was significantly lower than that in the open excision group (20.7%). Each procedure led to unsuccessful outcomes for two patients.

**Conclusion:** SACS closed curettage was safer than open excision for AO. Both procedures were extremely effective. Although decision-making for surgical treatment options for AO is affected by such other factors as discomfort in dressing, recovery time, scar formation, and cost, our results should be helpful for both surgeons and patients.

**Keywords:** Axillary osmidrosis, retrospective chart review, open excision, suction-assisted cartilage shaver system



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



## INTRODUCTION

Axillary osmidrosis (AO) is characterized by an offensive odor resulting from bacterial interaction with excessive apocrine secretions<sup>[1]</sup>. Especially in East Asian societies, AO impairs a patient's psychosocial functioning because of malodor and unsightly yellowish staining of clothing<sup>[1]</sup>.

Various nonsurgical and surgical treatments have been reported for AO. Nonsurgical treatments include the use of topical deodorant, subcutaneous injection of botulinum toxin-A, and endoscopic thoracic sympathectomy. Surgical treatments include laser technology, axillary skin excision, suction curettage, and conventional open excision of apocrine glands<sup>[2-5]</sup>.

In general, less invasive treatments such as lasers have low complication rates, but are less effective. More invasive treatments such as open surgery are more effective and have a lower recurrence rate, but they have a higher complication rate<sup>[2]</sup>.

Inaba and Ezaki<sup>[6]</sup> reported that surgical treatments have been traditionally performed for patients with AO in Japan since the 1950s. Since open excision of axillary sweat glands was first reported as a surgical treatment for hyperhidrosis by Skoog and Thyresson<sup>[7]</sup> in 1962, many surgeons and dermatologists have performed similar procedures for AO. Various modifications on types of incision, drainage methods, suture techniques, and tie-over dressings have been attempted<sup>[1,8,9]</sup>. Open excision is still commonly performed in Asian countries because the entire procedure can be completed using a basic set of surgical instruments that include forceps, skin hooks, dissecting scissors, and suture materials. It is also cost effective, compared with other treatment options<sup>[1,10]</sup>.

The suction-curettage approach for AO was first introduced by the Taiwanese surgeons Ou *et al.*<sup>[11]</sup> in 1998. It has been subsequently modified by many Asian surgeons and dermatologists. The modifications include a curette provided with a vacuum system<sup>[12]</sup>, Fatemi/Cassio cannulae<sup>[13]</sup>, and a suction-assisted cartilage shaver (SACS) system<sup>[14,15]</sup>. To date, subdermal excision as an open surgical procedure and suction curettage as a closed surgical procedure are the two major approaches for AO.

While other suction-curettage techniques manually remove sweat glands, SACS uses a double-sheathed electric oscillating cutter to aggressively remove sweat glands. The effectiveness of SACS closed curettage was reported to be comparable to that of open excision<sup>[14-20]</sup>. Shin *et al.*<sup>[21]</sup> recently performed a meta-analysis of the safety and efficacy of closed surgery compared with open surgery for AO and concluded that suction curettage was safer than open surgery, but less effective than open surgery. Their analysis evaluated more than one type of closed surgical procedure, as described in the previous paragraph, and open surgery included not only conventional techniques but also *en bloc* skin excision and endoscopic surgery. To our knowledge, no studies in the English-language literature comparing the safety and efficacy of SACS closed curettage versus conventional open excision have been published.

Since 2006, we have used, based on the patient's choice, either SACS closed curettage or open surgery to treat more than 600 patients with AO<sup>[1]</sup>. Our impression has been that SACS is preferable to open excision regarding its safety and efficacy. The aim of this retrospective cohort study was to determine whether our impression of the usefulness of SACS was accurate based on statistical analysis compared with conventional open surgery.

## METHODS

### Patients

The Showa University Hospital institutional research board approved this study (approval No. 2928). A retrospective chart review was conducted for consecutive patients with AO who underwent either open

excision or SACS closed curettage at Showa University Hospital or affiliated private clinics. Patients who had previously undergone other surgical treatments for AO were excluded from this study. Prior to surgery, the odor level of each patient was determined by a previously described gauze test<sup>[1]</sup>. The scale ranged from Level 1 (no odor) to Level 5 (severe odor), and patients with Levels 3 (moderate) to 5 were considered suitable for surgical treatments. The treatment option was chosen by each patient and his/her family instead of by the operating surgeon. Surgical procedures for more than 90% of patients were performed by a single surgeon (D.M.), and the other procedures were performed by surgeons under his supervision.

### **SACS system closed curettage**

Patients lay in the supine position, with their arms abducted 90°-120°. The region of hair-bearing skin plus a 5-mm margin was delineated and 30-40 mL of a solution of 0.25% lidocaine with 1:200,000 epinephrine was injected for local anesthesia. A 5-mm skin incision was made at either the medial or lateral edge of the marked skin. Suction of the marked area was first performed by a conventional liposuction cannula (3-mm diameter) inserted through the incision, and the subdermal layer containing the sweat glands (mainly apocrine glands) was removed by the SACS system (TPS; Stryker, Kalamazoo, MI, USA), equipped with an electric shaver (Formula; Stryker). This system is normally used for endoscopic arthroplasty in orthopedic surgery. It is composed of an outer cannula equipped with a grid that safely removes apocrine glands, and an inner cannula equipped with a serrated blade that strongly oscillates to remove apocrine glands [Figure 1]. The shaver was also connected to a vacuum pump (S200; Kakinuma Medical Inc., Tokyo, Japan) by a suction tube, which immediately removed the tissues containing the apocrine glands and discarded them into a container. After the subdermal tissues of the entire marked area were evenly excised, 2-6 quilting sutures (depending on the size of operated area) were used to anchor the loosened skin to the underlying fascia, and a drainage tube was inserted to extend through the skin incision. Compression by tie-over dressings and elastic bandages was applied for 3-5 days postoperatively. Anchoring sutures and sutures closing the incision were removed one week after surgery. The patient was allowed to perform full range-of-motion of the shoulder two weeks postoperatively.

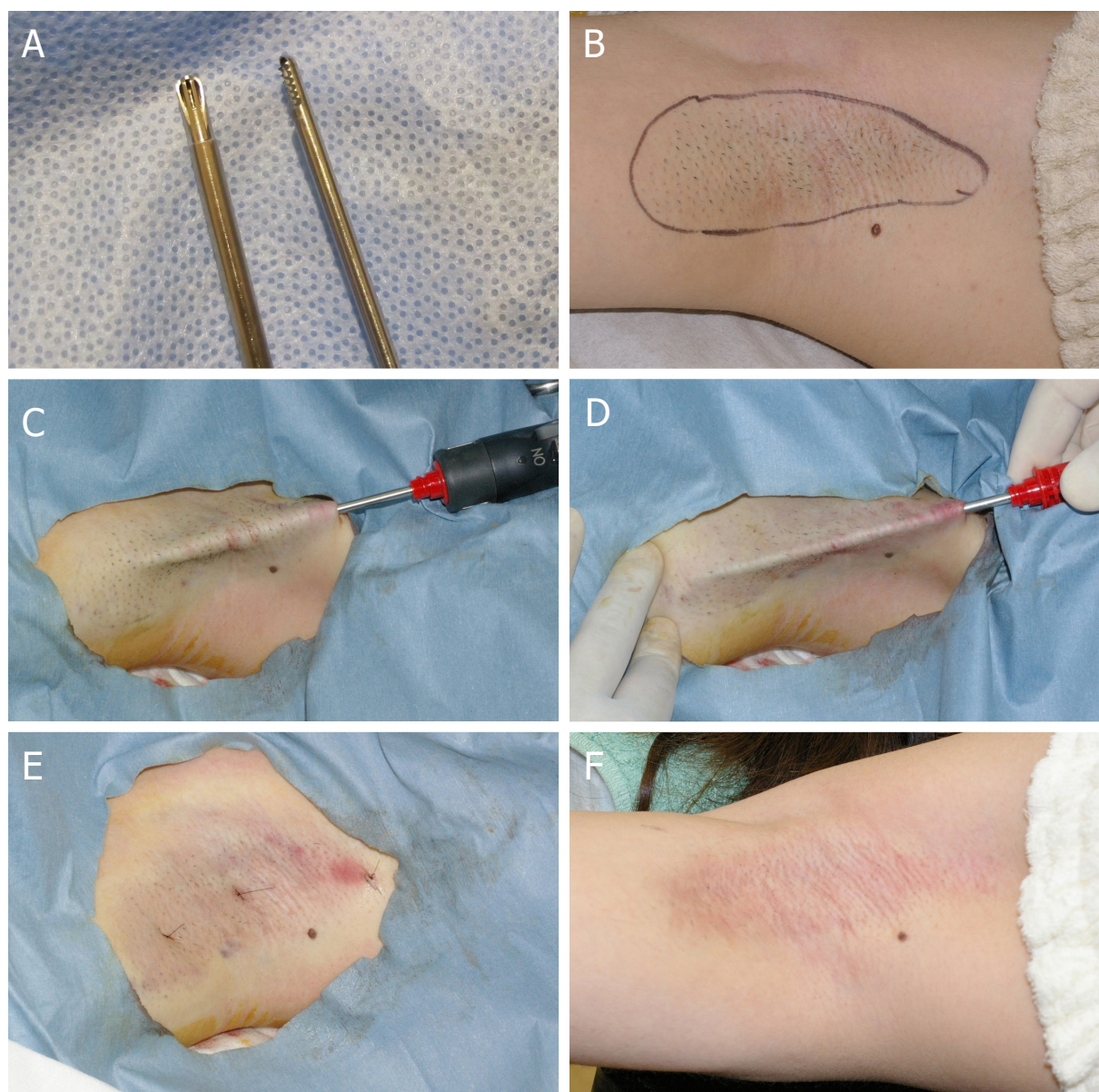
### **Open excision**

The operating position, local anesthesia, and operating area for conventional open excision were the same as for patients undergoing SACS curettage. According to a previously reported technique<sup>[22]</sup>, a small elliptical section of skin (3-6 cm long) in the center of the hair-bearing region was removed. The axillary skin was undermined from the incision both medially and laterally above the fascia. A skin hook was used to turn over the undermined skin, and the layer mainly containing the apocrine glands was identified [Figure 2]. Subdermal tissues containing the apocrine glands were pruned evenly from the center up to the edge of the marked area by dissecting scissors, and bleeding was controlled by electrocauterization. Following wound closure with 5/0 nylon sutures, 2-6 quilting sutures were used to anchor the skin flap to the underlying fascia, and a drainage tube was inserted to extend from the edge of the incision. Compression was achieved using tie-over dressings and elastic bandages, which were applied for 5-7 days postoperatively. Quilting sutures were removed one week after surgery, and incision sutures were removed between two and three weeks after surgery. The patient was usually allowed to perform full range-of-motion of the shoulder four weeks postoperatively.

### **Data assessments**

Safety was assessed according to the occurrence of acute moderate-to-severe adverse events, which included hematoma, seroma, and delayed wound healing. The events were assessed for each axilla. When more than two adverse events occurred in the same axilla (e.g., skin necrosis plus wound infection), the major event was used to determine the overall rate of occurrence for patients undergoing either closed or open surgery. Mild adverse events such as erosion, contact dermatitis due to dressing tape, and transient erythema were excluded from the assessment.



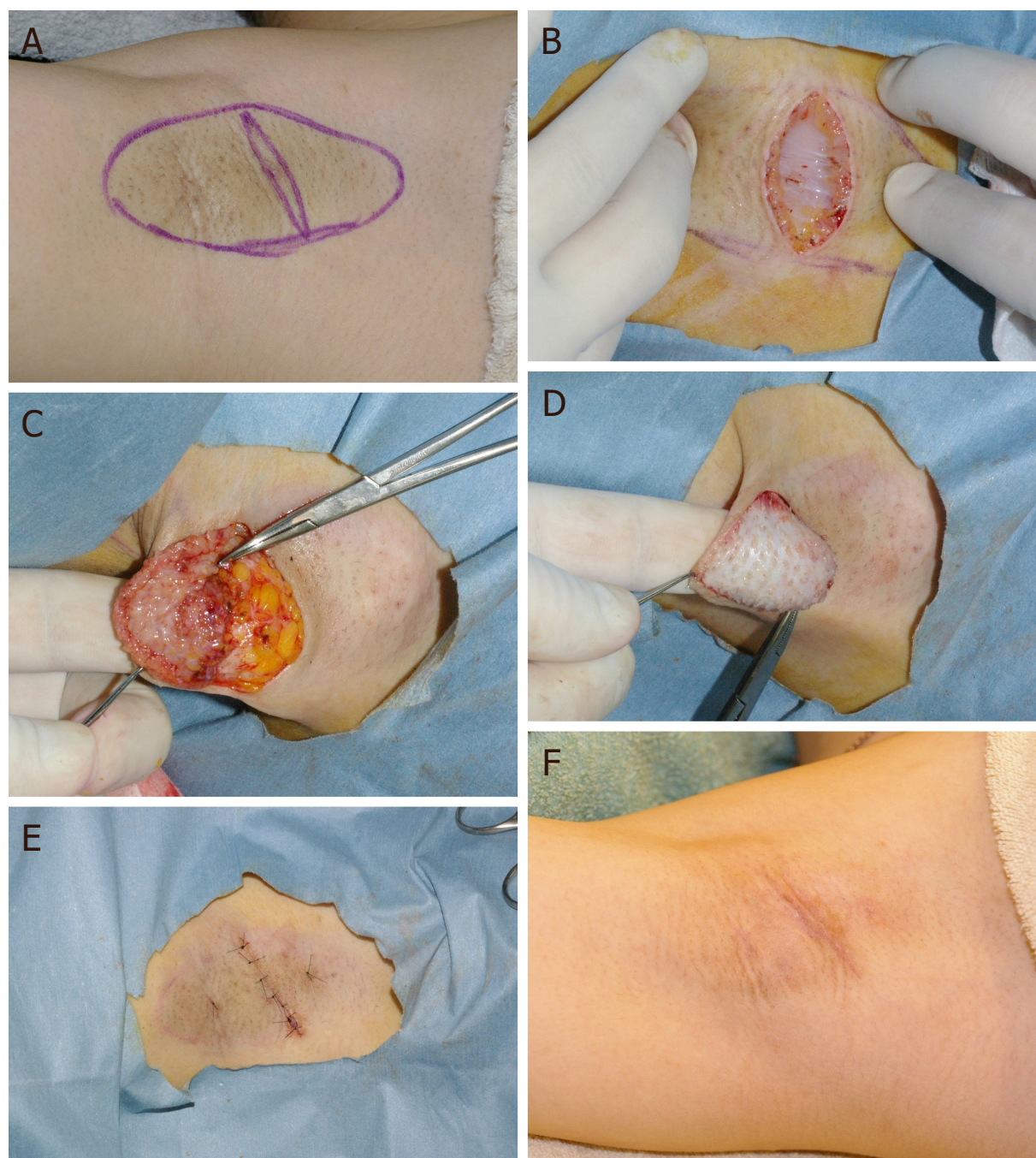


**Figure 1.** Closed-curettage surgery using the suction-assisted cartilage shaver system. A: the shaver is composed of an outer cannula with grid (left) and serrated inner cannula (right); B: the operating area is hair-bearing skin with a 5-mm margin; C: following subdermal undermining, the shaver is inserted from the edge of the marked region; D: the axillary skin is so thin that the shaver can be seen through the skin; E: the wound is closed with nylon sutures after insertion of a drainage tube, and two quilting sutures were made in this case; F: ten-month postoperative photograph. Note that axillary hair appears to have been almost permanently removed

The efficacy of each procedure was assessed by determining the rate of recurrence, which was mainly based on the patient's self-reported complaint or occasionally assessed by a physician performing the gauze test. The recurrence rate included overall assessments such as "not improved" or "ineffective", as well as "recurrence". The complication and recurrence rates of the patients who underwent SACS closed curettage were compared with those rates of the patients who underwent open excision.

Patients followed-up postoperatively for  $\geq 3$  months were included in this survey. Some patients graded the level of their subjective satisfaction for the entire treatment program (factors including surgical procedures, postoperative pain, discomfort, outcome, scar appearance, and cost) with a visual analog scale ranging from 0 (not satisfied) to 10 (fully satisfied).





**Figure 2.** Open excision procedures: A: the incision plan according to Rigg<sup>[22]</sup>; B: a small elliptical sliver of skin is removed, and the apocrine glands lined above the fascia are seen; C: after the subdermal layer of the axilla is undermined, the apocrine gland layer is seen; D: the apocrine gland layer is completely pruned, leaving the sebaceous glands; E: the wound is closed by 5/0 nylon sutures; two quilting sutures were made in this case. A drainage tube is inserted from the lower edge of incision wound; F: six-month postoperative photograph

### Statistical analysis

The mean ages of the two patient groups were compared by the Mann-Whitney *U*-test. Other categorical variables were compared by the chi-squared test. When the expected values were too small to be analyzed by the chi-squared test, the Fisher exact test or Yates continuity correction was used for the comparison. Statistical analysis was performed by Microsoft Excel 2010 (Microsoft, Redmond, WA, USA).  $P < 0.05$  was considered statistically significant.

**Table 1. Patient characteristics and postoperative outcomes of closed curettage by the SACS and open excision for axillary osmidrosis**

Procedure	SACS	Open excision	P
Number (male:female), <i>n</i>	91 (21:70)	168 (56:112)	0.09
Mean age $\pm$ SD, m	28.6 $\pm$ 7.6	28.0 $\pm$ 7.3	0.51
Odor levels, <i>n</i> (%) <sup>*</sup>			0.06
3	12 (13.2)	42 (25.0)	
4	53 (58.2)	90 (53.7)	
5	26 (28.6)	36 (21.3)	
Total complications, <i>n</i> (%) <sup>**</sup>	19 (10.4)	70 (20.8)	0.002
Hematoma	9 (4.9)	34 (10.1)	0.06
Wound dehiscence	0 (0.0)	21 (6.3)	< 0.001
Skin necrosis	3 (1.6)	11 (3.3)	0.09
Seroma	5 (2.7)	1 (0.3)	0.02
Others <sup>***</sup>	2 (1.1)	2 (0.6)	0.28
Recurrence, <i>n</i> (%)	2 (2.2)	2 (1.2)	0.61
Mean patient satisfaction <sup>****</sup>	8.5 ( <i>n</i> = 48)	8.8 ( <i>n</i> = 78)	0.10

<sup>\*</sup>Odor levels 1 (no odor) and 2 (faint odor) were not indicative for surgery; <sup>\*\*</sup>complications were assessed by axilla (*n* = 182 for SACS, *n* = 336 for open excision groups); <sup>\*\*\*</sup>other rare events included infection, sensory disturbance, and arterial injury; <sup>\*\*\*\*</sup>patient satisfaction was not assessed for all patients (*n* = 48 for SACS, *n* = 78 for open excision groups). SACS: suction-assisted cartilage shaver system

## RESULTS

### Patient characteristics

The characteristics of the patients are summarized in Table 1. A total of 188 patients underwent open excision and 91 patients underwent closed curettage by the SACS system. The male/female ratio, mean age, and odor levels were similar between the SACS and open-surgery patients. The duration of postoperative follow-up was 5.2 months (minimum 3 months, maximum 64 months), and the follow-up periods were statistically similar between the two patient groups.

### Safety and efficacy of the procedures

The complication rate of the patients treated by the SACS system was significantly lower than the complication rate of the patients undergoing open surgery (10.4% vs. 20.7%; *P* = 0.002). Table 1 shows detailed comparisons, and Figure 3 shows examples of the complications. The most frequent complication in either group was hematoma [Figure 3A], with no significant difference between the rates (*P* = 0.06). Wound dehiscence [Figure 3B] was seen only in patients undergoing open surgery. Delayed wound healing including skin necrosis and wound dehiscence was more frequently seen in patients undergoing open surgery (*P* < 0.001). Seroma [Figure 3C] was significantly more frequent in the patients treated by the SACS system (10.4%, *P* = 0.002).

In each treatment group, two patients had a recurrence or thought the outcome was unsuccessful, representing 1.1% of patients undergoing SACS closed curettage and 2.2% of patients undergoing open surgery. The difference was not significant (*P* = 0.614). Patient satisfaction for both procedures was very high (8.5/10 for SACS vs. 8.8/10 for open excision), with no significant difference (*P* = 0.010).

## DISCUSSION

### Previously reported cohorts

Several retrospective cohort studies have reported the treatment options for AO. For example, Park and Shin<sup>[23]</sup> compared conventional open excision, liposuction, laser vaporization, and ultrasonic aspiration, and they concluded that the best option was open excision. Chen *et al.*<sup>[24]</sup> compared laser coagulation and suction curettage, and concluded that suction curettage produced a higher incidence of complications than laser coagulation, but was more effective than laser coagulation. Yang *et al.*<sup>[5]</sup> recently compared





**Figure 3.** Examples of postoperative complications: A: hematoma after open excision; B: wound dehiscence after open excision; and C: seroma after closed curettage by the suction-assisted cartilage shaver system

the outcomes of microwave coagulation, suction curettage, and laser coagulation. They concluded that microwave coagulation and suction curettage were superior to laser coagulation, but the complication rate of suction curettage was much higher than the rates of the other two procedures. In the English-language literature, however, no studies have compared the two major surgical treatments (suction curettage and conventional open surgery). We believe that our study was meaningful because we compared the outcomes of these two procedures, 90% of which were performed by a single surgeon.

### SACS system

To our knowledge, only one comparative observation of the efficacy of curettage by the SACS system versus AO treatment by open excision has been published in Japanese<sup>[15]</sup>. The seven other English-language reports on AO treatment by closed-curettage SACS<sup>[2,14,16-20]</sup> were uncontrolled clinical observations or case series, as listed in Table 2. During early SACS procedures, the tip of the outer cannula was open, which often accidentally perforated the axillary skin during the procedure<sup>[14,15]</sup>. Therefore, curettage by SACS was performed under endoscopy. Later, most surgeons modified the procedure and used an outer cannula tip equipped with a grid, so that the apocrine glands could be safely removed while skin perforation and damage to the subdermal plexus were avoided [Figure 1A]. There are two different types of inner cannula tips, consisting of smooth or serrated blades. Most surgeons, including our group, have preferred the latter type of tip, because it more thoroughly removes the apocrine glands.

As shown in Table 2, the complication rate for the SACS system in our study (10.4%) was relatively higher than the rate in previous studies (0%-7.7%). The higher rate might be because we extensively undermined the hair-bearing skin plus a 5-mm margin to remove the apocrine glands thoroughly. A few authors spared fibrous cords and perforating vessels during curettage by the SACS system. Chern *et al.*<sup>[18]</sup> preserved fibrovascular bands and found a single adverse event involving 1 of 60 axillae. There were no recurrences. They found, however, that the mean efficacy rate was relatively low, since excellent results were obtained from only 67% of patients. Similarly, Hsu and Wang<sup>[19]</sup> preserved the subcutaneous fibrous septa, and did not observe any adverse events. However, 3 of 19 (15.8%) patients developed recurrence and underwent revision surgery. We concluded that the greater is the number of preserved fibrovascular bands, the greater is the number of remaining apocrine glands around the bands.

### Complications

Among the acute adverse events seen in this study, only the incidence of seroma in the patients undergoing SACS closed curettage was higher than the incidence in the patients undergoing open surgery (2.7% vs. 0.3%). Interestingly, in patients undergoing abdominoplasty, the incidence of seromas in the patients undergoing abdominoplasty combined with closed liposuction is higher than in patients undergoing conventional open abdominoplasty<sup>[25]</sup>. A seroma results from the rupture of lymph vessels, but the associated factors

**Table 2. Reports on closed curettage by suction-assisted cartilage shaver system for axillary osmidrosis**

Ref.	Patients (male:female), n	Mean age, year	Follow-up period, month	Complication, n (%)	Recurrence, n (%)	Type of shaver, inner/outer
Tung <sup>[14]</sup>	64 (21:43)	NR	6-13	5/128 (3.9)*	3/128 (2.3)	Serrated/open
Matsuda <sup>[15]</sup>	77 (20:57)	27	3-36	7/154 (4.5)*	2/77 (2.6)	Serrated/open + grid
Lee <i>et al.</i> <sup>[2]</sup>	89 (15:74)	NR	14-28	0/89 (0)	0/89 (0)	Smooth/grid
Wu <sup>[16]</sup>	156 (26:130)	23	6-59	12/156 (7.7)	4/156 (2.6)	Smooth/grid
Huang <i>et al.</i> <sup>[17]</sup>	70 (8:62)	26	6-35	NR	1/70 (1.4)	NR
Chern <i>et al.</i> <sup>[18]</sup>	30 (10:20)	22	3-13	1/60 (1.7)*	0/30 (0)	Smooth/grid
Hsu and Wang <sup>[19]</sup>	19 (4:15)	34	3	0/38 (0)*	3/19 (15.8)	NR
Tseng <i>et al.</i> <sup>[20]</sup>	39 (11:28)	26	12-69	0/39 (0)	0/39 (0)	NR
Current study	91 (21:70)	28.2	3-60	19/182 (10.4)*	2/91 (2.2)	Serrated/grid

\*Evaluation by axilla. NR: not reported

are thought to be multifactorial, including subdermal space, lymphatic drainage, the use of a cauterization device, and skin shearing<sup>[25]</sup>. Since the axillary region is well supplied with lymph vessels, we speculate that the mechanism involved in an axillary seroma is similar to that for an abdominal seroma. Seroma and hematoma in SACS closed-curettage procedures as well as in abdominoplasty might be avoided with an increased number of anchoring sutures and a longer drainage period. In fact, Tseng *et al.*<sup>[20]</sup> created multiple drainage holes and quilting sutures following the use of a shaver and did not find any adverse events. However, such additional procedures resulted in unsightly scarring.

### Efficacy

With regard to the assessment of efficacy, our study has some limitations. Postoperative efficacy was primarily assessed based on the patient's opinion, and complaints such as "recurrent odor", "not improved", or "not effective" were further reconfirmed by our gauze test. However, the perception of satisfaction varied based on the individual patient. Even for some patients who were satisfied with their procedure, recurrence might have been diagnosed by the gauze test. Second, although our minimum follow-up period was three months, which is similar to previously reported studies, the follow-up period might be too short. Wang *et al.*<sup>[4]</sup> compared the efficacy of their suction-curettage procedure with that of conventional open surgery, and they concluded that an evaluation of the final outcomes at three months after surgery might be too early. In fact, several authors have reported patients with axillary odor that recurred six months or longer after their procedure<sup>[1,5,15]</sup>. Thus, the recurrence rates in this study were possibly underestimated for both treatments.

### Emerging treatments

Recently, emerging treatments for axillary hyperhidrosis, such as microwave and radiofrequency technologies, have been reported to have some positive effects for AO as well<sup>[2,5]</sup>. Although the procedures using these new technologies are more costly than suction curettage procedures, the procedures are non-surgical and might provide permanent effects. Yang *et al.*<sup>[5]</sup> performed a retrospective cohort study that compared microwave technology and suction curettage and found that the recurrence rate was higher with microwave than with suction curettage, although the complication rate after microwave treatment was much lower than the rate after suction curettage. Further comparative studies between novel technologies and SACS closed curettage are needed with respect to complications and recurrence, as well as regarding cost, postoperative discomfort, recovery time, and patient satisfaction.

In conclusion, the results of previous studies have suggested that suction-curettage techniques for AO are much safer than conventional open surgery, but are inferior regarding recurrence rate. This study found that SACS closed curettage was as safe as other suction-curettage techniques, and as effective as conventional open surgery. In addition, the periods of discomfort due to dressings and limited range of motion in patients undergoing SACS closed curettage were half as long compared to those periods

in patients undergoing open surgery. Although further comparative studies are needed on emerging technologies versus SACS closed curettage, to date, SACS closed curettage should be considered an ideal surgical treatment for AO; however, decision-making regarding surgical treatment options for AO is affected by other factors, such as dressing discomfort, recovery time, scar formation, and cost. Our results should be helpful for both surgeons and patients.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study: Tanaka R, Morioka D, Akamine S, Kadomatsu K

Performed data analysis and interpretation: Tanaka R, Shimizu T

Performed data acquisition: Tanaka R, Morioka D

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

The Showa University Hospital institutional research board approved this study (approval no. 2928).

### Consent for publication

Written consent was obtained for publication for patient images.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Morioka D, Ohkubo F, Amikura Y. Clinical features of axillary osmidrosis: a retrospective chart review of 723 Japanese patients. *J Dermatol* 2013;40:384-8.
2. Lee JC, Kuo HW, Chen CH, Juan WH, Hong HS, et al. Treatment for axillary osmidrosis with suction-assisted cartilage shaver. *Br J Plast Surg* 2005;58:223-7.
3. Morioka D, Nomura M, Lan L, Tanaka R, Kadomatsu K. Axillary osmidrosis: past, present, and future. *Ann Plast Surg* 2019; Epub ahead of print. doi: 10.1097/SAP.0000000000002111.
4. Wang C, Wu H, Du F, Le S, Zheng S. Axillary osmidrosis treatment using an aggressive suction-curettage technique: a clinical study on paired control. *Aesthet Plast Surg* 2015;39:608-15.
5. Yang HH, Miao Y, Chen YT, Hu ZQ. Minimally invasive approaches to axillary osmidrosis treatment: a comparison between superficial liposuction with automatic shaver curettage, subcutaneous laser treatment, and microwave-based therapy with a modified technique. *J Cosmet Dermatol* 2018;18:594-601.
6. Inaba M, Ezaki T. New instrument for hircismus and hyperhidrosis operation: subcutaneous tissue shaver. *Plast Reconstr Surg* 1977;59:864-6.
7. Skoog T, Thyresson N. Hyperhidrosis of the axillae, a method of surgical treatment. *Acta Chir Scand* 1962;124:531-8.
8. Li ZR, Sun CW, Zhang JY, Qi YQ, Hu JZ. Excision of apocrine glands with preservation of axillary superficial fascia for the treatment of axillary bromhidrosis. *Dermatol Surg* 2015;41:640-4.
9. Dai Y, Xu AE, He J. A refined surgical treatment modality for bromhidrosis: Subcutaneous scissor with micropore. *Dermatol Ther* 2017;30:e12484.
10. Liu Q, Zhou Q, Song Y, Yang S, Zheng J, et al. Surgical subcision as a cost-effective and minimally invasive treatment for axillary



- osmidrosis. *J Cosmet Dermatol* 2010;9:44-9.
11. Ou LF, Yan RS, Chen IC, Tang YW. Treatment of axillary bromhidrosis with superficial liposuction. *Plast Reconstr Surg* 1998;102:1479-85.
  12. Shi Z, Yan X, Ye X. Modified tumescent superficial suction with curettage treatment for axillary bromhidrosis: clinical experience of 280 cases. *Aesthet Plast Surg* 2014;38:151-5.
  13. Kim WO, Song Y, Kil HK, Yoon KB, Yoon DM. Suction-curettage with combination of two different cannulae in the treatment of axillary osmidrosis and hyperhidrosis. *J Eur Acad Dermatol Venereol* 2008;22:1083-8.
  14. Tung TC. Endoscopic shaver with liposuction for treatment of axillary osmidrosis. *Ann Plast Surg* 2001;46:400-4.
  15. Matsuda K. Surgical treatment of axillary osmidrosis with the shaver system. *Jpn J Plast Surg* 2004;47:1253-9. (in Japanese)
  16. Wu WH. Ablation of apocrine glands with the use of a suction-assisted cartilage shaver for treatment of axillary osmidrosis. An analysis of 156 cases. *Ann Plast Surg* 2009;62:278-83.
  17. Huang YH, Yang CH, Chen YH, Chen CH, Lee SH. Reduction in osmidrosis using a suction-assisted cartilage shaver improves the quality of life. *Dermatol Surg* 2010;36:1573-7.
  18. Chern E, Yau D, Chuang FC, Wu WM. Arthroscopic shaver with refinement for axillary osmidrosis. *Int J Dermatol* 2010;49:813-7.
  19. Hsu KC, Wang KY. Sparing subcutaneous septa avoids skin necrosis in the treatment of axillary bromhidrosis with suction-curettage shaving. *J Cosmet Dermatol* 2019;18:892-6.
  20. Tseng YJ, Lee CH, Lin SH. Modified suction-assisted cartilage shaver for axillary osmidrosis. *Biomed Res Int* 2019;2019:7314753.
  21. Shin JY, Roh SG, Lee NH, Yang KM. Osmidrosis treatment approaches. A systematic review and meta-analysis. *Ann Plast Surg* 2017;78:354-9.
  22. Rigg BM. Axillary hyperhidrosis. *Plast Reconstr Surg* 1977;59:334-42.
  23. Park YJ, Shin MS. What is the best method for treating osmidrosis? *Ann Plast Surg* 2001;47:303-9.
  24. Chen YT, Shih PY, Chen HJ, Chen TJ. Treatment of axillary osmidrosis: a comparison between subcutaneous laser and superficial liposuction curettage. *J Eur Acad Dermatol Venereol* 2015;29:2019-23.
  25. Vidal P, Berner JE, Will PA. Managing complications in abdominoplasty: a literature review. *Arch Plast Surg* 2017;44:457-8.

Review

Open Access



# Flap reconstruction of the abdominal wall

Sneha Patel, Alexander F. Mericli, Sahil K. Kapur, Margaret S. Roubaud, Charles E. Butler

Department of Plastic Surgery, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA.

**Correspondence to:** Dr. Alexander F. Mericli, Department of Plastic Surgery, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030, USA. E-mail: [afmericli@mdanderson.org](mailto:afmericli@mdanderson.org)

**How to cite this article:** Patel S, Mericli AF, Kapur SK, Roubaud MS, Butler CE. Flap reconstruction of the abdominal wall. *Plast Aesthet Res* 2020;7:18. <http://dx.doi.org/10.20517/2347-9264.2019.15>

**Received:** 26 Aug 2019 **First Decision:** 24 Mar 2020 **Revised:** 31 Mar 2020 **Accepted:** 10 Apr 2020 **Published:** 17 Apr 2020

**Science Editor:** Raúl González-García **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

Large abdominal cutaneous defects may occur in association with complex ventral hernias, trauma, tumor resection, necrotizing infections or septic evisceration. Soft tissue reconstruction of the abdominal wall is performed when there is insufficient adipocutaneous tissue to permit standard, primary closure. A number of reconstructive techniques are available, the choice of which is based on a number of factors, including the size and location of the defect, etiology, and timing of closure. In general, local fasciocutaneous advancement flaps and adjacent tissue rearrangement are the workhorse techniques, followed by regional myocutaneous flaps and free tissue transfers for the most complex and extensive of defects. Herein, we describe our approach to abdominal soft tissue reconstruction, indications, technical nuances, and management of complications.

**Keywords:** Abdominal wall reconstruction, surgical flaps, pedicled flap, free flap, bioprosthetic mesh, hernia, reconstructive surgical procedures

## INTRODUCTION

Abdominal wall defects can occur in association with ventral hernias, trauma, tumors, infections or septic evisceration. The abdominal wall is best conceptualized as a trilaminar structure, with each layer serving a specific purpose: the muscular layer provides dynamic support and function, the fascial layer confers strength and durability, and the skin and subcutaneous tissue serves as a barrier to infection and provides a uniform, aesthetic contour. In abdominal wall reconstruction, each layer must be addressed and repaired to obtain an optimal result. Contemporary methods of abdominal wall reconstruction emphasize synthetic or bioprosthetic mesh for fascial repair and reinforcement, primary myofascial coaptation for a functional, durable and dynamic repair, and a variety of soft tissue flaps and rearrangement techniques to address skin deficits.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Optimizing skin and soft tissue coverage is of utmost importance in order to reduce the risk of developing a surgical site infection or surgical site occurrences and to expedite recovery<sup>[1-3]</sup>. This is particularly important in the oncologic population, in which a healed wound is imperative prior to initiating adjuvant chemotherapy or radiation therapy. Because the requirement for skin and soft-tissue reconstruction of the abdominal wall intimates a deficiency of local tissue available for resurfacing a defect, most abdominal wall defects are reconstructed using flaps from redundant adjacent tissue in the torso; however, certain cases may require pedicled regional flaps or even free tissue transfer depending on the size of the defect, location, and the patient's body habitus. The reconstructive ladder is a useful framework for guiding abdominal soft tissue reconstruction in most clinical scenarios. However, we have found the M.D. Anderson oncologic abdominal wall reconstruction classification system to be particularly beneficial for the unique needs of the oncologic population<sup>[4]</sup>. At a minimum, planning for abdominal soft tissue reconstruction must take into consideration the defect type, location, and the viability and perfusion of the surrounding tissues. Although a variety of options are available to the reconstructive surgeon for abdominal soft tissue defects, there are a number of key points and technical nuances that, when implemented appropriately, can help to ensure an acceptable result with minimal complications.

## LOCAL FLAP OPTIONS

Local flaps involve recruiting tissue adjacent to the wound defect. Well-planned incisions and a thorough understanding of the abdominal wall angiosomes is necessary to execute this collection of techniques. Regarding perfusion, local flaps can be designed as having either random-pattern or axial blood supply. There are numerous local flap types, including rotation/advancement, interpolation, V-Y advancement, keystone flaps, propeller flaps, and bipedicled flaps. Most commonly, the flap donor site is closed primarily, however a skin graft can alternatively be employed for this purpose, as in the bipedicled flap.

Pre-existing scars and closure tension are two important factors to consider in local flap reconstruction. For instance, a midline laparotomy scar may preclude designing a local flap along the contralateral abdominal wall. Regarding tension, the area of the local flap that is most important for the reconstruction is also the area that is most vulnerable to reduced perfusion: the most distal point. Reduced perfusion is exacerbated by excessive flap inset tension, therefore it is important to design wide-based, large local flaps, recruiting tissue from areas of relative redundancy, in order to mitigate this possibility. In addition, the thoughtful surgeon should account for expected postoperative edema and abdominal distention in the flap design and inset technique.

A propeller flap is a local fasciocutaneous flap that can be rotated up to 180 degrees in relation to its perforator. Perforator propeller flaps have been well described for extremity and chest wall reconstruction, however their use in abdominal reconstruction is more limited<sup>[5]</sup>. These flaps can be used to cover abdominal defects, recruiting flap tissue from an area with relative adipocutaneous redundancy compared to the recipient site. Propeller flaps are best used for smaller defects, as the donor site should be able to close primarily [Figure 1]. Familiarity with perforator dissection and microsurgical technique is also necessary, which may limit the applicability of this flap type.

A keystone flap is a fasciocutaneous flap composed of two V to Y advancements<sup>[6]</sup>. Unlike most other local flaps, keystone flap mobility is not facilitated by undermining. Instead, undermining should be minimized, in order to keep all underlying cutaneous perforators intact. For any defect, unilateral or bilateral flaps can be designed depending on the size and location of the area of skin deficit. The width of each flap should be at least as wide as the defect and the flap and defect length should be equal. The double VY closures work to advance the flap toward the defect, facilitating a tension-free defect closure.



**Figure 1.** Fasciocutaneous defect of lateral abdominal wall after resection of an irradiated sarcoma. Reconstructed with fasciocutaneous propeller flap from lumbar segmental perforating vessel

## REGIONAL FLAP OPTIONS

When local tissue is insufficient to fill the defect, a regional flap can be considered. Regional flaps rely on a named blood vessel (pedicle) for perfusion and are commonly harvested from adjacent anatomic areas such as the chest, groin, thigh or contralateral abdomen. They can be designed as a fasciocutaneous, myocutaneous or muscle-only flaps, depending on the defect requirements. Options for reconstruction of the abdominal wall with a regional flap include use of the external oblique muscle<sup>[7]</sup>, tensor fascia lata myocutaneous (TFL)<sup>[8,9]</sup>, rectus abdominis myocutaneous, rectus femoris myocutaneous<sup>[10]</sup>, anterolateral thigh fasciocutaneous (ALT) with or without a portion of vastus lateralis muscle<sup>[11]</sup>, latissimus dorsi muscle or myocutaneous and omental flaps<sup>[12]</sup>. Each flap has associated advantages and disadvantages which must be taken into consideration when designing the reconstruction [Table 1].

Since many regional flaps include a muscle, it is important to consider donor morbidity. Although a contralateral rectus abdominis muscle can be used to reconstruct a portion of the abdominal wall, the weakness and hernia potential incurred by moving this muscle may preclude its use. Similarly, the rectus femoris can be used to reconstruct the infraumbilical abdomen, however using this muscle may limit the terminal fifteen degrees of knee extension of the donor leg; this may be acceptable for some patients but not for others. This can be limited or eliminated by performing a tenorrhaphy between the vastus lateralis and medialis tendons superior to the patella. A regional flap's arc of rotation must be carefully measured to ensure it will reach the desired location without pedicle tension. Furthermore, the path the pedicle takes from its origin to the defect location must be planned in order to avoid compression and kinking. If the flap is to be passed through a subcutaneous tunnel from donor site to recipient site, the tunnel must be wide to allow for swelling without pedicle compression. For the pedicled ALT flap, the flap must be passed deep to the rectus femoris and sartorius muscles to allow adequate reach and minimize pedicle compression [Figure 2]. This is critical in preventing compression of the flap or pedicle postoperatively when the patient is mobile and exerting rotational, flexion, and extension forces on the torso.

## FREE FLAP OPTIONS

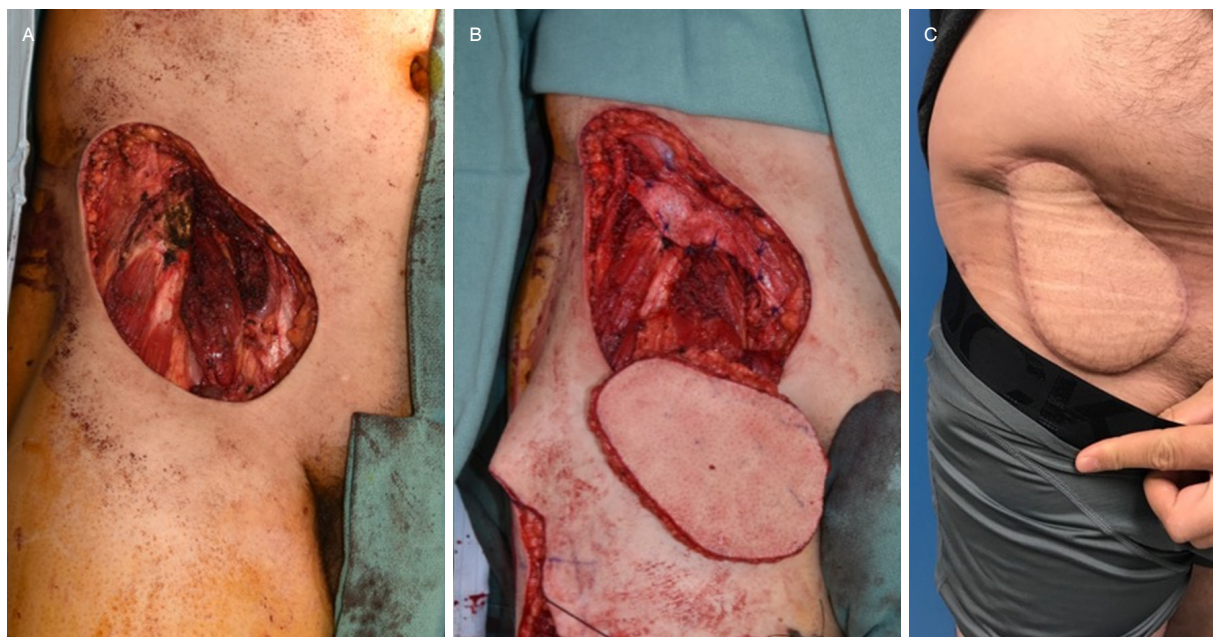
Free tissue transfer for abdominal wall reconstruction is relatively uncommon, considering the adequate local soft tissue and numerous regional flap options present in most patients. However, in certain clinical



**Table 1. Regional flap options for abdominal wall soft tissue reconstruction**

Flap name	Donor site	Possible recipient sites	Pedicle	Components	Disadvantages
ALT <sup>[11]</sup>	Anterolateral thigh	Infraumbilical abdomen	Descending branch of the lateral femoral circumflex	Fasciocutaneous	Limited arc of rotation Flap width limited to 8 cm to permit primary closure of thigh donor site
External oblique <sup>[7]</sup>	Anterolateral abdominal wall	Upper two thirds of the abdominal wall	Lateral branches of posterior intercostal vessels	Myocutaneous	Limited arc of rotation Distal flap tip perfusion unreliable in fasciocutaneous design
TFL <sup>[8,9]</sup>	Lateral thigh	Lower two thirds of the abdominal wall	Ascending branch of lateral femoral circumflex artery	Myocutaneous	Unreliability of distal one third of skin paddle
Rectus femoris <sup>[10]</sup>	Anterior thigh	Lower two thirds of the abdominal wall	Descending branch of lateral femoral circumflex artery	Myocutaneous	Donor site morbidity and limited terminal knee extension
Omentum <sup>[12]</sup>	Omentum	Entire abdominal wall	Right or left gastroepiploic arteries	Fat, connective tissue and lymphatics	Omentum must be resurfaced with a skin graft Potential for intraabdominal injury
Subtotal thigh <sup>[13]</sup>	Thigh	Entire abdominal wall	Lateral circumflex femoral artery	Fasciocutaneous or myocutaneous	Skin grafted donor site Limited terminal knee extension with inclusion of rectus femoris

ALT: anterolateral thighfasciocutaneous; TFL: tensor fascia lata myocutaneous



**Figure 2.** 9 cm × 18 cm defect of right inferolateral abdominal wall with resected inguinal ligament (A). Inguinal ligament and abdominal wall reconstructed with partial underlay-bridged bioprosthetic mesh and soft tissue reconstructed with pedicled anterolateral thigh fasciocutaneous flap (B). Fully healed reconstruction six months postoperatively (C)

scenarios involving particularly large full thickness defects or in patients with many prior surgeries, a free flap may be the most optimal choice for abdominal soft tissue reconstruction [Figure 3]. Such a flap can be designed with varying size, dimension, and composition, lending more flexibility than local or regional flaps. Free flaps should be considered when local or regional flaps are not present within reach of the defect or too small to cover the defect. Most commonly, free flaps for abdominal wall reconstruction are designed from the thigh or back<sup>[14]</sup>.

In cases requiring a large skin paddle, a free flap based on the subscapular vessel system may be ideal. This particular flap can include the fasciocutaneous scapular or parascapular tissues as well as the latissimus and/or serratus anterior muscles, if a chimeric design is needed<sup>[15]</sup>. Indeed, this chimeric, conjoined





**Figure 3.** 20 cm × 20 cm full thickness defect of abdominal wall with exposed viscera (A). Myofascial defect reconstructed with a bridged bioprosthetic mesh and free anterolateral thigh myocutaneous flap anastomosed to the right deep inferior epigastric vessels (B). Healed and viable reconstruction 1 month postoperatively; anterolateral thigh flap donor site closed with skin graft (C)

flap provides well-vascularized soft tissue to resurface a defect of up to a 1500 cm<sup>2</sup>. However, it bears mentioning that if the latissimus muscle is included, donor site morbidity must be considered as it relates to the weakened abdominal wall. Patients who have decreased core muscle strength as a result of a composite abdominal wall resection will depend upon upper extremity strength and range of motion more so than is usual. Simple actions - such as rising from a seated or supine position - extensively utilize the trunk musculature. If core strength has been reduced, the upper extremities will be needed to compensate for the lack of trunk stability. Therefore, in patients requiring a large free flap, preference should be given to lower-morbidity fasciocutaneous flap options, such as the thoracodorsal artery perforator, scapular/parascapular, or fasciocutaneous subtotal thigh. In addition, if a posterior trunk flap is chosen, the patient must undergo an intra-operative position change to elevate the flap on the posterior chest wall, then another position change to supine to inset the flap. The surgeon must be aware of these logistical issues, since intraoperative repositioning adds complexity, prolongs the operative duration, and extends flap ischemia time.

The thigh represents the mainstay for free flap donor sites for the abdominal wall [Figure 3]. Pedicled thigh flaps can reach the infraumbilical abdomen, however if the defect is larger or located outside the arc of rotation, the thigh flap must be designed as a free tissue transfer. The descending branch of the lateral circumflex femoral system provides blood supply to the vastus lateralis, rectus femoris muscles and anterolateral thigh skin. The ascending or transverse branch of the lateral circumflex femoral system provides blood supply to the tensor fascia lata muscle. These flaps can be harvested as muscle only flaps or as myocutaneous flaps with overlying skin paddles. The anterolateral thigh flap is particularly versatile and can be designed with or without a segment of vastus lateralis muscle. The tensor fascia lata flap can be designed to include the distal fascia of the iliotibial tract and a smaller proximal skin paddle if needed; this fascia can be used to reconstruct the fascial component of an abdominal wall defect, providing vascularized tissue for reconstruction in lieu of mesh<sup>[9,16]</sup>. The anteromedial thigh flap can be designed on medial perforators from the descending branch of the lateral circumflex femoral system. The rectus femoris muscle is more commonly designed as a muscle flap however a skin island can be included over the central muscle when appropriate sized cutaneous perforators are present<sup>[13]</sup>. Similar to the subscapular system, the lateral femoral circumflex vessels allow for chimeric flap design, i.e., ALT with anteromedial thigh flaps, ALT with TFL, vastus lateralis with TFL. For massive abdominal wall defects the vastus lateralis, tensor fascia lata, and the rectus femoris can be harvested with all overlying skin territory as a subtotal thigh flap for increased volume and skin coverage<sup>[13]</sup>.

Easily accessible free flap recipient vessels along the abdominal wall include the deep inferior epigastric, internal mammary, and deep circumflex iliac vessels. However, healthy recipient vessels may be absent

or inadequate, due to injury from prior surgery, trauma, or radiation. In this situation, vein grafts can be used to increase pedicle length. Common distant recipient vessels necessitating vein grafting include the superficial femoral, descending branch of the lateral femoral circumflex, internal mammary, and thoracodorsal.

As is true for any free tissue transfer, regardless of the area reconstructed, the flap should be examined and evaluated with pencil Doppler ultrasonography every hour for the first 48-72 h. Any changes in flap perfusion warrant a return to the operating room for exploration of the microvascular anastomosis.

## **POSTOPERATIVE CARE AND COMPLICATION MANAGEMENT**

A patient with abdominal wall reconstruction is managed similarly to a patient with any major abdominal surgery in the postoperative period. Diet is advanced in accordance with the return of bowel function, as is standard fashion. Although an abdominal binder is commonly used in cases of ventral hernia repair and abdominal wall reconstruction, a binder may be detrimental in flap cases due to the additional extrinsic pressure it transmits to the flap and vascular pedicle. Therefore, binder utilization may be delayed until the flap has developed adequate collateral circulation. Antiemetics, and if necessary, nasogastric tube decompression, are employed to minimize postoperative nausea and retching which may stress the repair. Similarly, aggressive postoperative pulmonary toilet is important to minimize coughing.

Closed-suction drains should be employed liberally for any surgery in which there is a significant amount of soft tissue undermining or dead space creation<sup>[2,17]</sup>. Postoperative prophylactic antibiotics should be considered in clean contaminated cases and/or in cases involving mesh<sup>[1-3]</sup>. Closed-incision negative pressure dressings should be considered, as they have been demonstrated to contribute to a reduced surgical site infection and surgical site occurrences rate in complex abdominal surgery<sup>[18]</sup>. We counsel all abdominal wall reconstruction patients to avoid lifting greater than 10 lbs. for 2-3 months following surgery in order to minimize hernia occurrence.

## **CONCLUSION**

A reliable skin closure is imperative for a durable, functional, and cosmetically-acceptable abdominal wall. Depending on the defect characteristics a variety of local, regional, or free flaps are available for reconstruction of the abdominal skin and subcutaneous tissue. Each flap has inherent advantages and disadvantages, necessitating a tailored approach to each individual patient.

## **DECLARATIONS**

### **Authors' contributions**

Participated in the accumulation of data, literature review, writing, and editing this manuscript: Patel S, Mericli AF, Kapur SK, Roubaud MS, Butler CE

### **Availability of data and materials**

Not applicable.

### **Financial support and sponsorship**

None.

### **Conflicts of interest**

All authors declared that there are no conflicts of interest.

### **Ethical approval and consent to participate**

Not applicable.

## Consent for publication

A written informed consent for publication was obtained.

## Copyright

© The Author(s) 2020.

## REFERENCES

1. Luijendijk RW, Hop WC, van den Tol MP, de Lange DC, Braaksma MM, et al. A comparison of suture repair with mesh repair for incisional hernia. *N Engl J Med* 2000;343:392-8.
2. Wong A, Lee S, Nathan NS, Wang F, Hansen SL, et al. Postoperative prophylactic antibiotic use following ventral hernia repair with placement of surgical drains reduces the postoperative surgical-site infection rate. *Plast Reconstr Surg* 2016;137:285-94.
3. Garvey PB, Martinez RA, Baumann DP, Liu J, Butler CE. Outcomes of abdominal wall reconstruction with acellular dermal matrix are not affected by wound contamination. *J Am Coll Surg* 2014;219:853-64.
4. Mericli AF, Baumann DP, Butler CE. Reconstruction of the abdominal wall after oncologic resection: defect classification and management strategies. *Plast Reconstr Surg* 2018;142:187-96S.
5. Teo TC. The propeller flap concept. *Clin Plast Surg* 2010;37:615-26.
6. Mohan AT, Rammos CK, Akhavan AA, Martinez J, Wu PS, et al. Evolving concepts of keystone perforator island flaps (KPIF): principles of perforator anatomy, design modifications, and extended clinical applications. *Plast Reconstr Surg* 2016;137:1909-20.
7. Alexander LG, Pavletic MM, Engler SJ. Abdominal wall reconstruction with a vascular external abdominal oblique myofascial flap. *Vet Surg* 1991;20:379-84.
8. Tukiainen E, Leppäniemi A. Reconstruction of extensive abdominal wall defects with microvascular tensor fasciae latae flap. *Br J Surg* 2011;98:880-4.
9. Dorai AA, Halim AS. Extended double pedicle free tensor fascia latae myocutaneous flap for abdominal wall reconstruction. *Singapore Med J* 2007;48:e141-5.
10. Landim FM, Tavares JM, Costa ML, Landim RM, Feitosa RG. Complex abdominal wall reconstruction after radiation therapy: a full-thickness defect was repaired with a rectus femoris myofasciocutaneous flap. *Am J Obstet Gynecol* 2009;200:116.e1-3.
11. Kimata Y, Uchiyama K, Sekido M, Sakuraba M, Iida H, et al. Anterolateral thigh flap for abdominal wall reconstruction. *Plast Reconstr Surg* 1999;103:1191-7.
12. Manay P, Khajanchi M, Prajapati R, Satoskar R. Pedicled omental and split skin graft in the reconstruction of the anterior abdominal wall. *Int J Surg Case Rep* 2014;5:161-3.
13. Lin SJ, Butler CE. Subtotal thigh flap and bioprosthetic mesh reconstruction for large, composite abdominal wall defects. *Plast Reconstr Surg* 2010;125:1146-56.
14. Sacks JM, Broyles JM, Baumann DP. Flap coverage of anterior abdominal wall defects. *Semin Plast Surg* 2012;26:36-9.
15. Hallock GG. The combined parascapular fasciocutaneous and latissimus muscle conjoined free flap. *Plast Reconstr Surg* 2008;121:101-7.
16. Williams JK, Carlson GW, Dechalain T, Howell R, Coleman JJ. Role of tensor fasciae latae in abdominal wall reconstruction. *Plast Reconstr Surg* 1998;101:713-8.
17. Janis JE, Khansa L, Khansa I. Strategies for postoperative seroma prevention: a systematic review. *Plast Reconstr Surg* 2016;138:240-52.
18. de Vries FEE, Atema JJ, Lapid O, Obdeijn MC, Boormeester MA. Closed incision prophylactic negative pressure wound therapy in patients undergoing major complex abdominal wall repair. *Hernia* 2017;21:583-9.

Review

Open Access



# Surgical debulking, lymphatico venous anastomosis, vascularised lymph node transfer in lower limb lymphoedema

Hari Venkatramani, Rajasabapathy Raja Shanmugakrishnan, Murugesan Senthil Kumaran, Shanmuganathan Raja Sabapathy

Plastic, Hand & Reconstructive Microsurgery & Burns, Ganga Hospital, Coimbatore 641043, India.

**Correspondence to:** Hari Venkatramani, Plastic, Hand & Reconstructive Microsurgery & Burns, Ganga Hospital, Coimbatore 641012, India. E-mail: drhariv@gmail.com

**How to cite this article:** Venkatramani H, Shanmugakrishnan RR, Kumaran MS, Sabapathy SR. Surgical debulking, lymphatico venous anastomosis, vascularised lymph node transfer in lower limb lymphoedema. *Plast Aesthet Res* 2020;7:19. <http://dx.doi.org/10.20517/2347-9264.2019.70>

**Received:** 7 Dec 2019 **First Decision:** 4 Mar 2020 **Revised:** 30 Mar 2020 **Accepted:** 7 Apr 2020 **Published:** 17 Apr 2020

**Science Editor:** Xiao Long **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

Lymphoedema is a chronic debilitating disease of the lymphatic system that occurs due to either abnormal development or damage of the lymphatics resulting from cancer or infection. The optimal treatment of lymphoedema is still elusive. Management is tailored according to clinical features, investigations and expectations of each patient. Lymphoedema patients should undergo a trial of conservative management with compression therapy, manual lymphatic drainage and external sequential compression devices. Early lymphoedema is treated by lymphovascular anastomosis, where the lymph vessels are connected to the subdermal veins by supermicrosurgery. In late cases when the limb is fibrotic, vascularised lymph node transfers are done, where lymph nodes are transferred from a healthy area to the affected area. In advanced cases, when the limb is fibrotic with cutaneous folds and skin changes, surgical debulking is done. In lymphoedema, along with accumulation of lymphatic tissue, there is also fat deposition, which can be removed by liposuction. One should be conversant with all treatment modalities to provide the lymphoedema patient with optimal care.

**Keywords:** Lymphoedema, lymphovascular anastomosis, vascularised lymph node transfers, liposuction, surgical debulking

## INTRODUCTION

Lymphoedema is a chronic debilitating disease of the lymphatic system that affects more than 250 million people worldwide<sup>[1]</sup>. Lymphoedema results from abnormal development or damage of the lymphatics due



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



to various causes such as filariasis or more commonly due to the various cancer treatment modalities. The resultant accumulation of lymphatic fluid causes persistent swelling of the limb, fat hypertrophy, tissue fibrosis, ulceration, infection and sepsis. Lymphoedema progresses through four stages<sup>[2]</sup>. Stage 0 indicates a clinically normal limb, but with abnormal lymph transport. Stage 1 shows early pitting oedema which improves with limb elevation. In stage 2, there is limb oedema that does not improve with limb elevation. Stage 3 represents pitting limb oedema with skin changes such as acanthosis, further deposition of fat and fibrosis and warty overgrowths. Currently, there is no cure for lymphoedema, and the optimal treatment is yet to be determined. The treatment for lymphoedema starts with conservative management followed by surgery if necessary. The various surgical modalities for lymphoedema are detailed in this review.

Lymphoedema management should ideally start with non-surgical modalities such as compression therapy, lymphoedema-specific manual lymphatic drainage and use of external sequential pneumatic compression devices<sup>[3]</sup>. This would not only reduce the amount of lymphoedema but would also facilitate surgical procedures by making the skin more pliable and soft. Lee recommends using non-surgical modalities for 2 years before attempting any surgical procedure<sup>[4]</sup>. The patient should be encouraged and counselled regarding the importance of conservative measures which can be difficult to achieve. Compliance is a problem as it can be costly, time-consuming and uncomfortable<sup>[5]</sup>.

Patients with early-stage lymphoedema benefit from physiological procedures such as lymphovenous anastomosis (LVA), where the lymphatics are connected to the veins or by vascularised lymph node transfers (VLNTs), in which lymph nodes from one part of the body are transferred to the affected area to drain excess lymphatic fluid. Liposuction is done for those patients whose swelling is mainly due to excess fat. For patients with end-stage disease and large fibrotic limbs, conservative therapy can be ineffective, and excisional surgery alone or in combination with other physiological procedures such as VLNT and LVA, is performed.

## LVA

LVA is a microsurgical approach used to augment the return of lymph to the blood circulation in which lymphatic vessels smaller than 0.8 mm are connected to the subdermal venules using fine microsurgical sutures, instruments and high resolution magnification microscopes<sup>[6]</sup>. LVA is used to treat early stage lymphoedema. Chang *et al.*<sup>[7]</sup> found that LVA was more effective when used for early stage lymphoedema (Stages 1 & 2) when compared to late stage lymphoedema (Stages 3 & 4) according to the M.D. Anderson Classification based on indocyanine green (ICG) lymphangiography. They postulated that this observation was because LVA needs intact functional lymphatic channels and minimal irreversible tissue fibrosis for it to be effective. In later stages, LVA could be combined with VLNT.

The patient is advised to wear compression stockings without exception during the day for at least 6 months to reduce oedema before LVA is attempted. The stockings are to be replaced with a new pair if they get loose. The patients are also told that they would have to continue wearing the compression stockings even after surgery to get the best results. Although LVA can be done without ICG lymphangiography, this imaging technique helps to assess the severity of lymphoedema and determine whether LVA would be effective, and it also aids in localising the position of the lymphatic channels in the limb<sup>[8]</sup>. The position of the lymphatic channels is marked using a pen on the skin. ICG lymphography can detect the position of the lymphatic channels only up to a depth of 10 mm from the skin surface. LVA can be done under regional or general anaesthesia under tourniquet control, or it can be done with a local anaesthetic containing adrenaline to limit bleeding from the dermal edges. A 3-cm incision is made where the lymphatic ducts are located by ICG lymphography. Although there is no consensus on the number of anastomoses needed to obtain a significant reduction in lymphoedema, it is believed that increasing the number of LVA can improve lymphoedema better<sup>[9]</sup>. If ICG lymphography is not available, 5 to 10 incisions can be made



on the medial and lateral side of the affected extremities as the lymphatics are more common in these areas. Isosulfan blue (Lymphazurin; Covidien) is injected just distal to the incision site. The dye easily gets absorbed into the lymphatic channels and facilitates easy visualisation of the lymphatic channels. In advanced lymphoedema, the visualisation of the dye can be less due to weaker staining because of reduced transport of the dye<sup>[10]</sup>. Small subdermal venules less than 0.5 mm with no backflow are preferably chosen for LVA since they have low intravascular pressure. Larger veins with backflow are associated with higher intravascular pressure and obstruction of the anastomotic site.

Recently, magnetic resonance lymphangiography (MRL) has been added to the armamentarium to help localise the lymphatic channels and venules for LVA<sup>[11]</sup>. A mixture of paramagnetic contrast medium containing gadobenate dimeglumine and lignocaine is injected subcutaneously/intradermally in the webspaces of both feet and MRI is performed. MRL diagnoses lymphoedema, grades the lymphoedema on the basis of the lymphatic drainage pattern and the delay of drainage, provides the number, diameter, course, and depth from the skin of both affected lymphatic vessels and the nearest veins, the distance between the lymphatics and the venules, and the location of the lymph nodes. Although the spatial orientation of the lymphatics that MRL provides along with no radiation is a highlight, it has some disadvantages in that it is costly, not freely available and time-consuming, and has occasional difficulty in distinguishing the affected lymphatic vessel from the adjacent vein when there is dye contamination in the venous system.

Once the appropriate lymphatic vessels and veins are identified, anastomosis is done between the lymphatic channels and the veins. This can be a technical challenge as the veins and the lymphatics are very small. Special instruments, sutures finer than 10-0 and high resolution microscopes with magnification of 20× to 30× are needed to perform this procedure. The anastomosis between lymphatics and veins are most commonly done in an end-to-end manner. The lymphatic vessel is smaller and thinner and often collapses, making it difficult to place the needle in the vessel. To increase the size of the lymphatic vessel, Yamamoto *et al.*<sup>[12]</sup> proposed clamping the proximal lymphatics and massaging distally. Narushima *et al.*<sup>[13]</sup> proposed using a 6-0 prolene suture as a stent within the lymphatic vessel to prevent suturing the backwall during anastomosis. When the vein is much smaller than the lymphatic vessel, the end of the vein can be sutured to the side of the lymphatic vessel in a side-to-end manner<sup>[14]</sup>. There is also a concern that the higher pressure in the vein can lead to obstruction of the anastomosis. Some proponents believe that the most optimal orientation would be to anastomose the end of the vein to the side of the lymphatic vessel, thus allowing the bidirectional flow of lymphatics to the veins<sup>[15]</sup>. This could also be achieved by anastomosing both the proximal and the distal cut ends of the lymphatics to veins<sup>[16]</sup>. Chen *et al.*<sup>[17]</sup> have proposed the “octopus lymphovascular anastomosis technique”, where multiple small lymphatic channels are anastomosed to a single vein. A 12-0 suture is placed transluminally on the vein and then through the adventitia only on the lymphatic vessels which will cause intussusception of the lymphatic vessels into the vein. After anastomosis, the patency and the flow direction can be assessed by using patent blue dye or ICG.

Postoperatively, Koshima advocates using an infusion of a vasodilator drug (prostaglandin E1) for 5 days and then using an oral form for several weeks<sup>[10]</sup>. As the incisions are only skin deep, the patient usually has very little pain and can go home the same day. Bandages are applied until the wounds heal. Compression stockings are used for 3 weeks after the operation and continued for at least 6 months after the procedure.

Scaglioni *et al.*<sup>[18]</sup> reviewed 18 studies of LVA involving 939 patients and found that all studies showed objective reduction in the circumference measurement. Subjective symptom relief was found in 50% to 100% of patients as well as reduction in the cellulitis episodes in all cases. Several studies also show a striking reduction in the episodes of cellulitis post-surgery<sup>[19-21]</sup>. Results of LVA were noted by Chang *et al.*<sup>[7]</sup> to be better in the upper limb (96% symptomatic improvement) compared to the lower limb (57%



**Figure 1.** A, B: pre-op and Post-op photo of a patient with lymphoedema of the right leg; C, D: supraclavicular flap harvested and anastomosed to the posterior tibial artery; E: lymphovascular anastomosis done in the right foot

symptomatic improvement), since the lower limb is dependent and associated with higher venous pressures. The efficacy of LVA treatment is dependent more on the severity of the lymphoedema than to the duration of the lymphedema, with later stage lymphedema being less responsive.

### VLNT

VLNT is a means to treat lymphoedema by replenishing the missing lymph nodes of the affected extremity by harvesting healthy vascularised lymph nodes from one area of the body and transplanting them to the affected extremity with the help of microsurgery<sup>[22]</sup>.

In early cases of lymphoedema, LVA is possible. However, in late cases, the limb is fibrotic and sclerosed, and VLNT is a physiological means to improve lymphoedema. The presence of significant backflow with few or no functioning lymphatic vessels on imaging using ICG, lymphography, lymphoscintigraphy or MRL suggests that VLNT may be indicated<sup>[23]</sup>. VLNT can be combined with LVA in certain cases if some good lymphatic channels are available, to give a better outcome [Figure 1A-E].

Several theories regarding how VLNT works have been postulated, since the precise mechanism of action of VLNT is incompletely understood. Honkonen *et al.*<sup>[24]</sup> used a swine model to propose that VLNT acts like a “lymphatic wick” between the proximal and distal lymphatic vessels at the recipient site. This theory seems to be attractive, especially in the early stages when functional lymphatic channels are retained in both the proximal and distal segment. It is also believed that lymphatic connections are established between the vascularised lymph node flaps and the surrounding tissues. Saaristo proposed that high levels of vascular endothelial growth factor C is produced by the transferred lymph nodes which induces lymphangiogenesis and facilitates recanalisation of the lymphatic vessels between the recipient and transferred lymph nodes<sup>[25]</sup>. These theories probably explain why VLNTs are transferred to proximal levels in the limbs. This is best illustrated by the case of using the deep inferior epigastric perforator (DIEP) flap along with the lymph nodes in the groin to treat post-mastectomy lymphoedema. Besides, the release of scar tissue in the axilla can result in early improvement by releasing the pressure on the axillary vein which will reduce capillary filtration. There is also abundant soft tissue to allow easy closure at the donor site, and the scar is well

**Table 1. Advantages and disadvantages of various types of vascularised lymph node transfers**

<b>Vascularised lymph node flaps</b>	<b>Advantages</b>	<b>Disadvantages</b>
Groin	Can be taken with DIEP Flap during breast reconstruction; well concealed scar; good cosmesis; commonly used for upper limb lymphoedema	Iatrogenic lower limb lymphoedema
Omentum	No iatrogenic lymphoedema; rich source of lymphatic tissue;	Laparoscopy/laparotomy needed; poor cosmesis; complications due to laparotomy; adhesions; hernias; DVT
Submental	Less iatrogenic lymphoedema	Injury to marginal mandibular branch of facial nerve; vessel is small; few nodes in the flap
Supraclavicular	Less iatrogenic lymphoedema	Vessel is small; few nodes; damage to brachial plexus and lymphatic duct
Lateral thoracic	Commonly used for lower limb lymphoedema	Iatrogenic upper limb lymphoedema; damage to the thoracodorsal nerve
Jejunum	No iatrogenic lymphoedema	Injury to the viscera, bowel adhesions, internal hernia; bowel ischaemia

DIEP: deep inferior epigastric perforator; DVT: deep vein thrombosis

hidden. If it is difficult to access the scar in the anatomical position, then the VLNTs can be placed just distal to the point of obstruction. For example, in patients who have had pelvic lymph nodes removed after a laparotomy, the vascularised lymph nodes would then be placed in the upper thigh medial to the femoral artery and the saphenous vein<sup>[1]</sup>.

Vascularised lymph nodes can also be transplanted distally in the limb. The theory behind placing the VLNT at non-anatomical sites is that they work like a “lymphatic pump”. The strong arterial pulsations in the flap provide a strong hydrostatic force in the flap. The flap veins, which have low pressure, act like a suction drawing the lymphatic fluid into the capillaries<sup>[26]</sup>. Due to gravity, the lymphatic collection is predominantly distal, and placing the VLNTs distally seems to have a “catchment effect”, thereby improving lymphatic drainage. However, distal placement of the flap can make the flap look bulky and non-aesthetic<sup>[27]</sup>. There are many potential donor lymph nodes for VLNTs, namely the groin, thoracic, submental, supraclavicular, omental and mesenteric lymph nodes. The advantages and disadvantages of the different donor options are listed in Table 1.

Breast cancer is the most common cancer among women and post-mastectomy lymphoedema occurs in 9%-41% of women who undergo axillary dissection and 4%-10% of women who undergo sentinel lymph node biopsy<sup>[28-30]</sup>. In such cases, the DIEP flap is the most commonly used. This flap not only helps in lymphoedema, but also helps to reconstruct the missing breasts at the same time. The superficial lymph node basin of the groin drains the lower abdomen and is the target for lymph node harvest, whereas deeper lymph nodes close to the femoral vessels drain the thigh and lower extremity. While harvesting the lymph nodes, it is preferable to harvest the lymph nodes lateral to the femoral vessels, since lymphatic drainage of the lower limb is predominantly medial to the femoral vessels and below the inguinal ligament<sup>[31]</sup>. The DIEP flap is raised as caudally as possible. The groin lymph nodes lateral to the femoral vessels are preferably harvested along with the superficial circumflex iliac vessels and are placed in the axilla, and the superficial circumflex iliac vein is anastomosed to either the thoracodorsal vein, the lateral thoracic vein or the serratus branch. The pedicle of the DIEP flap is attached either to the thoracodorsal vessels or the internal mammary vessels. The major concern about this flap is about donor site lymphoedema and can be prevented by reverse lymphatic mapping using preoperative lymphoscintigraphy, ICG and methylene blue dye<sup>[32]</sup>.

The supraclavicular flap based on the transverse cervical vessels can be used as a VLNT. Donor site lymphoedema is not common in this flap, as evidenced by the fact that the lymph nodes in this area are commonly removed during elective neck dissections after cancer<sup>[33]</sup>. The other benefit of the flap is that it generally heals well with a good scar. The flap dimensions and size are generally small compared to other donor sites, and this flap is generally placed distally in the limbs to treat lymphoedema, since the soft tissue cover around the wrist and ankles is much less. Safe harvesting of the supraclavicular flap would need

thorough knowledge of the local anatomy and the consequent complications that can arise. The transverse cervical vessels can have a variable course. It can take its origin from either the thyrocervical trunk (80%) or directly from the subclavian artery (20%) or rarely from the internal mammary artery<sup>[34]</sup>. Careful surgical technique is necessary to avoid damage to the carotid artery, internal jugular vein, phrenic nerve and the thoracic duct<sup>[35]</sup>. The other concern about this flap is that it is thought to have fewer lymph nodes that can be transferred<sup>[36]</sup>.

The submental flap based on the submental artery is a commonly used flap for head and neck reconstruction<sup>[37]</sup>. This flap can be raised as a free flap containing the submental lymph nodes and used to treat upper and lower limb lymphoedema. Similar to the supraclavicular flap, removal of the lymph nodes in the neck is inconsequential regarding donor site lymphoedema as evidenced by their routine harvest during oncological lymph node dissections. A small elliptical skin paddle can also be included. The upper border of the incision is along the lower border of the mandible and extends from the angle to the symphysis. Dissection is performed deep to the platysma. The anterior belly of the digastric muscle can be included to avoid damage to the perforators supplying the flap. Soft tissue around the junction of the submental and facial vessels is included to provide more lymph nodes in the neck. The main concern regarding the submental flap is the possible injury to the marginal mandibular nerve that one should be wary of while raising the flap<sup>[38]</sup>. Furthermore, the pedicle can be very short, which may need including the facial vessels to make it longer<sup>[39]</sup>.

The lateral thoracic flap involves the transfer of lymphatics between the anterior and posterior axillary folds lateral to the pectoralis minor<sup>[40]</sup>. The dominant vascular supply to these nodes is from the lateral thoracic vessels. The artery can be absent in 12.5% of cases, in which case the thoracodorsal vessels provide the vascular supply to these nodes<sup>[40]</sup>. The main advantage of this flap is the inconspicuous scar which is well hidden in the axillary fold and the longer pedicle length when compared to the other peripheral lymph node flaps. The main disadvantage of the flap is that lymphoedema can occur in the potential donor site in the upper limb.

The greater omentum can be harvested as a free flap based on the gastroepiploic vessels to treat lymphoedema. The omentum has abundant lymph nodes and helps to initiate absorption from the peritoneal cavity<sup>[41]</sup>. Hence, the omentum makes an ideal flap for draining stagnant lymphatic fluid. The large size of the omentum can be used to cover larger areas and can even be divided into two to treat bilateral lymphoedema<sup>[42]</sup>. Raising the omental flap via a laparotomy can result in abdominal wound infections, hernias, prolonged ileus and bowel obstruction<sup>[43]</sup>. Harvesting the omental flap by laparoscopy obviates the disadvantages that a laparotomy has by decreasing pain, discomfort, blood loss, wound infections, chest infections, prolonged ileus and bowel obstruction and deep vein thrombosis<sup>[44]</sup>. The major drawback of this flap is the need for laparoscopy and poor aesthesis associated with the bulk and skin grafting of the flap.

The jejunal mesenteric lymph node flap is also a very good option to avoid donor site lymphoedema following lymph node transfer. The mesentery in the jejunum is preferred to that in the ileum as it has more lymph nodes in the flap. The longest loop of the third part of the jejunum is identified and a flap based on the second, third or fourth mesenteric branch of the superior mesenteric artery is designed<sup>[45]</sup>. The proximal segment has significantly more lymph nodes than the other segments do, and the flap is raised preferably close to the root of the mesentery. To avoid a risk of internal hernia, only the anterior peritoneum containing the mesenteric lymph nodes and adjacent branches of the superior mesenteric vessels is raised, leaving behind the posterior peritoneum intact. Disadvantages of the flap include the risk of injury to the viscera, bowel adhesions, internal hernia and the need for bowel resection in case of bowel ischaemia.

The outcomes of VLNTs seem very promising according to various reports<sup>[45-47]</sup>. However, one needs to take steps to avoid complications. The paramount concern that one has while raising these flaps is not



only to avoid damage to the vital structures, but also to avoid inducing lymphoedema in the donor limbs. Viitanen *et al.*<sup>[48]</sup> demonstrated in their series that although none of their patients developed donor site lymphoedema, lymphoscintigraphy in over half the patients showed a slightly slower lymphatic flow in the donor limb compared to the non-operated limb. Donor site lymphoedema can be reduced by reverse lymphatic mapping<sup>[32]</sup>. The lymphatic drainage of the flap to be harvested and the potential donor site lymphoedema area are evaluated by two different methods. For example, before raising the DIEP flap containing the groin lymph nodes, ICG is injected intradermally in the lower abdomen and technetium injections in the first and second webspaces of the foot. A gamma probe is used to localise the lymph nodes draining the lower extremity which is avoided. Similarly, technetium can be injected into the hand and ICG injected into the back and lateral chest in cases of VNLT.

Postoperatively, the use of drains, antibiotics, anticoagulation and therapy is largely based on anecdotal experience and surgeon preference, as there is no uniform consensus on this subject, and a conclusive study can be difficult to devise considering the many variables and confounding issues involved. Compression over the flap is avoided for 2 to 3 weeks. Later, the patient is advised to wear compression garments throughout the day. Six months later, the patient can be reviewed, and if necessary, the excess fat in the limbs can be removed by liposuction.

## LIPOSUCTION

In lymphoedema there is a physiological imbalance of blood flow and lymphatic drainage, resulting in the impaired clearance of lipids and the uptake by macrophages<sup>[49]</sup>. Besides, it is believed that chronic inflammation in lymphoedema results in adipose tissue hypertrophy<sup>[50]</sup>. Several studies have found an association with fat hypertrophy along with lymphatic stasis. The findings are indicated below:

1. In Crohn's disease, there was increased fat deposition in the areas of the bowel that were inflamed, suggesting that inflammation plays an important role in increasing fat<sup>[51]</sup>.
2. Analysis of the content of the aspirate from liposuction showed a high content of adipose tissue (90%)<sup>[52]</sup>.
3. Dual energy X-ray absorptiometry in 18 women with post-mastectomy lymphoedema showed a significant increase in adipose tissue in the oedematous limb<sup>[53]</sup>.
4. Marked mononuclear cell response was found with adipogenesis in response to lymphatic fluid stasis<sup>[54]</sup>.
5. Lymphatic fluid stasis was shown to upregulate fat differentiation markers<sup>[55]</sup>.

The limbs affected with lymphoedema are swollen due to the accumulation of lymphatic fluid along with excess fat deposition. The accumulated lymphatic fluid can be decreased by conservative therapies such as complex decongestive therapy and controlled compression therapy along with microsurgical techniques such as LVA or VLNT. After lymphatic fluid has been decreased by the above procedures, the accumulated fat can be removed by liposuction. Liposuction is a method to remove fat and should be used only when the conservative or microsurgical techniques have been used before. The patient should be on compression garments. The absence of fluid collection is found when the limbs are not pitting even on applying pressure to them for a minute. Liposuction should only be done when there is no pitting and when the pitting cannot be reduced further even after regular physiotherapy<sup>[50]</sup>.

Under appropriate anaesthesia, power-assisted liposuction is used to reduce surgeon fatigue and also to facilitate liposuction especially in fibrous areas. Initially, the "dry technique" has been used<sup>[56]</sup>. To minimise blood loss, a combination of tourniquet and tumescence liposuction has been used<sup>[56]</sup>. A sterile tourniquet is tied proximally on the limb. Through appropriate 3-mm long incisions, liposuction cannulas 15 and 25 cm long and with diameters of 3 and 4 mm are used to aspirate the excess fat. A sterilised compressive dressing or a glove is worn, and the tourniquet is removed. The proximal part of the limb in which the tourniquet was removed is infiltrated with tumescence solution containing adrenaline and mild



local anaesthetic, and liposuction is continued proximally. Finally, the proximal part of the arm is also compressed. The incisions are left open to drain. The hand is rested on a large pillow at the level of the heart. The next day, the dressings are replaced with compression garments.

After initial compression therapy, the garments need to be reassessed for loss of elasticity in the garment and for reduction in the size of the limb. This is very important especially in the first 3 months after surgery. The patient is assessed every 3 months for the first year to look out for change in the volume and also to inspect the condition of the compression garments. Maximum reduction of limb size is usually achieved in the upper limb at 3 months and in the lower limb at 6 months, but it may take longer. For best results, it is advised to wear the garment lifelong<sup>[57]</sup>. Hoffner *et al.*<sup>[58]</sup> have shown a mean 5-year postoperative reduction of  $117\% \pm 26\%$  in the limb with lymphoedema compared with the healthy arm.

## SURGICAL DEBULKING

Surgical debulking of lymphoedema has been used for a long time. In spite of the popularity of surgical debulking decreasing due to the introduction of microsurgical techniques, surgical debulking remains the procedure of choice in carefully selected patients with Class III lymphoedema and skin changes, lymphoedema secondary to filariasis and in places where a microsurgical facility is unavailable.

In patients with filariasis, the adult worms of *Wuchereria bancrofti*, *Brugia malayi* and *Brugia timori* invade the lymphatic system and cause dilatation of the lymphatic channels, incompetence of the lymphatic valves and obliteration of the lymphatic channels<sup>[59]</sup>. This destruction of the lymphatic channels causes severe oedema and fibrosis in the limbs with resultant skin changes such as warty outgrowths, acanthosis and ulcers. When the limb and the toes become big, maintaining hygiene becomes difficult. This predisposes to many fungal infections in the interdigital spaces and fissures in the feet. These fungal infections act as entry points for many secondary bacterial infections, which produce acute dermatolymphangioadenitis<sup>[60]</sup>. Surveys estimate a frequency of 4.47 episodes of acute dermatolymphangioadenitis per year for bancroftian filariasis and 2.2 episodes for brugian filariasis<sup>[61]</sup>. Each episode of acute dermatolymphangioadenitis worsens the lymphoedema and produces more fibrosis, scarring and more swelling. Destruction of the lymphatic channels and severe fibrosis of the limbs precludes performing LVA and liposuction, respectively, in such patients. Furthermore, many patients with filarial lymphoedema and severe lymphoedema have many skin changes that are best treated by surgical debulking in the limbs. Microsurgical procedures such as LVA and VLNTs are generally beneficial in the early stages of the disease, when the lymphatics are relatively healthy and when the tissues are still soft and pliable. Chronic accumulation of lymphatic tissue in the subcutaneous tissues cause thickening of the skin, hypercellularity, progressive fibrosis, increased fat deposition and irreversible damage to the lymphatic vessels. For these patients with end-stage lymphatic disease, excisional surgical procedures remain the mainstay of patient management. Large folds of skin and subcutaneous tissue can be excised, which leads to improved outcomes. We will look at the different excisional techniques in detail.

## CHARLES PROCEDURE

The Charles procedure is the prototype of all excisional procedures<sup>[62]</sup>. Although Sir Richard Henry Havelock Charles who described the Charles procedure, described the technique mainly for lymphoedema of the scrotum, the procedure bears his name for the excisional surgery for lymphoedema of the lower limbs as a result of series of questionable references<sup>[63]</sup>. The Charles procedure involves excision of the skin, subcutaneous tissue and deep fascia of the legs involved with lymphoedema and grafting the raw areas on the bare exposed muscle. This procedure is done in advanced lymphoedema with skin changes [Figure 2A-E]. After excision of the skin, due to the unavailability of subdermal lymphatic drainage, worse lymphoedema is expected distally in the foot. Severe secondary changes in the skin such as ulceration,



**Figure 2.** Charles procedure. A: pre-op photo of a patient with severe lymphoedema of the right leg; B: skin and subcutaneous tissue removed from the right leg; C: tissue removed; D: after skin grafting; E: long term result

papillomatosis, hyperkeratosis, weeping dermatitis and chronic cellulitis are commonly seen in the distal feet and toes<sup>[64]</sup>. Such skin changes in the toes can be very uncomfortable for the patient, and many patients may find it difficult to maintain personal hygiene. Karonidis *et al.*<sup>[65]</sup> thought it advisable to preserve the toes if there was only swelling without previous cellulitis or verrucous hyperkeratosis and neither deformity nor osteomyelitis of the toes. Some surgeons have modified the original technique to preserve the deep fascia to improve lymphatic drainage of the leg. When this procedure is selected for the right patient, it results in considerable reduction in size, improvement in function and satisfactory results. However, due to the poor cosmesis, associated bottleneck deformity and distal lymphoedema, this procedure is not very commonly done. Van der Walt *et al.*<sup>[66]</sup> applied negative pressure wound therapy after excisional surgery to prepare the bed better for grafting. Negative wound pressure therapy is also commonly used after applying the grafts to keep the grafts in place and for better take. The Charles procedure can be associated with complications such as poor graft take, delayed healing, distal lymphoedema and recurrence of lymphoedema, especially at the foot, which may need resurfacing, re-grafting and toe amputations. The patient needs to be taught good nail care and foot care, since infections in the nails can lead to repeated cellulitis and worsening lymphoedema.

### STAGED SUBCUTANEOUS EXCISION BENEATH SKIN FLAPS

This surgery is performed in two stages both over the medial and lateral aspect of the limbs<sup>[67]</sup>. Usually the medial aspect of the limb is removed first as more tissue can be removed. An incision is made over the medial aspect of the limb. Flaps are raised on either side of the incision, and the excess tissue in the subcutaneous area is removed. The excess skin can be closed, trimmed or de-epithelialised. This is then repeated in the lateral aspect as well at least 3 months after the initial surgery.

### CHARLES PROCEDURE ALONG WITH VASCULARISED LYMPH NODE FLAP (CHEN-MODIFIED CHARLES PROCEDURE)

To reduce the risks associated with lymphoedema, such as repeated cellulitis and recurrence and worsening of lymphoedema, VLNTs can be done along with the Charles procedure. While doing the Charles procedure, the superficial veins are to be kept intact so that they can be used as a recipient vein

for the vascularised lymph node flaps. The vascularised lymph node flap is done distally in the limb and anastomosed to vessels around the ankle and the wrist. This helps to reduce the worsening distal oedema and recurrent cellulitis of the limbs<sup>[68]</sup>.

## **RADICAL REDUCTION OF LYMPHOEDEMA WITH PRESERVATION OF PERFORATORS**

With knowledge of the anatomical position of the perforators of the limb and with various investigations such as handheld Doppler and CT scan, we are able to raise flaps with better vascularity. This helps us to excise tissue more confidently leaving behind good vascularised tissue. Accordingly, incisions in the limbs are made, and flaps based on the perforators are raised, while the excess tissue is radically removed. In the upper limb, the blood supply of the tissue left behind is based on the perforators from the brachial artery in the arm and from the radial, ulnar and posterior interosseous artery perforators. In the lower limb, the blood supply of the tissue left behind is mainly from the posterior tibial artery and peroneal artery. This technique is particularly useful for lower limb lymphoedema (52% reduction)<sup>[69]</sup> compared to upper limb lymphoedema (15% reduction)<sup>[70]</sup>.

## **CONCLUSION**

The management of lymphoedema is an evolving science. Every patient with lymphoedema needs a detailed clinical evaluation, which may include investigations such as ICG lymphangiography to decide upon the best treatment. Understanding the benefits and limitations of various procedures will help us choose the optimal line of management for every patient. The results could be very gratifying both to the patient and the surgeon.

## **DECLARATIONS**

### **Authors' contributions**

Lead surgeon: Venkatramani H

Preparation of manuscript and collection of data: Shanmugakrishnan RR

Assistant surgeon participated in most of the surgeries: Kumaran MS

Correction of manuscript and scientific advice: Sabapathy SR

### **Availability of data and materials**

Not applicable.

### **Financial support and sponsorship**

None.

### **Conflicts of interest**

All authors declared that there are no conflicts of interest.

### **Ethical approval and consent to participate**

Ethical approval obtained from the institutional review board (IRB). Written consent was obtained from all patients to take photographs and use their data for publication.

### **Consent for publication**

The authors give consent for publication. Photographic consent was obtained for all patients.

### **Copyright**

© The Author(s) 2020.

## REFERENCES

1. Silva AK, Chang DW. Vascularized lymph node transfer and lymphovenous bypass: novel treatment strategies for symptomatic lymphedema. *J Surg Oncol* 2016;113:932-9.
2. International Society of Lymphology. The diagnosis and treatment of peripheral lymphedema: 2013 Consensus Document of the International Society of Lymphology. *Lymphology* 2013;46:1-11.
3. Tiwari A, Cheng KS, Button M, Myint F, Hamilton G. Differential diagnosis, investigation, and current treatment of lower limb lymphedema. *Arch Surg* 2003;138:152-61.
4. Lee BB. Contemporary issues in management of chronic lymphedema: personal reflection on an experience with 1065 patients. *Lymphology* 2005;38:28-31.
5. Shih YC, Xu Y, Cormier JN, Giordano S, Ridner SH, et al. Incidence, treatment costs, and complications of lymphedema after breast cancer among women of working age: a 2-year follow-up study. *J Clin Oncol* 2009;27:2007-14.
6. Koshima I, Inagawa K, Urushibara K, Moriguchi T. Supermicrosurgical lymphaticovenular anastomosis for the treatment of lymphedema in the upper extremities. *J Reconstr Microsurg* 2000;16:437-42.
7. Chang DW, Suami H, Skoracki R. A prospective analysis of 100 consecutive lymphovenous bypass cases for treatment of extremity lymphedema. *Plast Reconstr Surg* 2013;132:1305-14.
8. Suami H, Chang DW, Yamada K, Kimata Y. Use of indocyanine green fluorescent lymphography for evaluating dynamic lymphatic status. *Plast Reconstr Surg* 2011;127:74-6e.
9. Yamamoto T, Kikuchi K, Yoshimatsu H, Koshima I. Ladder-shaped lymphaticovenular anastomosis using multiple side-to-side lymphatic anastomoses for a leg lymphedema patient. *Microsurgery* 2014;34:404-8.
10. Koshima I, Narushima M, Mihara M, Yamamoto Y, Iida T. Treatments for leg lymphedema. *J Jpn Soc Gynecol Oncol* 2009;27:10-18.
11. Mazzei FG, Gentili F, Guerrini S, Cioffi Squitieri N. MR lymphangiography: a practical guide to perform it and a brief review of the literature from a technical point of view. *Biomed Res Int* 2017;2017:2598358.
12. Yamamoto T, Yoshimatsu H, Yamamoto N, Narushima M, Iida T, et al. Side-to-end lymphaticovenular anastomosis through temporary lymphatic expansion. *PLoS One* 2013;8:e59523.
13. Narushima M, Mihara M, Yamamoto Y, Iida T, Koshima I, et al. The intravascular stenting method for treatment of extremity lymphedema with multiconfiguration lymphaticovenous anastomoses. *Plast Reconstr Surg* 2010;125:935-43.
14. Yoshimatsu H, Yamamoto T, Narushima M, Iida T, Koshima I. The guide wire method: a new technique for easier side-to-end lymphaticovenular anastomosis. *Ann Plast Surg* 2014;73:231-3.
15. Yamamoto T, Chen WF, Yamamoto N, Yoshimatsu H, Tashiro K, et al. Technical simplification of the supermicrosurgical side-to-end lymphaticovenular anastomosis using the parachute technique. *Microsurgery* 2015;35:129-34.
16. Mihara M, Hara H, Iida T, Todokoro T, Yamamoto T, et al. Antegrade and retrograde lymphaticovenous anastomosis for cancer-related lymphedema with lymphatic valve dysfunction and lymphatic varix. *Microsurgery* 2012;32:580-4.
17. Chen WF, Yamamoto T, Fisher M, Liao J, Carr J. The "octopus" lymphaticovenular anastomosis: evolving beyond the standard supermicrosurgical technique. *J Reconstr Microsurg* 2015;31:450-7.
18. Scaglioni MF, Fontein DBY, Arvanitakis M, Giovanoli P. Systematic review of lymphovenous anastomosis (LVA) for the treatment of lymphedema. *Microsurgery* 2017;37:947-53.
19. Chen WF, Zhao H, Yamamoto T, Hara H, Ding J. Indocyanine green lymphographic evidence of surgical efficacy following microsurgical and supermicrosurgical lymphedema reconstructions. *J Reconstr Microsurg* 2016;32:688-98.
20. Soran A, D'Angelo G, Begovic M, Ardic F, Harlak A, et al. Breast cancer-related lymphedema--what are the significant predictors and how they affect the severity of lymphedema? *Breast J* 2006;12:536-43.
21. Yamamoto T, Koshima I. Supermicrosurgical anastomosis of superficial lymphatic vessel to deep lymphatic vessel for a patient with cellulitis-induced chronic localized leg lymphedema. *Microsurgery* 2015;35:68-71.
22. Chang DW, Masia J, Garza R, Skoracki R, Neligan PC. Lymphedema: surgical and medical therapy. *Plast Reconstr Surg* 2016;138:209-18S.
23. Schaverian MV, Badash I, Patel KM, Selber JC. Vascularized lymph node transfer for lymphedema. *Semin Plast Surg* 2018;32:28-35.
24. Honkonen KM, Visuri MT, Tervala TV, Halonen PJ, Koivisto M, et al. Lymph node transfer and perinodal lymphatic growth factor treatment for lymphedema. *Ann Surg* 2013;257:961-7.
25. Saaristo AM, Niemi TS, Viitanen TP, Tervala TV, Hartiala P, et al. Microvascular breast reconstruction and lymph node transfer for postmastectomy lymphedema patients. *Ann Surg* 2012;255:468-73.
26. Lin CH, Ali R, Chen SC, Wallace C, Chang YC, et al. Vascularized groin lymph node transfer using the wrist as a recipient site for management of postmastectomy upper extremity lymphedema. *Plast Reconstr Surg* 2009;123:1265-75.
27. Patel KM, Lin CY, Cheng MH. From theory to evidence: Long-term evaluation of the mechanism of action and flap integration of distal vascularized lymph node transfers. *J Reconstr Microsurg* 2015;31:26-30.
28. Suami H, Chang DW. Overview of surgical treatments for breast cancer-related lymphedema. *Plast Reconstr Surg* 2010;126:1853-63.
29. Warren AG, Brorson H, Borud LJ, Slavin SA. Lymphedema: a comprehensive review. *Ann Plast Surg* 2007;59:464-72.
30. DiSipio T, Rye S, Newman B, Hayes S. Incidence of unilateral arm lymphoedema after breast cancer: a systematic review and metaanalysis. *Lancet Oncol* 2013;14:500-15.
31. Van der Ploeg IM, Kroon BB, Valde's Olmos RA, Nieweg OE. Evaluation of lymphatic drainage patterns to the groin and implications for the extent of groin dissection in melanoma patients. *Ann Surg Oncol* 2009;16:2994-9.
32. Dayan JH, Dayan E, Smith ML. Reverse lymphatic mapping: a new technique for maximizing safety in vascularized lymph node transfer. *Plast Reconstr Surg* 2015;135:277-85.



33. Mardonado AA, Chen R, Chang DW. The use of supraclavicular freeflap with vascularized lymph node transfer for treatment of lymphedema: a prospective study of 100 consecutive cases. *J Surg Oncol* 2017;115:68-71.
34. Sapountzis S, Singhal D, Rashid A, Ciudad P, Meo D, et al. Lymph node flap based on the right transverse cervical artery as a donor site for lymph node transfer. *Ann Plast Surg* 2014;73:398-401.
35. Ooi ASH, Chang DW. 5-step harvest of supraclavicular lymph nodes as vascularized free tissue transfer for treatment of lymphedema. *J Surg Oncol* 2017;115:63-7.
36. Bank J, Chang DW. Microsurgical procedures: vascularized lymph node transfer from the supraclavicular region. Principles and practice of lymphedema surgery. Philadelphia, PA: Elsevier; 2016. pp. 148-54.
37. Martin D, Pascal JF, Baudet J, Mondie JM, Farhat JB, et al. The submental island flap: anew donor site. Anatomy and clinical applications as a free orpedicled flap. *Plast Reconstr Surg* 1993;92:867-73.
38. Cheng MH, Patel KM. Micro surgical procedures: vascularized lymph node transfer from the submental region. Principles and practice of lymphedema surgery. Philadelphia, PA: Elsevier; 2016. pp. 138-47.
39. Cheng MH, Huang JJ, Nguyen DH, Saint-Cyr M, Zenn MR, et al. A novel approach to the treatment of lower extremity lymphedema by transferring a vascularized submental lymph node flap to the ankle. *Gynecol Oncol* 2012;126:93-8.
40. Barreiro GC, Baptista RR, Kasai KE, dos Anjos DM, Busnardo Fde F, et al. Lymph fasciocutaneous lateral thoracic artery flap: anatomical study and clinical use. *J Reconstr Microsurg* 2014;30:389-96.
41. Nguyen AT, Suami H. Laparoscopic free omental lymphatic flap for the treatment of lymphoedema. *Plast Reconstr Surg* 2015;136:114-8.
42. Nguyen AT, Suami H, Hanasono MM, Womack VA. Long term outcomes of the minimally invasive free vascularised omental lymphatic flap for the treatment of lymphedema. *J Surg Oncol* 2017;115:84-9.
43. Hultman CS, Carlson GW, Losken A, Jones G, Culbertson J, et al. Utility of the omentum in the reconstruction of complex extraperitoneal wounds and defects. Donor site complications in 135 patients from 1975 to 2000. *Ann Surg* 2002;235:782-95.
44. Lasso JM, Pinilla C, Castellano M. New refinements in greater omentum free flap transfer for severe secondary lymphedema surgical treatment. *Plast Reconstr Surg Glob Open* 2015;3:e387.
45. Corididi M, Wee C, Meyerson J, Eiferman D, Skoracki R. Vascularized jejunal mesenteric lymph node transfer: a novel surgical treatment for extremity lymphedema. *J Am Coll Surg* 2017;225:650-7.
46. Becker C, Vasile JV, Levine JL, Batista BN, Studinger RM, et al. Microlymphatic surgery for the treatment of iatrogenic lymphedema. *Clin Plast Surg* 2012;39:385-98.
47. Basta MN, Gao LL, Wu LC. Operative treatment of peripheral lymphedema: a systematic meta-analysis of the efficacy and safety of lymphovenous microsurgery and tissue transplantation. *Plast Reconstr Surg* 2014;133:905-13.
48. Viitanen TP, Mäki MT, Seppänen MP, Suominen EA, Saaristo AM. Donor-site lymphatic function after microvascular lymph node transfer. *Plast Reconstr Surg* 2012;130:1246-53.
49. Brorson H. Liposuction in arm lymphedema treatment. *Scand J Surg* 2003;92:287-95.
50. Clayton DN, Clayton JN, Lindley TS, Clayton JL. Large volume lipoplasty. *Clin Plast Sur* 1989;16:305-12.
51. Borley NR, Mortensen NJ, Jewell DP, Warren BF. The relationship between inflammatory and serosal connective tissue changes in ileal Crohn's disease: evidence for a possible causative link. *J Pathol* 2000;190:196-202.
52. Brorson H, Aberg M, Svensson H. Chronic lymphedema and adipocyte proliferation: clinical therapeutic implications. *Lymphology* 2004;37:153-5.
53. Brorson H, Ohlin K, Olsson G, Karlsson MK. Breast cancer related chronic arm lymphedema is associated with excess adipose and muscle tissue. *Lymphat Res Biol* 2009;7:3-10.
54. Zampell JC, Aschen S, Weitman ES, Yan A, Elhadad S, et al. *Plast Reconstr Surg* 2012;129:825-34.
55. Aschen S, Zampell JC, Elhadad S, Weitman E, De Brot M, et al. Regulation of adipogenesis by lymphatic fluid stasis: part II. Expression of adipose differentiation genes. *Plast Reconstr Surg* 2012;129:838-47.
56. Wojnikow S, Malm J, Brorson H. Use of a tourniquet with and without adrenaline reduces blood loss during liposuction for lymphoedema of the arm. *Scand J Plast Reconstr Surg Hand Surg* 2007;41:243-9.
57. Schaverien MV, Munnoch DA, Brorson H. Liposuction treatment of lymphedema. *Semin Plast Surg* 2018;32:42-7.
58. Hoffner M, Ohlin K, Svensson B, Manjer J, Hansson E, et al. Liposuction gives complete reduction of arm lymphedema following breast cancer treatment-A 5-year prospective study in 105 patients without recurrence. *Plast Reconstr Surg Glob Open* 2018;6:e1912.
59. Shenoy RK. Clinical and pathological aspects of filarial lymphedema and its management. *Korean J Parasitol* 2008;46:119-25.
60. Shenoy RK, Suma TK, Rajan K, Kumaraswami V. Prevention of acute adenolymphangitis in brugian filariasis: comparison of the efficacy of ivermectin and diethylcarbamazine, each combined with local treatment of the affected limb. *Ann Trop Med Parasitol* 1998;92:587-94.
61. Panicker KN, Sebasan S. Socioeconomic perspectives. Miscellaneous publications of VCRC. 1990;16:42-7.
62. Charles RH. Elephantiasis scroti. In: Latham AC, English TC, editor. A system of treatment. London: Churchill; 1912.
63. Dumanian GA, Futrell JW. The Charles procedure: misquoted and misunderstood since 1950. *Reconstr Surg* 1996;98:1258-63.
64. Taylor GW. Surgical management of primary lymphoedema. *Proc R Soc Med* 1965;58:1024-6.
65. Karonidis A, Chen HC. Preservation of toes in advanced lymphedema: an important step in the control of infection. *Ann Plast Surg* 2010;64:446-50.
66. van der Walt JC, Perks TJ, Zeeman BJ, Bruce-Chwatt AJ, Graewe FR. Modified Charles procedure using negative pressure dressings for primary lymphedema: a functional assessment. *Ann Plast Surg* 2009;62:669-75.
67. Miller TA, Wyatt LE, Rudkin GH. Staged skin and subcutaneous excision for lymphedema: a favourable report of long-term results. *Plast Reconstr Surg* 1998;102:1486-98.
68. Sapountzis S, Ciudad P, Lim SY, Chilgar RM, Kiranantawat K, et al. Modified Charles procedure and lymph node flap transfer for advanced



- lower extremity lymphedema. *Microsurgery* 2014;34:439-47.
69. Salgado CJ, Mardini S, Spanio S, Tang WR, Sassu P, et al. Radical reduction of lymphedema with preservation of perforators. *Ann Plast Surg* 2007;59:173-9.
70. Salgado CJ, Sassu P, Gharb BB, Spanio di Spilimbergo S, Mardini S, et al. Radical reduction of upper extremity lymphedema with preservation of perforators. *Ann Plast Surg* 2009;63:302-6.

Commentary

Open Access



# Evaluation and management of acquired ptosis

Stephanie E. Farber<sup>1</sup>, Mark A. Codner<sup>2</sup>

<sup>1</sup>Department of Plastic Surgery, University of Pittsburgh, PA 15261, USA.

<sup>2</sup>Division of Plastic Surgery, Emory University, Atlanta, GA 30322, USA.

**Correspondence to:** Dr. Mark A. Codner, Mark Codner Plastic Surgery, 1800 Howell Mill Rd, Suite 140, Atlanta, GA 30318, USA.  
E-mail: macodner@gmail.com; mark@markcodnermd.com

**How to cite this article:** Farber SE, Codner MA. Evaluation and management of acquired ptosis. *Plast Aesthet Res* 2020;7:20.  
<http://dx.doi.org/10.20517/2347-9264.2020.05>

**Received:** 7 Jan 2020 **First Decision:** 20 Mar 2020 **Revised:** 24 Mar 2020 **Accepted:** 10 Apr 2020 **Published:** 23 Apr 2020

**Science Editors:** Allen M. Putterman, Chau Pham **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

## Abstract

Periorbital rejuvenation is a common aesthetic goal sought by patients presenting to the plastic or oculoplastic surgeon. For this reason, it is critical that the surgeon understand the functional considerations, such as preexisting blepharoptosis, which will contribute to the ultimate aesthetic outcome. This article will review the anatomy of the normal and ptotic lid and will discuss the approach to diagnosing and characterizing the type and degree of lid ptosis. High-yield surgical techniques for ptosis correction will then be described, including the indications for and steps of each procedure. Finally, the diagnosis and management of common complications that follow ptosis surgery will be discussed. Our main objective is to arm the surgeon with the preoperative and operative planning tools to successfully manage comorbid ptosis and thereby improve blepharoplasty outcomes.

**Keywords:** Functional eyelid surgery, eyelid ptosis, acquired ptosis, blepharoplasty, blepharoptosis, periorbital rejuvenation

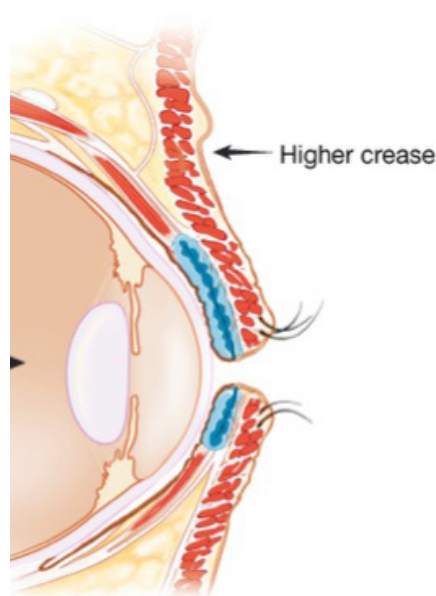
## INTRODUCTION

The eyes are the focal point of the face. For this reason, it is common for patients to present to plastic surgeons seeking an aesthetic, youthful periorbital appearance. Upper and lower lid blepharoplasty are key operations intended to rejuvenate the periorbital region. However, given the many functional considerations involved in eyelid and periorbital surgery, a thorough preoperative evaluation may reveal other functional issues that must be addressed at the time of aesthetic eyelid surgery.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** Attachments of the levator aponeurosis to the lid crease, which elevates with ptosis and Mueller's muscle originating from the undersurface of the levator inserting into the superior tarsal plate

In addition to xerophthalmia, lower eyelid malposition, and other eyelid problems that cause functional disability and may be addressed surgically, upper lid ptosis, or abnormal drooping of the upper lid, is a common comorbidity encountered in patients presenting for periorbital rejuvenation. This complex anatomic problem should be treated concurrently to achieve optimal periorbital aesthetics and function.

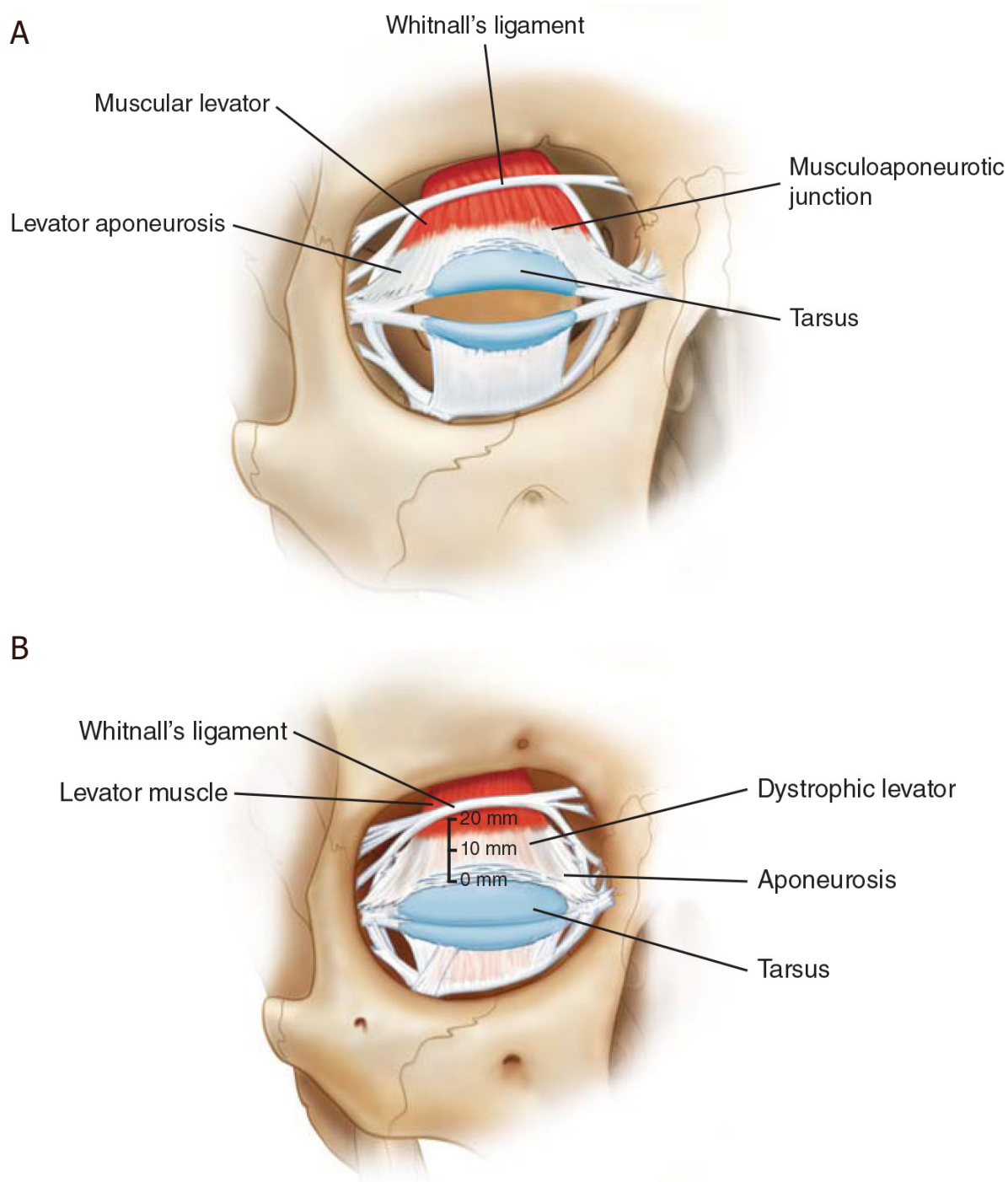
This review will provide the plastic surgeon with a structured approach to the evaluation and treatment of lid ptosis. Following a basic summary of normal and aged upper lid anatomy, we will outline the preoperative workup of a patient presenting for upper eyelid rejuvenation, including techniques for identifying the presence and etiology of lid ptosis. We will then review the indications for particular surgical procedures. These operative techniques will be outlined in detail, followed by a description of the evaluation and management of common complications resulting from surgical correction of lid ptosis.

## ANATOMY

### Normal

The upper eyelid consists of an anterior and posterior lamella separated by the orbital septum. The anterior lamella includes the thin upper lid skin and the orbicularis oculi muscle. The posterior lamella contains the tarsal plate, lid retractors, and the conjunctiva [Figure 1]<sup>[1]</sup>.

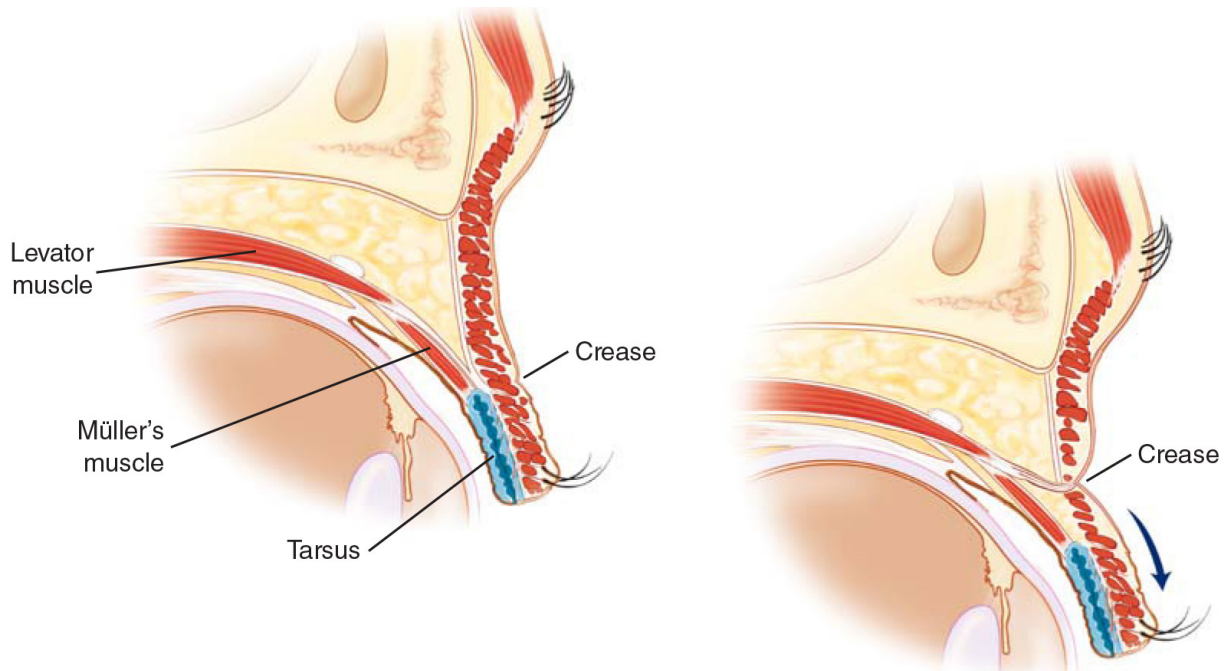
The upper lid retractors include the levator palpebrae superioris and Müller's muscle. These are the structures responsible for elevating the lid and are most relevant to the development of ptosis. The levator muscle is a striated muscle innervated by cranial nerve III (oculomotor nerve) and extends from the lesser wing of the sphenoid to the level of Whitnall's ligament. Whitnall's ligament is a fascial sheath of the levator muscle that serves as a check ligament and upper lid support structure. At this level, the levator muscle becomes the levator aponeurosis and attaches to the anterosuperior tarsus, orbital septum, and upper lid skin, creating the upper lid crease [Figure 2]. Müller's muscle, innervated by the sympathetic nervous system, originates from the undersurface of the levator aponeurosis and attaches to the superior tarsus [Figure 3]<sup>[1]</sup>.



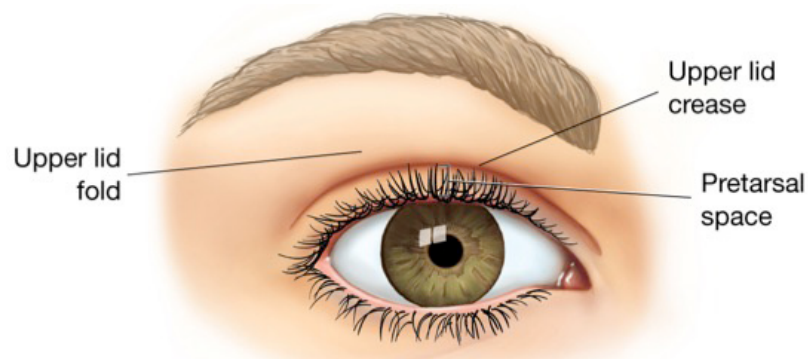
**Figure 2.** A: the relationship of the levator muscle to the musculoaponeurotic junction and tarsal plate; B: the distance from the superior border of the tarsal plate and the levator muscle increases with a dystrophic muscle and aponeurosis

### Ideal eyelid

The aesthetic upper lid consists of a well-defined supratarsal crease with a visible pretarsal space that is not covered by overhanging upper lid skin [Figure 4]. The upper lid sulcus should be a visible concave area between the lid crease and the superior orbital rim without prominent hollows or bulges. The ideal upper lid margin should lie just below the upper corneal limbus<sup>[2]</sup>.



**Figure 3.** As the lid becomes ptotic, the levator pulls the crease superiorly



**Figure 4.** The relationship of the lid fold which folds over the crease. The terms should not be used interchangeably. The pretarsal space and lid margin are in good position with a symmetrical arch

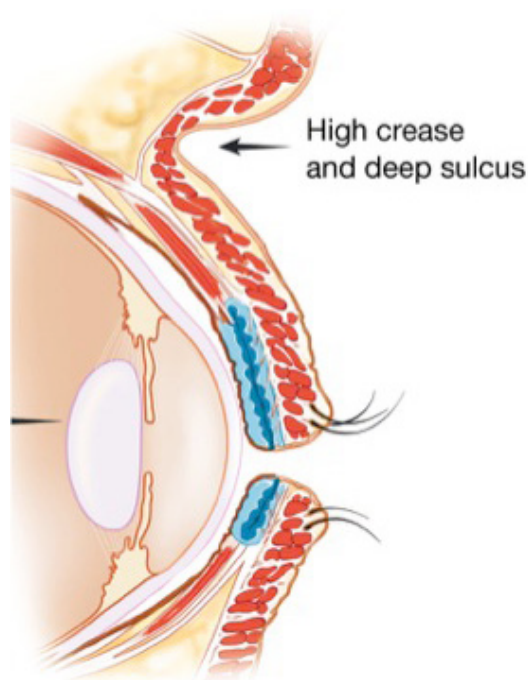
### Photic eyelid

The ptotic lid is characterized by a decreased margin to reflex distance 1 and a reduced palpebral fissure [Figure 5]. These measures will be reviewed in more detail in the “Patient Evaluation” section. Myogenic, neurogenic, mechanical, and involutional ptosis all have distinct anatomic etiologies that may lead to these examination findings. Myogenic ptosis occurs secondary to myasthenia gravis, neurologic ptosis secondary to dysfunction of the third cranial nerve (such as in Horner’s syndrome), and mechanical ptosis secondary to trauma, hard contacts, or prior blepharoplasty. The focus of this article is on involutional ptosis, which is caused by dehiscence of the levator aponeurosis from the tarsal plate. As the lid falls, the levator attachments to the skin remain intact, causing an elevation of the upper lid crease [Figure 6]<sup>[3]</sup>.

### DIFFERENTIAL DIAGNOSIS

First, it must be determined whether lid ptosis is congenital or acquired. A diagnosis of congenital ptosis requires alternative techniques for surgical correction, since the levator is characteristically fibrotic. The key physical examination finding in congenital ptosis is lid lag on downgaze [Figure 7]<sup>[4]</sup>.





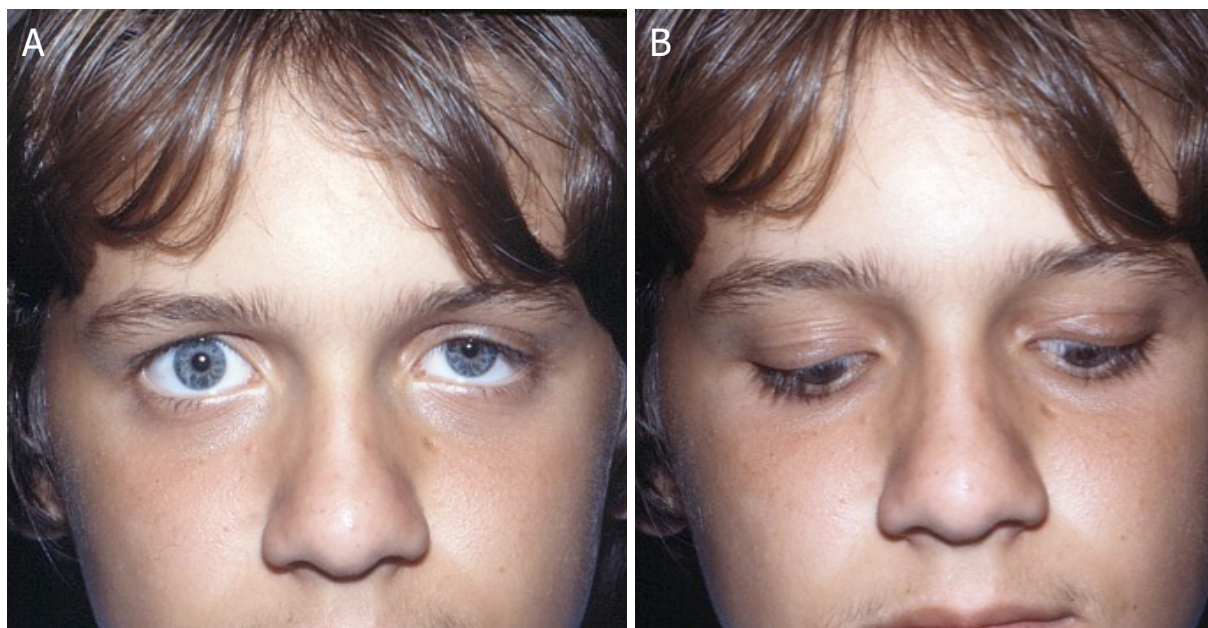
**Figure 5.** Close-up view of the deris of the lid crease moving higher as the levator withdraws and the lid drops



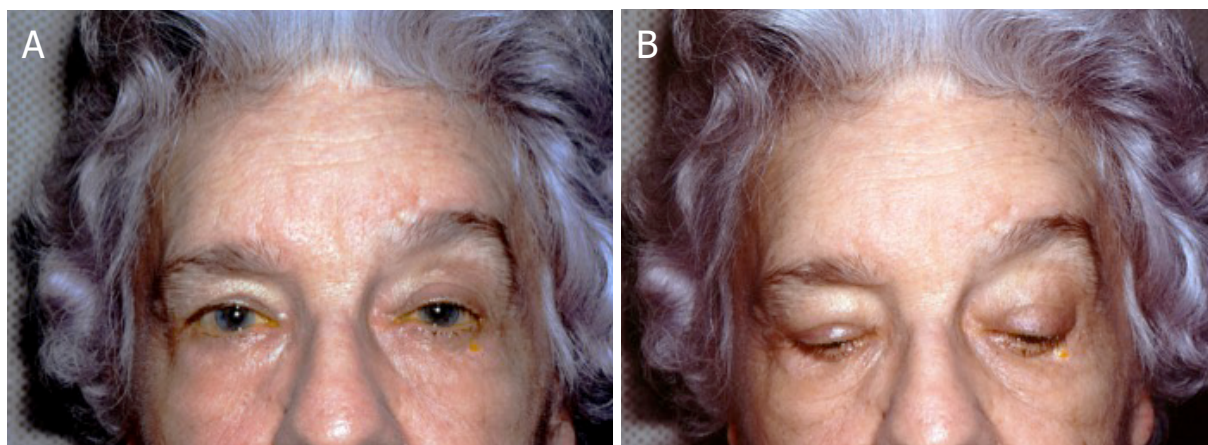
**Figure 6.** Patient 1 year after upper blepharoplasty and early postoperative left hematoma managed conservatively

Acquired ptosis can be due to forces extrinsic or intrinsic to the upper lid [Figure 8]. Extrinsic lid ptosis is secondary to factors outside of the upper lid, such as mechanical forces on the upper lid (brow ptosis, dermatochalasis, or tumor), contralateral lid retraction (most commonly secondary to thyroid disease), enophthalmos, orbicularis spasm, or facial nerve abnormalities<sup>[4]</sup>.

Intrinsic lid ptosis can be aponeurotic, myogenic, or neurogenic. The management of neurogenic and myogenic lid ptosis requires a specialized neurological evaluation. Aponeurotic ptosis will be the focus of this article and can be secondary to attenuation of the aponeurosis, trauma, eyelid swelling, or ocular surgery<sup>[4]</sup>. One form of trauma after which patients may develop lid ptosis is following cataract surgery due to retraction injury and stretching of the levator muscle.



**Figure 7.** A: congenital ptosis in a child with left ptosis; B: the pathognomonic sign of left lagophthalmos in downgaze to restriction of the lid from fibrosis of the levator and Mueller's muscle



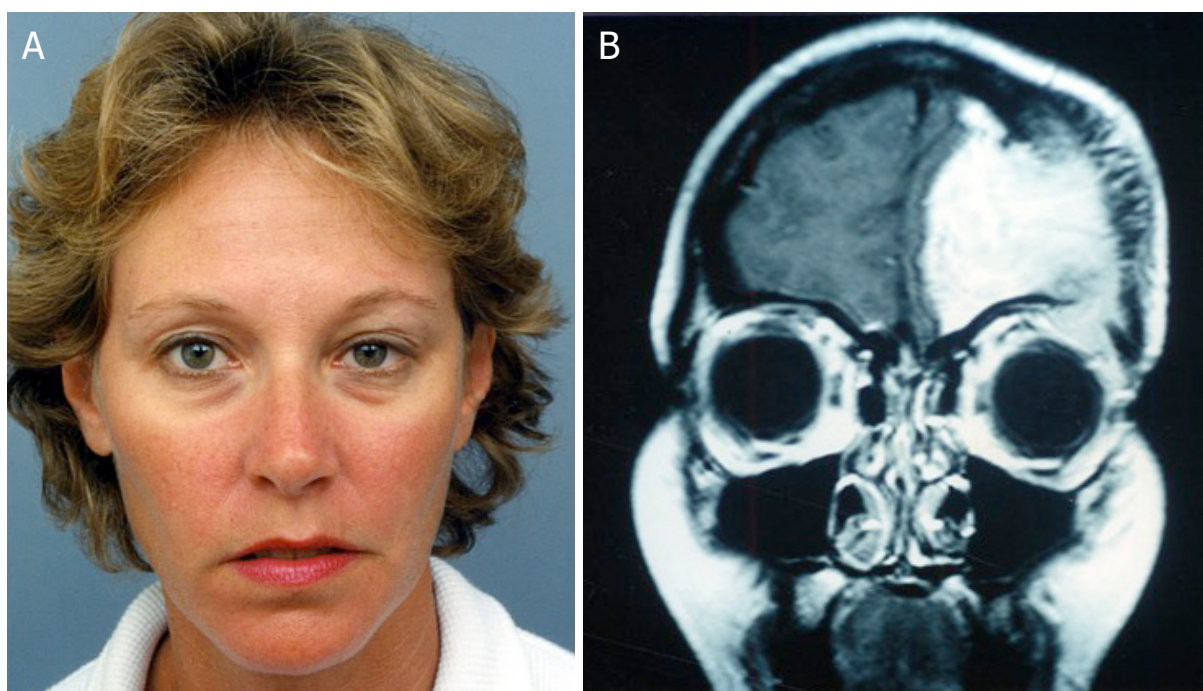
**Figure 8.** A: an elderly woman with bilateral ptosis worse on the left side from dehiscence of the levator aponeurosis; B: on downgaze, there is no restriction or lagophthalmos

## PATIENT EVALUATION

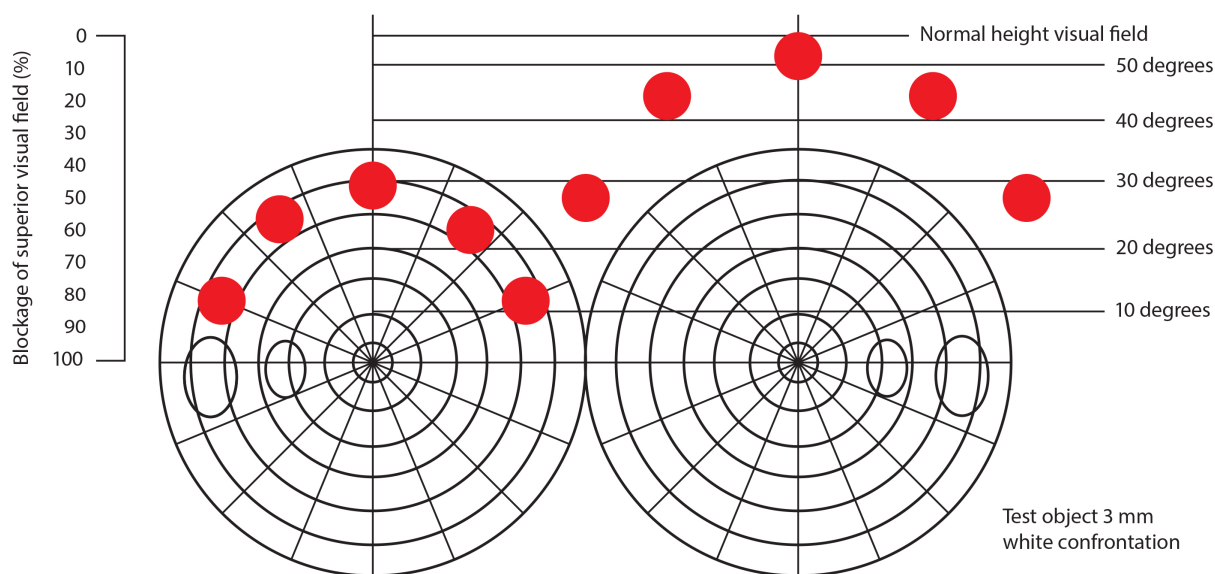
### History

As with any preoperative patient evaluation, the assessment must begin with a thorough medical history. In the case of an adult patient with new onset ptosis presenting for eyelid surgery, special attention must be paid to risk factors other than aging, including thyroid disease, diabetes, bleeding diatheses, periorbital surgery or trauma, other ocular conditions, and an orbital or brain tumor, which must be excluded by MRI or CT scan. These patients should be cleared for ptosis surgery by a neuro-ophthalmologist [Figure 9]. Adult patients who have acute acquired unilateral ptosis without a history of trauma, recent cataract or vision surgery when a speculum was used, or long-standing history of unilateral contact use should also be ruled out for development of an intraorbital or intracranial tumor.





**Figure 9.** A: an otherwise healthy patient presents with newly acquired ptosis of the left eye with no significant history of a possible cause; B: MRI reveals a large left frontal meningioma eroding through the sphenoid wing causing ptosis and orbital dystopia



**Figure 10.** Example of a visual field test performed in the office with a Q-tip not a visual field machine, documenting the loss of 50% of the right superior visual field by confrontation test

Patients with acquired ptosis often complain of a progressive decrease in the size of their eyes, a tired appearance, and even visual field loss [Figure 10]. Risk factors for this finding include advanced age, contact lens use, cataract surgery, or history of lid edema such as blepharochalasis and floppy upper lid syndrome [Figure 11]<sup>[3]</sup>. Another medical disease that often presents as ptosis is myasthenia gravis. Specific questions should be asked, including if the patient has worsening of the ptosis at night and if there are other signs such as muscle weakness or difficulty breathing or swallowing. Physical examination will reveal



**Figure 11.** Large male with the hallmark appearance of floppy upper lid syndrome and ptosis

eyelid fatigability when the patient is instructed to close his or her eyes tightly, fatiguing the orbicularis and inhibiting the levator, and to open them rapidly. A positive test is defined by immediate upward movement of the lid secondary to the levator muscle, followed by downward drift. An adult with new onset signs and symptoms, including the ones just mentioned, should have a neuro-ophthalmologic evaluation including a Tensilon test.

Another important consideration is the presence of xerophthalmia, or dry eye syndrome. This syndrome is characterized by a disruption in the tear film either due to decreased production or increased evaporation. Symptoms such as burning, itching, foreign body sensation or other types of eye discomfort should be elucidated. Risk factors for dry eye syndrome include laser eye surgery, smoking, and certain medications. These risk factors should be mitigated prior to surgery and eye lubricating drops and ointments may also be used as adjuncts<sup>[3]</sup>.

Importantly, ptosis can be a key presenting sign in patients with the onset of neurological conditions. Therefore, in an adult presenting with ptosis, the absence of levator dehiscence, and the presence of other red flag symptoms such as diplopia, abnormal pupillary reflexes and difficulty with speech or swallowing, neurological evaluation is critical to rule out other potentially life-threatening conditions<sup>[1]</sup>.

### Physical examination

Any ocular and periorbital examination should begin with an assessment of the globe, including visual acuity, extraocular movements, and pupillary response. Additionally, Bell's phenomenon should be documented to ensure that the patient's cornea will be protected postoperatively [Figure 12]<sup>[5]</sup>.





**Figure 12.** Bell's test showing a good superior position of the eye on forced opening

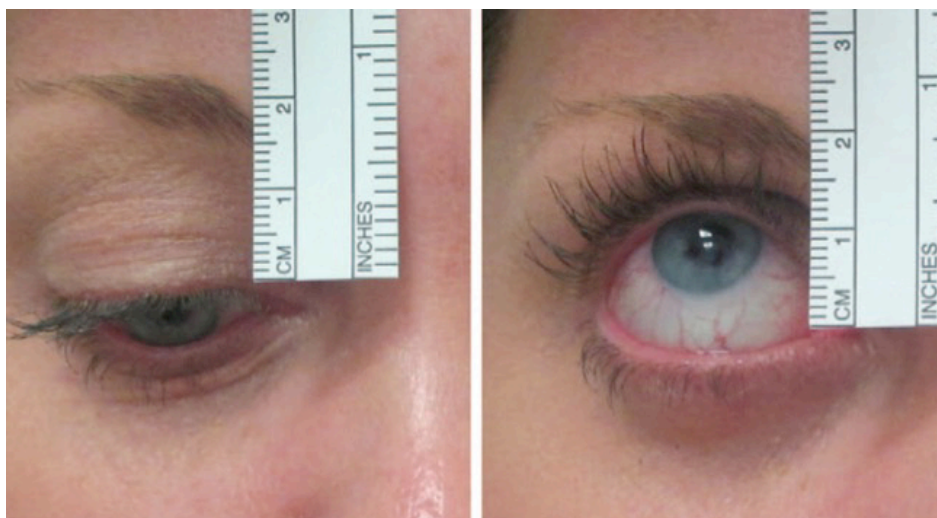


**Figure 13.** A patient with left upper lid ptosis with a high lid crease and elevation of the left brow for compensation

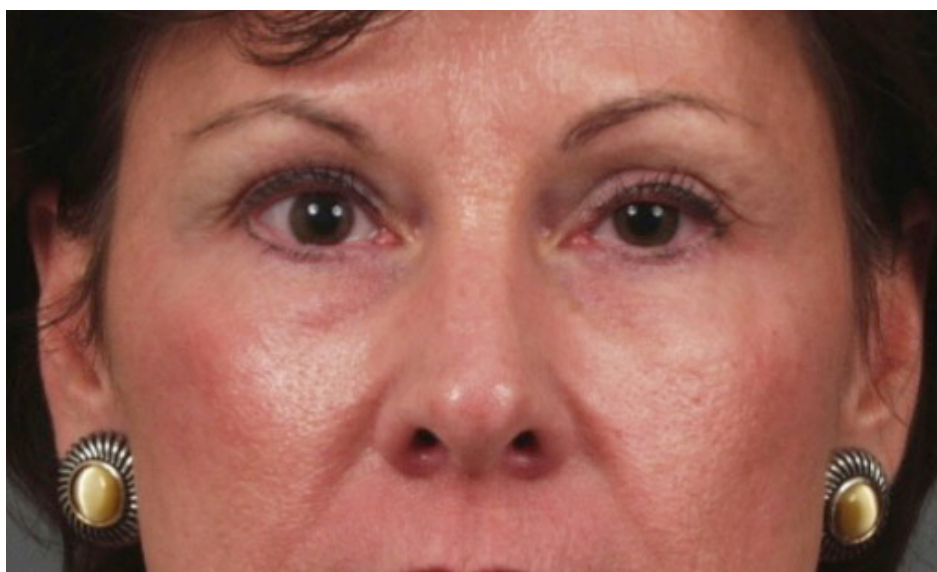
Next, the etiology of the patient's lid ptosis should be determined as an extrinsic factor, such as brow ptosis and pseudoptosis, or dermatochalasis, which can also give the appearance of a ptotic lid. In the case of brow ptosis, the brow rests below its normal position, which is at the level of the supraorbital rim in men or up to one centimeter above the rim in women. Attention must be paid to the presence of transverse forehead rhytids and a unilateral elevated brow which is compensating for unilateral ptosis, as these may be an indication of compensated brow or lid ptosis [Figure 13]. Dermatochalasis is characterized by an excess of upper lid skin and soft tissue, which can cause pseudoptosis of the upper lid<sup>[6]</sup>. In the presence of true involutional lid ptosis, in addition to a low lid margin, other examination findings might include an elevated lid crease and thinned upper eyelid, signifying attenuation of the levator aponeurosis<sup>[7]</sup>.

There are many techniques for quantifying the degree of lid ptosis. First, the margin reflex distance 1 is the distance from the central corneal light reflex to the upper eyelid margin. Normally, this measurement is between 3-4 mm and is reduced in patients with upper lid ptosis. In the worst cases, the upper lid may partially obstruct the corneal light reflex. Next the measurement of the palpebral fissure, or the eyelid





**Figure 14.** Measurement of levator function by the excursion distance from downgaze to maximum upgaze



**Figure 15.** An example of a patient with left upper lid ptosis and right upper lid retraction caused by Herring's Law

aperture measured from the lower to upper lid margin in the midpupillary axis, should normally be 8-10 mm. However, in patients with ptosis, it is reduced. Additionally, lid crease height measures the distance from the lash line to the lid crease. Due to the dehiscence of the levator from the tarsus in patients with involutional ptosis, the lid crease height is often elevated in these patients. Finally, levator function is measured as the amount of lid excursion from extreme upgaze to extreme downgaze [Figure 14]. In patients with concomitant brow ptosis, this finding must be quantified while stabilizing the brow on the supraorbital rim. Normal excursion should be greater than 10 mm. Patients with involutional ptosis characteristically have preserved levator function. Importantly, asymmetries in the degree of ptosis should be noted, as correction of only the more ptotic eye will result in worsening contralateral ptosis postoperatively due to equal bilateral innervation of the levator by cranial nerve III - a phenomenon known as Herring's Law [Figure 15]. It is generally recommended to patch the ptotic eye for 5 min and the contralateral lid will usually descend, and therefore, bilateral ptosis repair is recommended<sup>[3]</sup>.



**Figure 16.** To assess the presence of dry eye, an anesthetized Schirmer's test is performed prior to surgery. One would be more conservative during upper blepharoplasty in a patient with poor tear production

In addition to eliciting a history of dry eye symptoms, these findings can also be quantified during the physical examination. The Schirmer test is performed by placing a piece of filter paper inside the lateral lower lid margin and waiting for five minutes [Figure 16]. At this point in time, a less than 5-mm length of wetting is used to diagnose dry eyes, while greater than 10 mm is normal. Positive findings would indicate the need for treatment of dry eye symptoms pre- and postoperatively<sup>[8]</sup>. Another useful test to consider prior to a posterior approach, such as a Mullerectomy, is improvement of the eyelid position in the office with a phenylephrine test. If a patient has isolated 1-2 mm of ptosis and no other indications for surgery, Phenylephrine can be given to both eyes. If this corrects the ptosis, this subgroup of patients can be considered for a posterior approach.

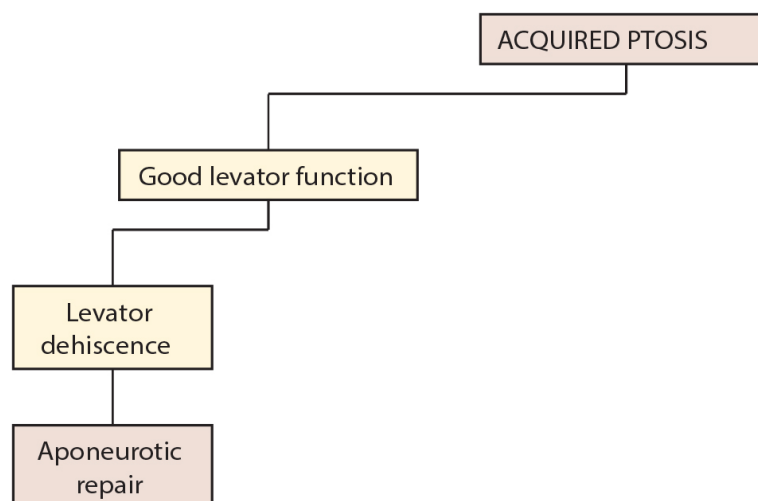
Other protective mechanisms should be assessed to ensure adequate postoperative ocular protection. These mechanisms are critical, as all ptosis procedures decrease the capacity of the upper lid to close. Therefore, in addition to Bell's phenomenon, eyelid closure strength, corneal sensation, and ocular lubrication must be verified preoperatively<sup>[3]</sup>.

## OPERATIVE APPROACH

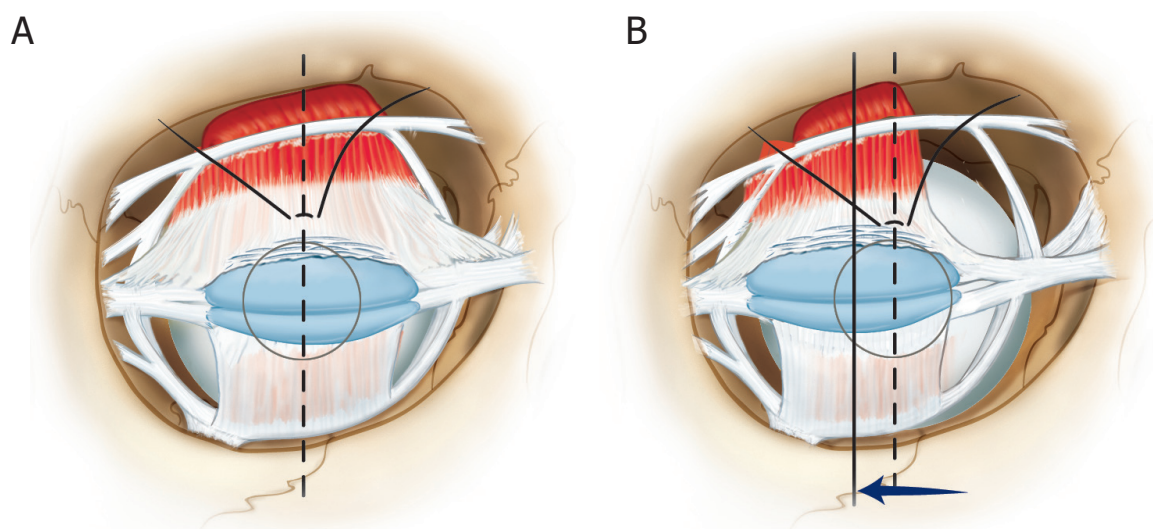
### Basic considerations

To address the needs of patients with lid ptosis, the surgeon treating these patients should be capable of performing a variety of types of ptosis repairs. As with most surgical problems, no single technique is best suited for all patients. Therefore, we will outline high-yield procedures that a surgeon treating patients with lid ptosis should have in his or her armamentarium. Ultimately, when deciding which ptosis repair to perform, the surgeon should consider the needs of the patient and which operation achieves the best results in his or her hands.

Upper eyelid surgery can be performed using either local or general anesthesia. Local anesthetic allows for patient participation in the assessment of upper lid function. However, it is important to note that local anesthetics may alter upper lid dynamics intraoperatively, complicating surgical decision making. In particular, epinephrine can stimulate the sympathetically innervated Müller's muscle, causing false lid elevation, and lidocaine can weaken the levator muscle, causing false lid depression. Therefore, a precise



**Figure 17.** Brief algorithm followed for adults with acquired ptosis and good levator function



**Figure 18.** A: the circle marks the pupil and the vertical midline is drawn which is near the center of the tarsal plate border in the youthful lid; B: with aging and weakening of the medial horn of the levator, the levator and tarsal plate complex shift laterally. The ptosis suture still needs to be placed in the midline of the pupil

quantification of upper lid ptosis may be possible under either sedation or general anesthesia with or without patient cooperation<sup>[9]</sup>.

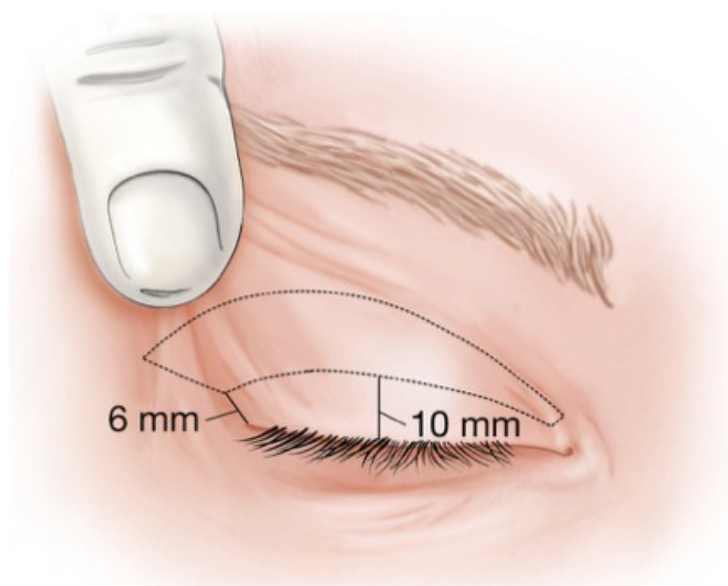
## Techniques

### *Levator aponeurosis repair*

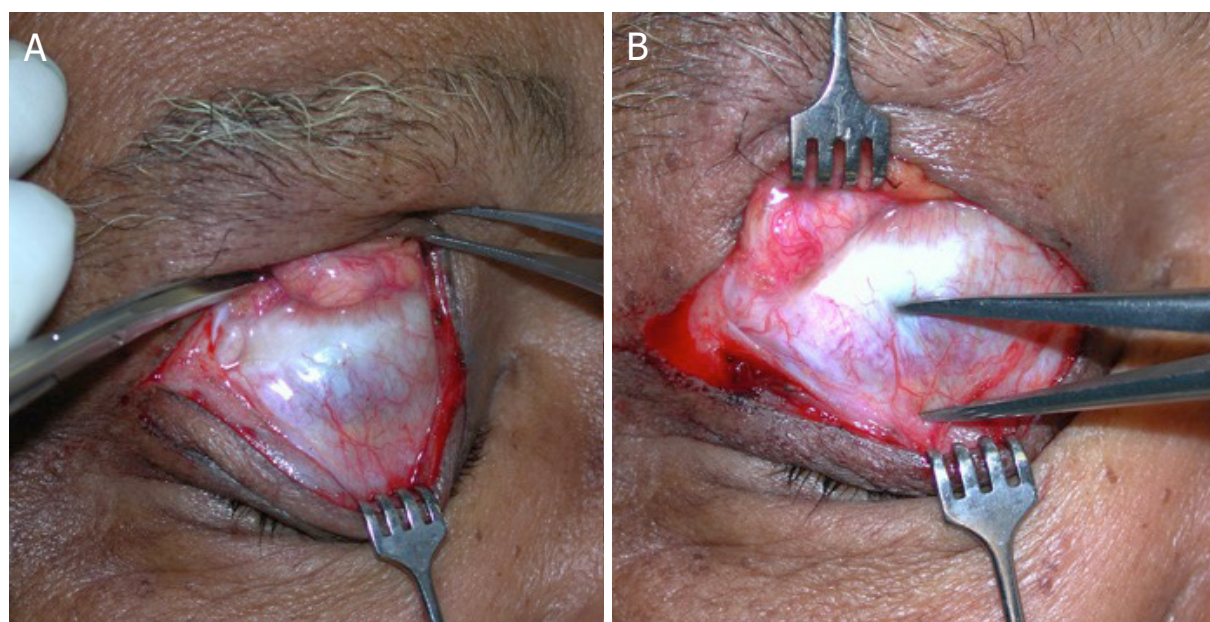
The most common type of lid ptosis results from a dehiscence of the levator aponeurosis. Therefore, levator aponeurosis repair is the most common levator procedure performed [Figure 17]. In addition to the dehiscence of the aponeurosis and resultant downward migration of the tarsus, there is also a more pronounced attenuation of the medial horn of the levator aponeurosis, leading to a lateral migration of the tarsus [Figure 18]<sup>[10]</sup>.

The levator aponeurosis repair procedure is indicated in patients who have normal levator function but appreciable dehiscence of the aponeurosis, as evidenced by an elevated lid crease, a thinned upper





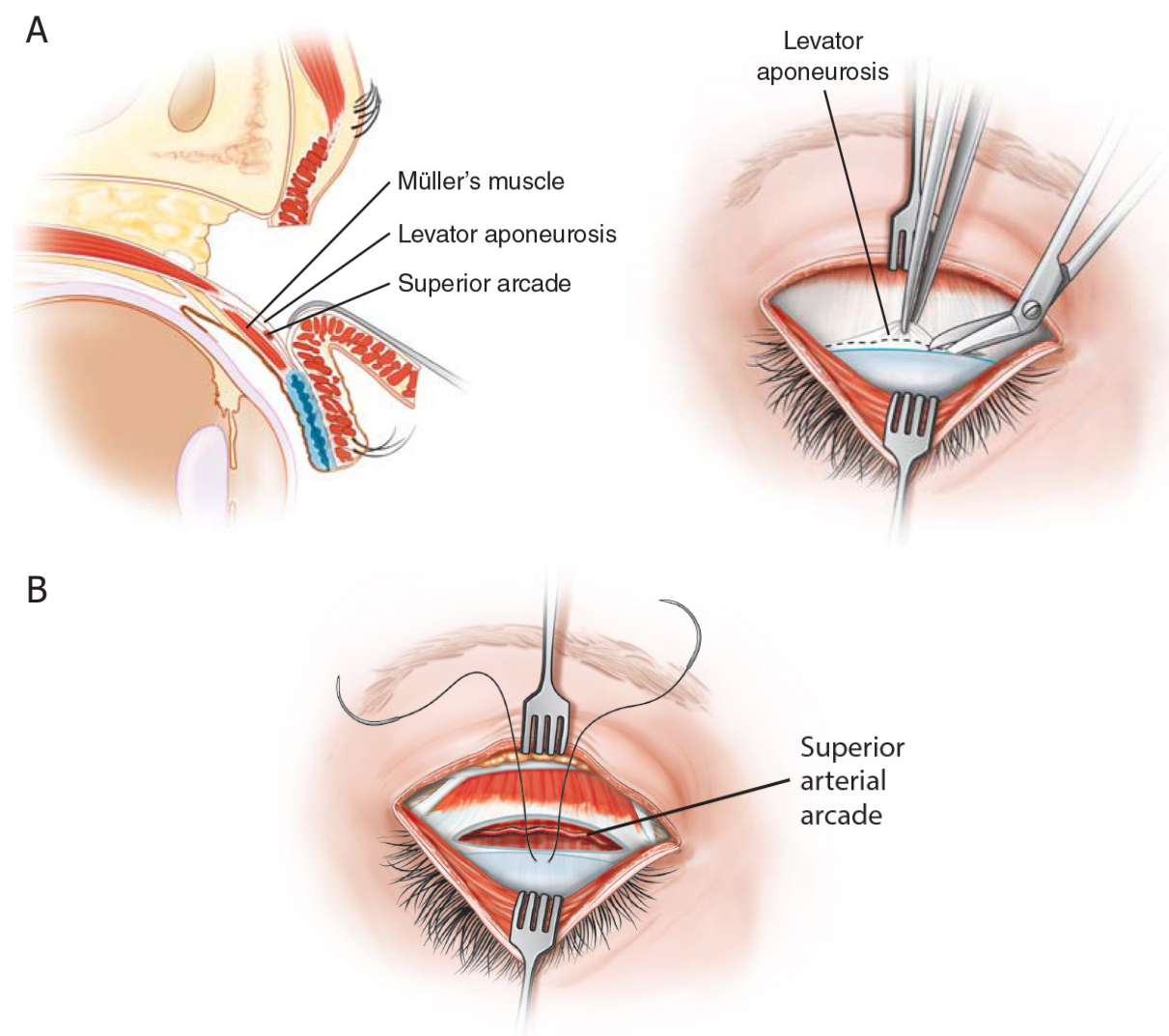
**Figure 19.** The approximate skin markings for tarslevator advancement uses an anterior approach similar to an upper blepharoplasty



**Figure 20.** A: the dystrophic, thin levator is demonstrated; B: the calipers are used to show 10 mm of dehiscence from the tarsal plate to the levator aponeurosis

lid, and a visible corneal outline through the attenuated lid. The basis of this technique is restoring the levator aponeurosis to its normal anatomic position, which involves both superior and potentially medial repositioning<sup>[1]</sup>.

Skin markings for the levator aponeurosis repair are performed as in a standard upper lid blepharoplasty [Figure 19]. The lid crease is marked where visible or 8 mm above the margin in a male or 8-10 mm in a female. Once incisions are made, the skin and muscle flap are excised, along with orbital septum, to expose the preaponeurotic fat - a helpful landmark for the underlying levator aponeurosis. Once the preaponeurotic fat is retracted, the aponeurosis and musculoaponeurotic junction are visible [Figure 20].

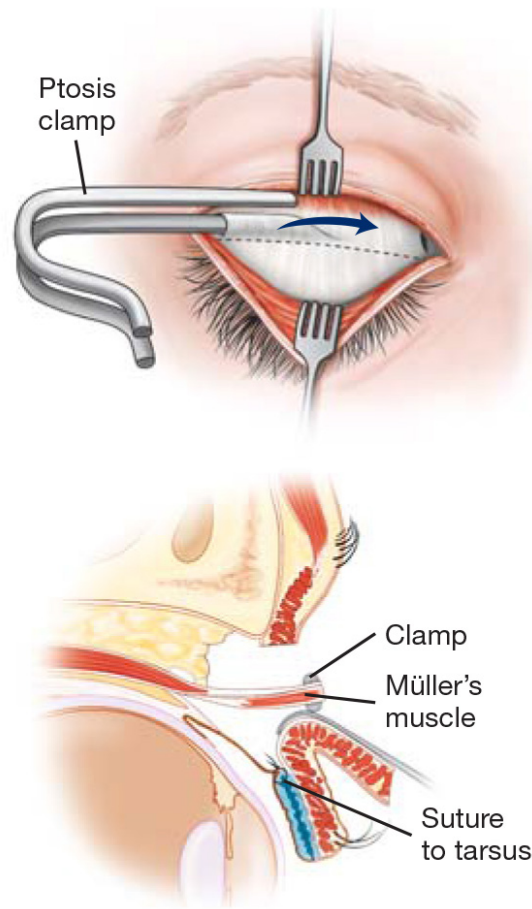


**Figure 21.** A: tarsal levator advancement is generally performed by initially dividing the central levator aponeurosis, sparing the medial and lateral horns; B: once the levator aponeurosis is elevated off Mueller's muscle, avoiding the superior arcade, a double-armed 6-0 silk single suture is placed through the midline of the superior tarsal plate and through the musculoaponeurotic junction with an adjustable knot anteriorly

An incision is made through the aponeurosis down to the superior border of the tarsus, and the aponeurosis is dissected off of the underlying Müller's muscle [Figure 21, Video 1]<sup>[1]</sup>.

At this point, the amount of aponeurotic repair must be determined, which can be done through patient cooperation and intraoperative adjustment or, more precisely, through the senior author's preferred three-step technique. This technique involves first approximating the superior border of the tarsal plate and the musculoaponeurotic junction with a single, double-armed suture at the midpupillary line in a horizontal mattress fashion, verifying that the suture has not passed through the underlying conjunctiva. Next, the suture is adjusted to ensure a symmetric amount of gapping between the two sides. Finally, the tension of the repair is tested by pulling down the lashes to close the lid and releasing to confirm equal velocity of snapback between the two sides [Video 2]. This particular technique has several advantages, including the ability to perform it under general anesthesia and combine it with other aesthetic operations, the exclusion of local anesthetic agents, which may confound the degree of ptosis repair, and the elimination of the need for patient cooperation. Other techniques of quantitating the degree of ptosis repair include the cookie





**Figure 22.** In congenital ptosis, primarily a ptosis clamp can be used to perform levator Mueller's resection

cutter technique (measuring an exact amount of tissue to be removed) and the voluntary cooperation technique. In our experience, these methods are not recommended as they have been associated with higher rates of revision<sup>[9]</sup>.

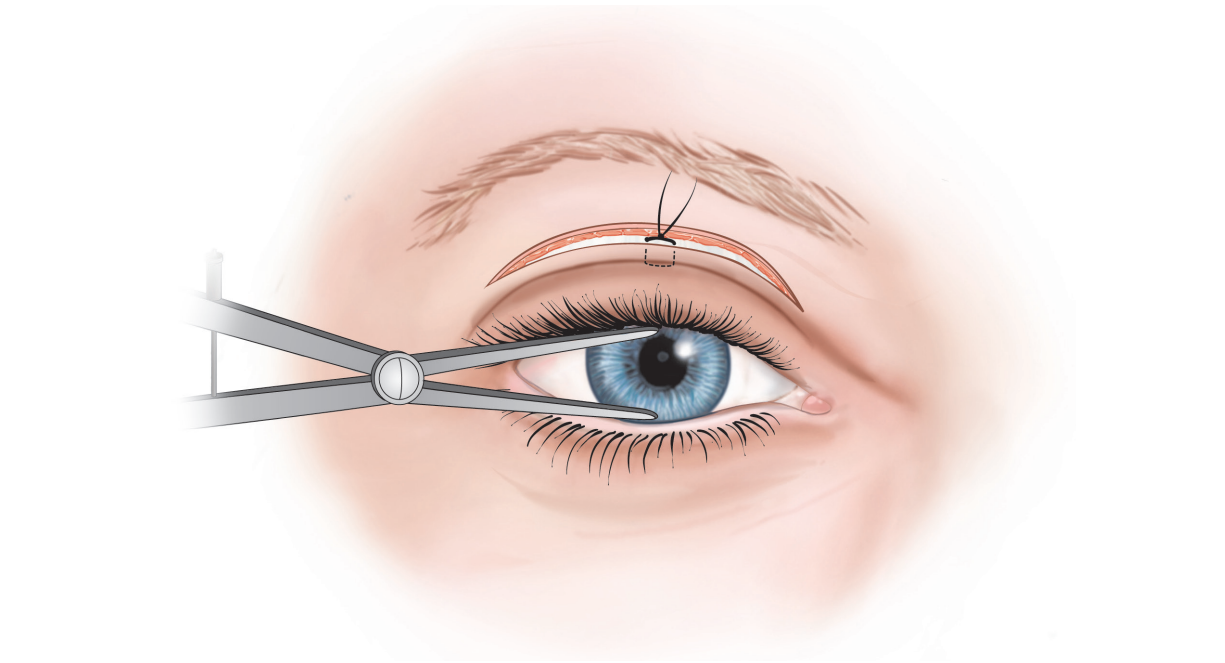
If the ptosis is unilateral, careful consideration must be given to ensure that this diagnosis is correct, as there is often a lesser degree of contralateral ptosis that might go untreated. If this is not the case, unilateral ptosis can be corrected solely with approximation of anatomic landmarks<sup>[9]</sup>. Levator plication is a simple but inaccurate technique for ptosis repair and is not recommended or used by the senior author.

#### *Levator-Müller's muscle resection*

Resection of both the levator and Müller's muscle is generally indicated in patients with decreased levator function secondary to scarring or muscle dysfunction. However, for this technique to be effective, there must be some residual levator function<sup>[1]</sup>.

The procedure begins with an identical marking and initial dissection to the levator aponeurosis repair. However, this approach also includes an exposure of Müller's muscle and dissection of the levator from its connective tissue attachments (lateral horn and Whitnall's ligament) to increase its stretch [Figure 22]<sup>[1]</sup>.

In this type of repair, the resection can be quantified by measuring the amount of levator resected or the amount of eyelid gapping, though the eyelid gapping technique has proven more predictable [Figure 23].



**Figure 23.** After ptosis repair while the knot is still adjustable, the gap between the upper and lower lid is measured with calipers and can be compared from each side

The degree of gapping is determined by the amount of levator function - poorer levator function requires a more aggressive repair. The central lifting suture is then placed in the tarsal plate and the double arms are run through the muscle complex near Whitnall's ligament and tightened to the desired gapping. This tightening generates redundant levator and Müller's muscle, which is subsequently resected. Additional sutures can then be placed to create a smooth contour<sup>[1]</sup>.

#### *Tarsoaponeurectomy*

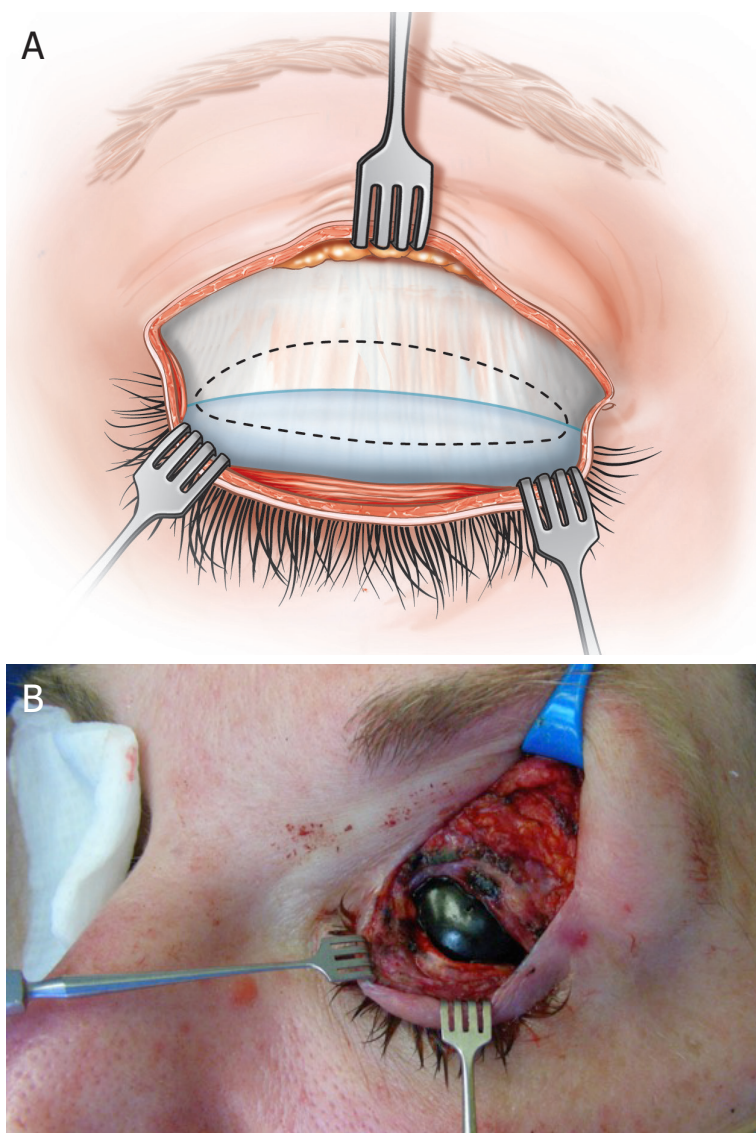
The tarsoaponeurectomy allows for a less precise anatomic resection of upper lid structures. It is indicated in patients who have scarring of anatomic planes due to trauma or prior surgery but still maintain excellent levator function. It is typically used as a last resort when anatomic planes cannot be identified<sup>[11]</sup>.

The operation is performed by making an upper lid crease incision and exposing the tarsal plate and levator aponeurosis. A predetermined amount of full thickness resection is performed, usually amounting to 0-2 mm plus the amount of ptosis [Figure 24]<sup>[1]</sup>.

#### *Tarsomullerectomy/Fasanella-Servat*

This procedure involves a posterior approach resecting Müller's muscle with or without tarsus. An advantage of this approach is the avoidance of a skin incision and preservation of lid contour<sup>[12,13]</sup>.

Following a skin and muscle resection where indicated, the operation proceeds by everting the lid and using two small mosquito clamps to grasp the tarsus and conjunctiva [Figure 25]. The amount of tissue to grasp within the clamp is determined by the degree of ptosis and amount of desired lift. Sutures are placed and passed through the eyelid 1 mm beneath the clamp carefully passing each suture through the previous suture hole to effectively bury the suture and prevent corneal irritation. Next, the excess conjunctiva, Müller's muscle and tarsus are excised and the sutures are externalized and tied down for removal around 1 week postoperatively. Essentially, this procedure attaches a disinserted levator to a shortened tarsus<sup>[1]</sup>.



**Figure 24.** A: markings on the levator aponeurosis and superior tarsal plate for excision of full thickness tissue during a tarsoplasty; B: the black eye protector can be seen during a tarsoplasty

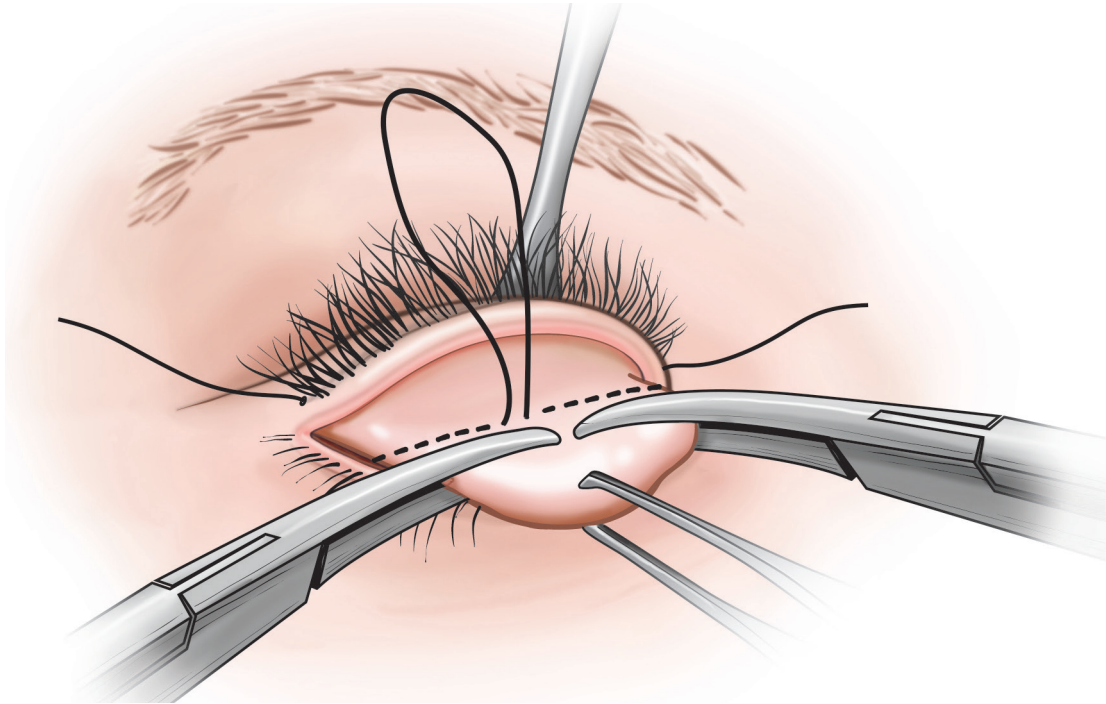
### *Frontalis suspension*

Frontalis procedures circumvent the levator when elevating the upper lid and are therefore indicated in patients with poor levator function. Typically, frontalis suspension is indicated in patients with congenital ptosis<sup>[1]</sup>.

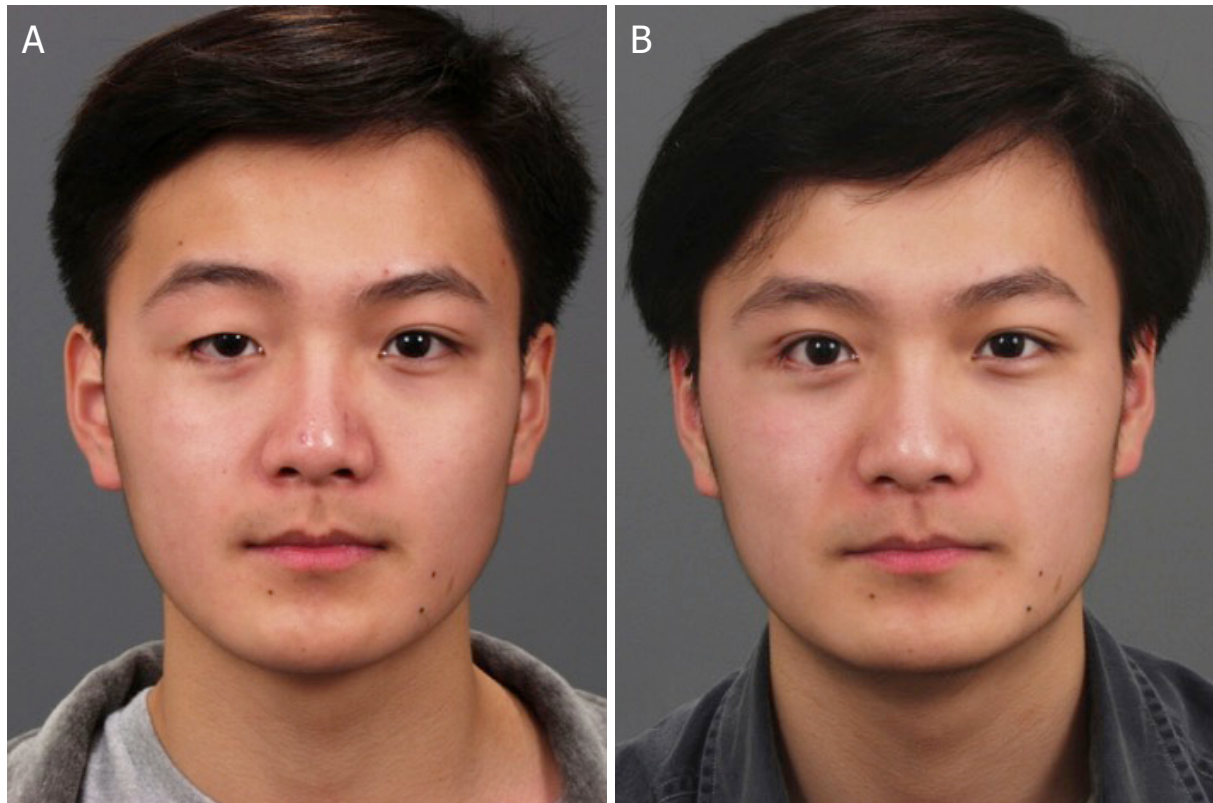
The operation can be performed with prosthetic or autologous material. Fascia lata is most commonly used in the adult patient with acquired ptosis. Silicone rods are reserved for pediatric patients who do not have sufficient donor site fascia<sup>[1]</sup>.

The technique begins with a single brow incision in the midpupillary line until the frontalis is identified. Next, a lid crease incision is made, and the superior edge of the tarsal plate is exposed to allow for sutures to be tied over a strand of suspensory material. This material is then passed deep to the orbicularis until it reaches the frontalis, generating a pentagonal configuration by placing the lateral and medial sutures inferiorly outside of the limbus [Figure 26]. The lid crease is closed prior to tightening the suspensory material at the brow level<sup>[1]</sup>.





**Figure 25.** Two small clamps are used in a posterior approach while the tarsal plate and Mueller's muscle are oversewn and resected to approximate the amount of tissue removed to correct the degree of ptosis



**Figure 26.** A: a young Asian man with asymmetrical creases and right upper lid ptosis; B: shown after surgery on the right eyelid only to create a fold and similar height crease



**Figure 27.** An elderly patient demonstrates bilateral ptosis with a high lid crease on the left shown before and after tarsal levator advancement

### Postoperative management

Patients should be instructed to sleep with their head elevated and to apply cold compresses. Lubricating eye drops or ointment should be prescribed and used liberally. Most surgeons also prescribe a topical ointment containing antibiotics or steroids to be applied to the lid incision. Sutures are normally removed within one week postoperatively.

## OUTCOMES

### Postoperative results

Although there are no randomized controlled comparison studies on ptosis repair techniques, there are studies reviewing the revision rates of individual techniques for involutional ptosis repair [Figures 26-31]. The outcomes and techniques in these studies are highly variable<sup>[8]</sup>. However, one consistent finding was the higher rate of revisions in patients with more severe preoperative ptosis and fibrosis<sup>[14]</sup>. Therefore, patients with more severe ptosis should be counseled about this risk preoperatively. The most important consideration is ultimately which technique is most reliable in each surgeon's own hands.

### Complications

#### *Overcorrection or undercorrection*

Rates of overcorrection and undercorrection vary on the basis of repair technique. Undercorrection is best treated with surgical revision if the degree of asymmetry or ptosis is sufficient to warrant operative intervention. The timing of surgical revision should allow for adequate resolution of acute postoperative swelling, but should also occur prior to the formation of significant scarring that would make identification of anatomic planes difficult.

If the eyelid is slightly overcorrected, as evidenced by incomplete lid closure or scleral show, this finding can oftentimes be corrected by stretching the lid downward [Figure 32]. Traction stretching can be performed while the patient is awake and should be initiated within the first postoperative week. If this





**Figure 28.** An elderly male with poor but adequate levator function before failed silicone slings were removed and an aggressive tarsalelevator advancement was performed



**Figure 29.** An elderly male with bilateral upper lid ptosis and lower lid ectropion from tarsoligamentous laxity shown before and after bilateral tarsalelevator advancement and bilateral lateral canthoplasties

is unsuccessful, lid stretching can also be performed under local anesthesia in the early postoperative period<sup>[2]</sup>. Patient dissatisfaction is higher in the aesthetic patient and can be upwards of 25%. Reassurance and time, as well as massage lid stretching exercises, are often used. Unless overcorrection is felt to be a technical problem that can be fixed with immediate surgery at one week, the senior author recommends



**Figure 30.** A cosmetic patient who felt she looked tired before and after tarsolevator advancement. Lower blepharoplasty, and facelift



**Figure 31.** A young woman with congenital ptosis and good levator function after right tarsolevator advancement

always trying to overcorrect by a millimeter and having the patient stretch the lid if needed and waiting six months before revision surgery is performed.

#### *Ocular injury*

Every attempt should be made intraoperatively and postoperatively to protect the globe from ocular injury. Corneal protectors should be used, and importantly, the integrity of the surface that contacts the cornea





**Figure 32.** Patient with acquired left ptosis after left upper lid hematoma treated conservatively for a year (A). She had left tarsoaponeurotomy and was slightly overcorrected, which can easily be treated with downward lid massage (B).

should be verified prior to application. Postoperatively, adequate ocular lubrication should be ensured if lid closure is incomplete. Should an ocular injury occur, diagnosis is critical. Once a diagnosis is made, ophthalmologic referral is necessary.

### *Xerophthalmia*

Transient dry eyes are a common finding following blepharoplasty with or without ptosis repair. However, if not appropriately treated, patients may suffer long-term morbidity. Lubricating eye drops and ointments should be prescribed. In more severe cases, patients can be prescribed a cyclosporine ophthalmic emulsion (i.e., Restasis; Allergan, Inc., Irvine Calif.) to stimulate tear production<sup>[15]</sup>.

## CONCLUSION

With periorbital rejuvenation and blepharoplasty being a common request among aesthetic surgery patients, it is critical that the plastic and oculoplastic surgeon understand the preexisting conditions and potential complications that will affect the ultimate postoperative result. Commonly, patients presenting for blepharoplasty also have blepharoptosis. Failure to diagnose and treat lid ptosis will result in a less than ideal result and a dissatisfied patient. In this article, we reviewed the preoperative workup, the operative approach, and the postoperative management of concomitant blepharoptosis. By comprehensively addressing all age-related periorbital changes, the aesthetic plastic surgeon can expect to enjoy optimized results and satisfied patients.

## DECLARATIONS

### Authors' contributions

Manuscript drafting: Farber SE

Manuscript revisions, figures, videos: Codner MA

**Availability of data and materials**

The data supporting our findings can be found stored securely in the office of Mark Codner, MD Plastic Surgery.

**Financial support and sponsorship**

None.

**Conflicts of interest**

All authors declared that there are no conflicts of interest.

**Ethical approval and consent to participate**

Not applicable.

**Consent for publication**

Written consent for publication was obtained for all images and videos.

**Copyright**

© The Author(s) 2020.

**REFERENCES**

1. Periorbital and eyelid anatomy. In: Codner MA, McCord DC, editors. *Eyelid and periorbital surgery*. 2nd ed. New York: Thieme; 2017. pp. 3-52.
2. Upper lid blepharoplasty. In: Codner MA, McCord DC, editors. *Eyelid and periorbital surgery*. 2nd ed. New York: Thieme; 2017. pp. 229-56.
3. Jindal K, Sarcia M, Codner M. Functional considerations in aesthetic eyelid surgery. *Plast Reconstr Surg J* 2015;134:1154-70.
4. Ptosis: evaluation and treatment. In: Codner MA, McCord DC, editors. *Eyelid and periorbital surgery*. 2nd ed. New York: Thieme; 2017. pp. 591-657.
5. Trussler AP, Rohrich RJ. MOC-PSSM CME article: blepharoplasty. *Plast Reconstr Surg J* 2009;121:1-10.
6. Lambros V. Observations on periorbital and midface aging. *Plast Reconstr Surg J* 2007;120:1367-76.
7. Codner MA, Kikkawa DO, Korn BS, Pacella SJ. Blepharoplasty and brow lift. *Plast Reconstr Surg J* 2010;126:1-17e.5
8. Chang S, Lehrman C, Itani K, Rohrich RJ. A systematic review of comparison of upper eyelid involutonal ptosis repair techniques: efficacy and complication rates. *Plast Reconstr Surg J* 2012;129:149-57.
9. McCord CD, Seify J, Codner MA. Transblepharoplasty ptosis repair: three-step technique. *Plast Reconstr Surg J* 2007;120:1037-44.
10. Jones LT, Quicken MH, Wobig JL. The cure of ptosis by aponeurotic repair. *Arch Ophthalmol* 1975;93:629-34.
11. McCord CD. External minimal ptosis procedure - external tarsoaponeurectomy. *Trans Sect Am Acad Ophtalmol Otolaryngol* 1975;79:683-6.
12. Putterman AM, Urist MJ. Müller's muscle-conjunctival resection. *Arch Ophthalmol* 1975;93:619-23.
13. Fasanella RM, Servat J. Levator resection for minimal ptosis: another simplified operation. *Arch Ophthalmol* 1961;65:493-6.
14. Codner MA. Discussion: a systematic review of comparison of upper eyelid involutonal ptosis repair techniques: efficacy and complication rates. *Plast Reconstr Surg J* 2012;129:158-9.
15. Pacella SJ, Codner MA. Minor complications after blepharoplasty: dry eyes, chemosis, granulomas, ptosis, and scleral show. *Plast Reconstr Surg J* 2010;125:709-18.

Perspective

Open Access



# Establishing a center of excellence in abdominal wall reconstruction

Jenny Shao, Sharbel Elhage, Eva Deerenberg, Vedra Augenstein, B. Todd Heniford

Gastrointestinal and Minimally Invasive Surgery, Department of Surgery, Carolinas Medical Center, Charlotte, NC 28204, USA.

**Correspondence to:** Dr. B. Todd Heniford, Gastrointestinal and Minimally Invasive Surgery, Department of Surgery, 1025 Morehead Medical Drive Suite 300, Charlotte, NC 28204, USA. E-mail: todd.heniford@gmail.com

**How to cite this article:** Shao J, Elhage S, Deerenberg E, Augenstein V, Heniford BT. Establishing a center of excellence in abdominal wall reconstruction. *Plast Aesthet Res* 2020;7:21. <http://dx.doi.org/10.20517/2347-9264.2020.04>

**Received:** 3 Jan 2020 **First Decision:** 10 Apr 2020 **Revised:** 13 Apr 2020 **Accepted:** 16 Apr 2020 **Published:** 25 Apr 2020

**Science Editor:** Sahil Kuldip Kapur **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

Building a tertiary referral center of excellence for complex abdominal wall reconstruction is a multi-step process that requires many elements to garner and promote success. Ultimately the creation of such a center is important for continual improvement of abdominal wall reconstruction outcomes by decreasing complications, recurrences, length of hospital stay, hospital readmissions, and overall costs. Establishing a center of excellence incorporates several key components including the surgeon's desires and expertise, institutional participation, multidisciplinary collaboration, outcomes research and innovation, and financial stability. This article outlines the principal elements of building a sustainable, functional, and successful center of excellence for complex abdominal wall reconstruction.

**Keywords:** Complex abdominal wall reconstruction, center of excellence

## INTRODUCTION

Over 1 million hernia surgeries are performed in the United States annually, making hernia repair the most common general surgery procedure performed by surgeons across the country<sup>[1-3]</sup>. Hernias occur due to a variety of factors, including prior abdominal surgery, increased intraabdominal pressure secondary to chronic cough or obesity, and compromised connective tissue integrity as a result of genetics, infection and other factors. The incidence of abdominal hernia needing surgery is estimated to be as high as 20%-30% in the literature<sup>[4-7]</sup>. Due to the large number of patients requiring hernia repair, it is estimated that a 1% reduction in hernia recurrence would save \$32 million annually in healthcare<sup>[1]</sup>. While personal health consequences are supremely important, these data highlight the significant financial implications for both hospitals and patients in hernia prevention and durability of repair. Of those patients undergoing



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





ventral hernia repair, it is estimated that as many as 12%-52% will have a hernia recurrence, which ultimately leads to more surgery, reduced quality of life, prolonged recovery, a greater chance of future failure, and increasing healthcare costs<sup>[8-12]</sup>. It is estimated that each failed hernia repair results in higher morbidity, cost, and risk of recurrence for each subsequent repair<sup>[13-15]</sup>. All these factors combined make a compelling argument for optimizing the index hernia repair for patients to decrease recurrence rates and cost, ultimately improving patient outcomes. This leads to preoperative optimization of modifiable patient comorbidities, increasing patient education, and streamlining operative and postoperative factors to provide the best care.

## **BUILDING A CENTER OF EXCELLENCE**

### **What does it mean to be a center of excellence?**

The trend towards building “centers of excellence” is not a new concept in surgery. Within recent years, driven by national policies considering outcome measures, there has been a drive towards improved outcomes and decreased complications, length of hospital stay, and hospital readmissions<sup>[16,17]</sup>. This is backed by data and evidence from across many surgical specialties, including oncologic, bariatric, orthopedic, and cardiovascular surgery, in which better surgical outcomes are seen at established high-volume specialized centers<sup>[18-28]</sup>. The trend of improved outcomes at high-volume specialty centers is especially noticeable for complex procedures. While the current literature does not provide a full explanation for this association, the improvement in outcomes is likely linked to an increased surgical skill set from high-volume repetition (practice makes perfect model), selective-referral patterns, and accessibility to additional specialized resources for more comprehensive perioperative care<sup>[27]</sup>. The literature also demonstrates that associated surgical fellowships also improve surgical outcomes<sup>[29-31]</sup>. This is most ardently demonstrated in bariatric surgery, which has reinforced the development of a bariatric center of excellence (COE)<sup>[32,33]</sup>. The term COE has been widely adopted by many fields of surgery, but what does it truly mean to be a COE? This article will describe the foundations of a COE for complex abdominal wall reconstruction (AWR) and the key components needed to build a multidisciplinary practice centered on improvement of patient outcomes and development of new techniques for advancement of surgical practice.

### **Establishing a multidisciplinary AWR center**

Establishing a tertiary referral and care center for AWR is a concept that has solidified in the past decade<sup>[17,34-36]</sup>. Recognition of hernia surgery as a challenging endeavor fraught with unique challenges has led to the development of several specialized AWR centers<sup>[34-36]</sup>. There is a paucity of literature available regarding which healthcare systems would qualify as a COE. From the literature, we see how the German Hernia Society has created a 3-tier accreditation system for hernia repair centers, stratifying centers based on volume, outcome evaluation, academic involvement, hernia research, and participation in multicenter databases<sup>[37]</sup>. In addition to traditional measures of success, such as hernia recurrence and pure clinical outcomes, there is a push to include patient quality of life, focus on research, and continual participation in ongoing academics to bolster the current advancements in surgery<sup>[37]</sup>. Williams *et al.*<sup>[35]</sup> described the establishment of a hernia referral center, which led to an increase in the complexity of the patients referred, including a 48% increase in recurrent hernias, increasing patient comorbidities, and overall complexity. Raigani *et al.*<sup>[36]</sup> describe a similar trend of increasing complexity in 2014, in which patients traveling over 100 miles were more likely to have active mesh infections and increased length of stay. Both studies highlight that increasing hernia complexity combined with patient comorbidity and operative difficulty should lead to increasing postoperative complications. However, in practice, complex patients treated at tertiary referral centers do not have a proportional increase in adverse outcomes or complications due to expertise provided at these centers<sup>[35,36]</sup>.

The European Hernia Society has also developed an expert consensus comprising 18 surgeons across Europe to form the ACCESS Group (Hernia Accreditation and Certification of Centers and Surgeons -

Working Group) to formulate scientifically based requirements for hernia centers and hernia specialists<sup>[38]</sup>. They propose that an adequately prepared hernia center will have the following components: accredited/certified by a national or international hernia society, perform higher case volume hernia surgery compared to average, experienced surgeons beyond their learning curve, evidence-based clinical treatment, document outcomes in hernia registry or database, and perform follow-up comparisons of outcomes with benchmark data for continual improvement<sup>[38]</sup>. These recommended requirements have been put forth so that healthcare systems can develop their own programs, while taking into account specific healthcare system constraints<sup>[38]</sup>.

At the authors' own institution, a hernia center has been established since 2004, due to increasing patient numbers, complexity of abdominal wall hernias, and outside referrals. Since that time, a growing network of general surgeons, plastic surgeons, infectious disease specialists, allergists, radiologists, orthopedic surgeons, geriatricians, and sports medicine, research, and support staff have been recruited in a multidisciplinary fashion to coordinate complex care for patients. This coordinated problem-solving approach has allowed the hospital system to increase annual case volume by 234%, annual billing by 713%, in-state referrals by 340%, and out of state referrals by 540%<sup>[35]</sup>. Using a model of concentrated, high-quality patient-centered, patient-specific care in both the inpatient and outpatient setting has led to a streamlined perioperative process that reduces preoperative modifiable comorbidities, increases patient education and high-volume technical operations, combined with specific goal-oriented postoperative management strategies, and ultimately smooth transitions of care. All these combined efforts have led to decreasing recurrence rates and improved patient outcomes and quality of life<sup>[39]</sup>. This comprehensive care has also allowed for continued academic improvement and research, to analyze outcomes for further improvement.

Regardless of the approach, whether a government-driven model like the one in Germany or a surgeon-driven model such as at the authors' institution, they both emphasize similar core characteristics encompassing high-quality, financially sustainable, multidisciplinary care, with the ability for continual improvement through academic research and outcomes analysis. An AWR COE should be able to confer the benefits of the institution to the patient in a standardized manner with reproducible and reliable outcomes for complex hernia patients. Cherla *et al.*<sup>[40]</sup> described a significant reduction in surgical site infections in over 600 patients from 13.5 to 1.5% over a period of one year, despite increasing complexity of cases, due to appropriate patient referral, improved preoperative management of patient comorbidities, and internal consistency of treatment principles across all surgeons participating in AWR. The true hallmark of a COE is the ability to mitigate increasing risk factors in complex patients and still provide successful clinical outcomes with low hernia recurrence, infections, length of stay, and cost, while simultaneously improving quality of life.

While no strict criteria exist indicating which patients should be referred to a AWR COE, some general considerations that may warrant referral are: surgeon experience and comfort performing hernia operations, patient complexity including presence of mesh infections or fistulas, loss of domain, need for components separation technique, lack of hospital resources such as Botox and plastic surgery, and multiple recurrent hernias with many prior operations. Ultimately, referral is dependent on a recognition for a higher level of care due to increasing patient complexity and the ability of a COE to provide patient-specific care with improved hernia outcomes and decreased recurrence rates<sup>[27,35,36,39,40]</sup>.

Having a COE is beneficial not only to patients but to parent institutions as well by increasing surgical volume, complexity, and reputational benefits, which will draw additional resources and talent and expand the infrastructure already implemented. Additionally, the treatment of a large volume of patients, as seen at our institution, can be and has been the driving force for innovative research focusing on preoperative optimization, operative techniques, and postoperative quality of life. Integration of academic research in

AWR allows for quality improvement as institutions can follow their own long-term outcomes for self-evaluation, and eventual growth and improvement.

## KEY COMPONENTS OF A SUCCESSFUL HERNIA CENTER

There are several key components that all successful tertiary care AWR COEs will require: surgeon experience and expertise, support of parent institution, interdisciplinary collaboration, commitment to current evidence-based practice and continual outcome assessment, and financial stability.

### Surgical expertise

#### *Operative technique and anatomical mastery*

Education and specialization in AWR and hernia surgery with good operative technique is a foundational building block of a successful hernia center. As with many other experts in their respective fields, practice makes perfect and having the appropriate experience and training is important in AWR<sup>[27,28]</sup>. Surgeons specializing in AWR may have training in general, minimally invasive, robotic, and plastic surgery, or a combination of all these techniques in their armamentarium. Proper knowledge of the relevant anatomy and of the appropriate surgical technique and tissue planes cannot be understated to perform the optimal surgery, and the ability to innovate and create new techniques for hernia repair is also important. Once mastery is achieved, continued education and involvement in academic research and conferences are also important to sustain a level of high-quality care. Having surgical fellows and training programs also fosters excellence within a center and improves outcomes<sup>[29-31]</sup>. Attending and hosting AWR courses can also be utilized as a tool for maintaining expertise.

#### *Perioperative management*

In addition to the appropriate technical skills, surgeons must also possess the clinical acumen to provide appropriate perioperative care for complex AWR patients. Being able to successfully navigate complex patient records with multiple prior abdominal surgeries, complications, and hernia repairs is very helpful in formulating the next clinical care steps. Routinely, outside hospital records and CT scans are obtained. Once an assessment has been made, along with a thorough history and physical examination, identification of the patient's specific risk factors is made, and pre-habilitation as needed precedes surgery<sup>[39]</sup>. Sometimes, a patient may not be ready for surgery for several months after their initial consultation to optimize their specific comorbidities.

Risk models commonly used include the Ventral Hernia Risk Score, the Ventral Hernia Working Group Grade, Centers of Disease Control and Prevention Wound Class, and Hernia Wound Risk Assessment tool. An application designed by our institution known as the Carolinas Equation for Determining Associated Risks is commonly employed to calculate a patient's postoperative cost and risk of wound complications<sup>[41-45]</sup>. Utilization of this application has decreased cost and wound complications specific to our center and has been recognized across the world as an accurate predictor of risk<sup>[46]</sup>.

Specifically, the risk factors and comorbidities addressed by the assessments are geared towards prevention of hernia recurrence, need for additional surgery, prevention of complications, and decreasing costs<sup>[39,45,46]</sup>. To accomplish this goal, associated complications or comorbidities that contribute to recurrence such as infection, obesity, tobacco use, and diabetes are factors that warrant careful attention. Having a comprehensive approach to patient care and tailored patient counseling prior to surgery is an important keystone in the patients' postoperative course and outcomes. Optimization is not limited to informing patients about their risk, it also includes patient-specific interventions and confirmation of success.

Strategies for optimization include weight loss for patients who are obese with BMI  $\geq 30$  kg/m<sup>2</sup> by not only providing patient education, but also dietary plans, multidisciplinary follow-up with bariatric dieticians,

establishing specific weight loss goals, and follow-up appointments with healthcare providers prior to scheduling surgery. Smoking cessation is mandatory and confirmed with nicotine tests on follow-up clinic visits and prior to surgery. Glycemic control defined as a hemoglobin A1c less than 7.2% is used on the basis of internal validation for risk calculations as well as international consensus<sup>[47-51]</sup>. All these interventions are achieved through a healthcare network in conjunction with a multidisciplinary team involving the primary care provider, dietician, bariatricians, bariatric surgeons, and other healthcare providers. Ultimately, these efforts are geared towards preventing wound complications postoperatively, since obesity, active smoking, and diabetes are all independent predictors for increasing wound complications and recurrence in AWR patients<sup>[39,47-54]</sup>. In addition, patients also undergo preoperative screening to make sure all preoperative work up is completed from a cardiopulmonary and anesthesia standpoint.

Patients who have had multiple hernia surgeries also undergo preoperative CT scans to assess hernia defect size and better understand surgical anatomy. Often, prior meshes, as well as other abnormalities that may impact the surgery are seen on CT and can be used for perioperative planning. In patients who have large defect sizes and loss of domain that may make fascial closure difficult, they can be considered for preoperative botulinum toxin injection and be counseled concerning the need for components separation. Preoperative planning for success is perhaps the most important component to providing a successful surgery. Appropriate patient selection, risk factor modification, and sound surgical planning prior to a procedure is crucial to a successful index operation or durable recurrent hernia repair.

### **Institutional participation**

Building a hernia COE requires many resources and buy in from all participants, most importantly from the parent institution. Understanding the value of having a hernia COE and the additional value it brings to an institution will allow the parent institution to participate, promote and use available resources to bring recognition to the AWR center<sup>[55]</sup>.

### *Creating visibility*

Marketing and creating a presence through advertising by various means will help facilitate referrals and increase patient volume. Additional promotion should include a dedicated website and social media presence, featured articles in local media, press releases, distribution of brochures, peer-reviewed publications, national conference presentation and recognition, and education of other healthcare providers within the system to increase the referral base.

### *Dedicated ancillary support*

Other keys to making a successful COE is having dedicated personnel specific to the program who can espouse the key principles of the hernia center and who understand how to support the program and help it grow. These are a team of advanced care practitioners (nurse practitioners and physician assistants) who are trained to specifically take care of hernia patients, surgical schedulers with knowledge of operative needs, insurance and billing specialists to help obtain coverage and payment for specific interventions that are hernia specific. In an academic center, having designated research personnel and statisticians, as well as staff assistants and coordinators are also important and can significantly improve care by simply associating behavior and outcomes in the center.

### *Coordination of care*

Having a nationally renowned AWR center with a wide referral base also means attracting patients from a larger catchment area with patients traveling from out of state for consultation, perioperative care, and surgery. Being able to coordinate alignment of clinic times for multiple consultants, operative scheduling and travel plans, and allowing for smooth integration of care in a new healthcare system by obtaining prior medical records and imaging are all tasks that will need to be completed for successful and streamlined care.

## Interdisciplinary collaboration

### *Building a network of specialists*

Surgery alone is not enough for success in complex AWR patients who often will require a multi-faceted approach to ensure they are prepared, optimized, and ultimately ready to undergo surgery. As previously discussed, having a support network of ancillary staff and physicians with aligned goals in a coordinated effort is essential. Building a network of physicians who intimately understand the unique challenges that AWR patients face and how to intervene in these problems will be beneficial.

### *Potential areas of collaboration*

Much of specialist collaboration is planned prior to the operation by recognizing areas of improvement. Preoperative consultation of bariatricians, dieticians, primary care doctors, and endocrinologists to help with management of comorbidities including weight loss, glycemic control, and smoking cessation is important. There should be consideration of a prior history of thromboembolism and the use of anticoagulation medications. This would lead to decisions concerning which patients would benefit from an inferior vena cava filter and the management of anticoagulated medications to limit the risk of adverse events including stroke, pulmonary embolism, while attempting to mitigate the chance of postoperative bleeding or hematoma. Consulting with radiology regarding CT scan findings can be helpful to decide which patients with larger hernia defects would benefit from preoperative Botox injection<sup>[56]</sup>. Radiologists can also participate greatly in drain placement, injection of steroids or other drugs in specific areas, and diagnosis and management of pain in patients. Patients might also benefit from a consult with plastic surgery regarding advancement flaps or perhaps the need for concomitant panniculectomy during the hernia repair itself. Some patients may also need simultaneous intraabdominal procedure such as enterocutaneous fistula takedown, biliary surgery, colon resection, ostomy revision or reversal, etc., and having the appropriate consulting surgeons available is necessary. Other patients who have had prior hernia repairs with complications of prior or existing infection or have extensive antibiotic allergies may also benefit from an infectious disease consult.

Intraoperatively, having an anesthesia team focused on multimodal pain management is extremely helpful to limit postoperative narcotic requirement, improve postoperative mobility, and ultimately reduce the length of hospital stay<sup>[57-59]</sup>. Setting forth an intraoperative protocol for pain management including the use of a lidocaine drip, dexmedetomidine, and liposomal bupivacaine by performing transversus abdominis plane blocks will be helpful in terms of limiting overall narcotic use (ERAS). Postoperative use of patient-controlled analgesia, early mobility, and introduction of scheduled Tylenol, gabapentin, and ibuprofen will also be key to decreasing ileus, decreasing narcotic use, and decreasing hospital length of stay<sup>[57-59]</sup>.

Postoperative consultation with geriatricians to manage older patients with frailty, limited mobility, declining mental status, and polypharmacy can significantly improve the postoperative recovery process for that subset of patients<sup>[60]</sup>. Managing inpatient and outpatient complications will also be important as the need arises. Also necessary will be utilizing resources of interventional radiology to drain any postoperative fluid collection, consultation with infectious disease physicians for treatment of surgical site occurrences, the need for physical therapy and social work, and rarely the need for intensivist care or other subspecialists.

The underlying vital concept is to generate harmony between care teams, and that predominantly relies on leadership, adequate communication of ideas and responsibilities, and a healthy relationship between all members of the healthcare network. Indeed, identifying experts and leaders in each field will influence the success of a patient's ultimate outcome and success of the AWR center. Great teamwork with careful coordination is indispensable in a well-run and organized complex AWR COE.



### Outcomes research and innovation

Analysis of outcomes and participation in clinical research has been demonstrated to have an association with improved postoperative outcomes. Involvement in multicenter hernia registry has also been linked to improved outcomes due to awareness of surveillance (Hawthorne effect), or self-selection bias in which participating centers have higher vested interest in overall hernia outcomes<sup>[37,38,61,62]</sup>. The ability to maintain a prospective database with long-term analysis of hernia recurrence rates, quality of life, and postoperative outcomes is the only way to understand if implemented techniques and interventions achieve their goal and meet patient satisfaction<sup>[63-66]</sup>.

Promotion of a rich research environment by having a diverse group of research personnel for each step of the process will streamline data collection, consent of patients, institutional review board approval, data analysis, and ultimately abstract submissions, presentations, and preparation of manuscripts for publication. These tasks can be done with dedicated research staff including research managers, data collection specialists, residents, and fellows. Having a surgical fellowship encourages continual learning, improvement, introduction of new techniques and the ability to sustain academic productivity<sup>[29-31]</sup>. In many ways, academic research can complement a clinically busy workload. Research can also be funded by obtaining sponsors and grants, including but not limited to national sponsors (National Institutes of Health, Department of Defense), surgical associations, industry, and hospital-specific internal funding.

Collaboration with other hernia centers encourages shared learning, data collection, and increased statistical power. It also allows for the identification of reproducible outcomes that can then be generalized. Participation in hernia registries can go a long way in terms of gaining new data and information. Collaboration can be more formalized through registered databases, at national and international academic meetings, but it can also be informal through social media and other various idea and information sharing platforms.

### Financial independence and sustainability

The financial investment needed for establishing an AWR COE can be substantial but will be offset by the financial benefits of having a tertiary care referral center that will generate revenue from a growing patient network, referral patterns, and decreasing cost of complications, recurrence, and reoperations. Negotiation of resources is unique in each institution and building a COE will be a long-term investment that will ultimately improve the reputation of the parent institution, establish patient care practices that will decrease postoperative cost, length of stay and financial burden of complex patients. Building a sustainable and streamlined process only leads to a more efficient and cost-effective system that focuses on decreasing healthcare costs. A recent review demonstrated that by modifying risk factors alone in over 700 patients, there was a decrease in postoperative wound-related complications from 40.8% to 20.6%, with an estimated savings of over \$4 million<sup>[46]</sup>. Postoperative billing should also reflect the complexity of the work performed by the healthcare team and should consider recurrence, incarceration, mesh excisions, components separation, and documentation of advancement flaps and soft tissue rearrangements needed after the hernia repair itself. These combined interventions of generating referral patterns, increasing productivity in clinical activity, research reputation, and decreasing healthcare costs are worthwhile institutional investments.

### CONCLUSION

The marker of a successful AWR program is multifactorial with many key components. In a system that works well, all participants should collaborate and benefit from the COE, including the institution, surgeons, physicians, care providers, and above all the patients. Increased case volume, complexity and referrals together will be one indication of success, but other measurable indicators include long-term patient outcomes and hernia recurrence rates.

## DECLARATIONS

### Acknowledgments

The intention of this article is to provide building blocks and a foundation for the successful establishment of a hernia specialty center. Any changes in referral or influence are secondary and unintended.

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Shao J, Elhage S, Deerenberg E, Augenstein V, Heniford BT

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

Dr. Heniford has received honoraria and research support from Allergan, W. L. Gore, and Stryker. Dr. Augenstein has received consultation fees from W. L. Gore, Allergan, and KCI. The authors have no other relevant financial or personal relationships that could inappropriately influence this work or its conclusions. None of the authors has a financial interest in any of the products, devices or drugs mentioned in the article.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Poulou BK, Shelton J, Phillips S, Moore D, Nealon W, et al. Epidemiology and cost of ventral hernia repair: making the case for hernia research. *Hernia* 2012;16:179-83.
2. Rutkow IM. Demographic and socioeconomic aspects of hernia repair in the United States in 2003. *Surg Clin North Am* 2003;83:1045-51.
3. Colavita PD, Tsirlane VB, Walters AL, Lincourt AE, Belyanksy I, et al. Laparoscopic versus open hernia repair: outcomes and sociodemographic utilization results from the nationwide inpatient sample. *Surg Endosc* 2013;27:109-17.
4. Deerenberg EB, Harlaar JJ, Steyerberg EW, Lont HE, van Doorn HC, et al. Small bites versus large bites for closure of abdominal midline incisions (STITCH): a double-blind, multicentre, randomised controlled trial. *Lancet* 2015;386:1254-60.
5. Jairam AP, Timmermans L, Eker HH, Pierik REGJM, van Klaveren D, et al. Prevention of incisional hernia with prophylactic onlay and sublay mesh reinforcement versus primary suture only in midline laparotomies (PRIMA): 2-year follow-up of a multicentre, double-blind, randomised controlled trial. *Lancet* 2017;390:567-76.
6. Aquina CT, Rickles AS, Probst CP, Kelly KN, Deeb AP, et al. Visceral obesity, not elevated BMI, is strongly associated with incisional hernia after colorectal surgery. *Dis Colon Rectum* 2015;58:220-7.
7. Trimbos JB, Smit IB, Holm JP, Hermans J. A randomized clinical trial comparing two methods of fascia closure following midline laparotomy. *Arch Surg* 1992;127:1232-4.
8. Davila DG, Parikh N, Frelich MJ, Goldblatt MI. The increased cost of ventral hernia recurrence: a cost analysis. *Hernia* 2016;20:811-7.
9. van Ramshorst GH, Eker HH, Hop WC, Jeekel J, Lange JF. Impact of incisional hernia on health-related quality of life and body image: a prospective cohort study. *Am J Surg* 2012;204:144-50.
10. Hesselink VJ, Luijendijk RW, de Wilt JH, Heide R, Jeekel J. An evaluation of risk factors in incisional hernia recurrence. *Surg Gynecol Obstet* 1993;176:228-234.
11. Stoppa RE. The treatment of complicated groin and incisional hernias. *World J Surg* 1989;13:545-554.

12. Luijendijk RW, Hop WC, van den Tol MP, de Lange DC, Braaksma MM, et al. A comparison of suture repair with mesh repair for incisional hernia. *N Engl J Med* 2000;343:392-8.
13. Flum DR, Horvath K, Koepsell T. Have outcomes of incisional hernia repair improved with time? A population-based analysis. *Ann Surg* 2003;237:129-35.
14. Heniford BT, Park A, Ramshaw BJ, Voeller G. Laparoscopic repair of ventral hernias: nine years' experience with 850 consecutive hernias. *Ann Surg* 2003;238:391-9.
15. Reynolds D, Davenport DL, Korosec RL, Roth JS. Financial implications of ventral hernia repair: a hospital cost analysis. *J Gastrointest Surg* 2013;17:159-66.
16. Ibrahim AM, Hughes TG, Thumma JR, Dimick JB. Association of hospital critical access status with surgical outcomes and expenditures among medicare beneficiaries. *JAMA* 2016;315:2095-103.
17. Colavita PD, Walters AL, Tsirlane VB, Belyansky I, Lincourt AE, et al. The regionalization of ventral hernia repair: occurrence and outcomes over a decade. *Am Surg* 2013;79:693-701.
18. Birkmeyer JD, Siewers AE, Finlayson EV, Stukel TA, Lucas FL, et al. Hospital volume and surgical mortality in the United States. *N Engl J Med* 2002;346:1128-37.
19. Champion JK, Pories WJ. Centers of excellence for bariatric surgery. *Surg Obes Relat Dis* 2005;1:148-51.
20. Cress D, Pelton J, Thayer SC, Bukrey C. Development of a center of excellence for joint replacement. *Orthop Nurs* 2010;29:150-68.
21. Hollenbeak CS, Rogers AM, Barrus B, Wadiwala I, Cooney RN. Surgical volume impacts bariatric surgery mortality: a case for centers of excellence. *Surgery* 2008;144:736-43.
22. Sharma A, Mehrotra M, Khullar R, Soni V, Bajjal M, et al. Laparoscopic ventral/incisional hernia repair: a single centre experience of 1,242 patients over a period of 13 years. *Hernia* 2011;15:131-9.
23. Mehrotra A, Sloss EM, Hussey PS, Adams JL, Lovejoy S, et al. Evaluation of centers of excellence program for knee and hip replacement. *Med Care* 2013;51:28-36.
24. Begg CB, Cramer LD, Hoskins WJ, Brennan MF. Impact of hospital volume on operative mortality for major cancer surgery. *JAMA* 1998;280:1747-51.
25. Fong Y, Gonen M, Rubin D, Radzyner M, Brennan MF. Long-term survival is superior after resection for cancer in high-volume centers. *Ann Surg* 2005;242:540-7.
26. Luft HS, Bunker JP, Enthoven AC. Should operations be regionalized? The empirical relation between surgical volume and mortality. *N Engl J Med* 1979;301:1364-9.
27. Luft HS, Hunt SS, Maerki SC. The volume-outcome relationship: practice-makes-perfect or selective-referral patterns? *Health Serv Res* 1987;22:157-82.
28. Shahian DM, Normand SL. The volume-outcome relationship: from Luft to Leapfrog. *Ann Thorac Surg* 2003;75:1048-58.
29. Kohn GP, Galanko JA, Overby DW, Farrell TM. High case volumes and surgical fellowships are associated with improved outcomes for bariatric surgery patients: a justification of current credentialing initiatives for practice and training. *J Am Coll Surg* 2010;210:909-18.
30. Kim PS, Telem DA, Altieri MS, Talamini M, Yang J, et al. Bariatric outcomes are significantly improved in hospitals with fellowship council-accredited bariatric fellowships. *J Gastrointest Surg* 2015;19:594-7.
31. Altieri MS, Yang J, Yin D, Frenkel C, Talamini M, et al. Presence of a fellowship improves perioperative outcomes following hepatopancreatobiliary procedures. *Surg Endosc* 2017;31:2918-24.
32. Livingston EH. Surgical volume impacts bariatric surgery mortality: a case for bariatric surgery centers of excellence. *Surgery* 2010;147:751-3.
33. Ibrahim AM, Ghaferi AA, Thumma JR, Dimick JB. Variation in outcomes at bariatric surgery centers of excellence. *JAMA* 2017;318:629-36.
34. Gilbert AI, Graham MF, Young J, Patel BG, Shaw K. Closer to an ideal solution for inguinal hernia repair: comparison between general surgeons and hernia specialists. *Hernia* 2006;10:162-8.
35. Williams KB, Belyansky I, Dacey KT, Yurko Y, Augenstein VA, et al. Impact of the establishment of a specialty hernia referral center. *Surg Innov* 2014;21:572-9.
36. Raigani S, De Silva GS, Criss CN, Novitsky YW, Rosen MJ. The impact of developing a comprehensive hernia center on the referral patterns and complexity of hernia care. *Hernia* 2014;18:625-30.
37. Köckerling F, Berger D, Jost JO. What is a certified hernia center? The example of the German hernia Society and German Society of General and Visceral Surgery. *Front Surg* 2014;1:26.
38. Köckerling F, Sheen AJ, Berrevoet F, Campanelli G, Cuccurullo G, et al. Accreditation and certification requirements for hernia centers and surgeons: the ACCESS project. *Hernia* 2019;23:185-203.
39. Cox TC, Blair LJ, Huntington CR, Colavita PD, Prasad T, et al. The cost of preventable comorbidities on wound complications in open ventral hernia repair. *J Surg Res* 2016;206:214-22.
40. Cherla DV, Holihan JL, Flores-Gonzalez JR, Lew DF, Escamilla RJ, et al. Decreasing surgical site infections after ventral hernia repair: a quality-improvement initiative. *Surg Infect (Larchmt)* 2017;18:780-6.
41. Liang MK, Goodenough CJ, Martindale RG, Roth JS, Kao LS. External validation of the ventral hernia risk score for prediction of surgical site infections. *Surg Infect (Larchmt)* 2015;16:36-40.
42. Berger RL, Li LT, Hicks SC, Davila JA, Kao LS, et al. Development and validation of a risk-stratification score for surgical site occurrence and surgical site infection after open ventral hernia repair. *J Am Coll Surg* 2013;217:974-82.
43. Breuing K, Butler CE, Ferzoco S, Hultman CS, Kilbridge JF, et al; Ventral Hernia Working Group. Incisional ventral hernias: review of

- the literature and recommendations regarding the grading and technique of repair. *Surgery* 2010;148:544-58.
44. Fischer JP, Wink JD, Tuggle CT, Nelson JA, Kovach SJ, et al. Wound risk assessment in ventral hernia repair: generation and internal validation of a risk stratification system using the ACS-NSQIP. *Hernia* 2015;19:103-11.
  45. Augenstein VA, Colavita PD, Wormer BA, Walters AL, Bradley JF, et al. CeDAR: Carolinas Equation for Determining Associated Risks. *J Am Coll Surg* 2015;221:S65-66.
  46. Otero J, Cox T, Huntington C, Prasad T, Davis BR, et al. Development of the carolinas equation for determining associated risks application and its effects on patient outcomes and potential financial savings in open ventral hernia repair. *Am Coll Surg* 2018.
  47. Hikata T, Iwanami A, Hosogane N, Watanabe K, Ishii K, et al. High preoperative hemoglobin A1c is a risk factor for surgical site infection after posterior thoracic and lumbar spinal instrumentation surgery. *J Orthop Sci* 2014;19:223-8.
  48. Shaw P, Saleem T, Gahtan V. Correlation of hemoglobin A1C level with surgical outcomes: can tight perioperative glucose control reduce infection and cardiac events? *Semin Vasc Surg* 2014;27:156-61.
  49. Liang MK, Holihan JL, Itani K, Alawadi ZM, Gonzalez JR, et al. Ventral hernia management: expert consensus guided by systematic review. *Ann Surg* 2017;265:80-9.
  50. Gatti G, Perrotti A, Reichart D, Maschietto L, Onorati F, et al. Glycated hemoglobin and risk of sternal wound infection after isolated coronary surgery. *Circ J* 2017;81:36-43.
  51. Arya S, Binney ZO, Khakharia A, Long CA, Brewster LP, et al. High hemoglobin A1c associated with increased adverse limb events in peripheral arterial disease patients undergoing revascularization. *J Vasc Surg* 2018;67:217-28.
  52. Sauerland S, Korenkov M, Kleinen T, Arndt M, Paul A. Obesity is a risk factor for recurrence after incisional hernia repair. *Hernia* 2004;8:42-6.
  53. Vidović D, Jurisić D, Franjić BD, Glavan E, Ledinsky M, et al. Factors affecting recurrence after incisional hernia repair. *Hernia* 2006;10:322-5.
  54. Newcomb WL, Polhill JL, Chen AY, Kuwada TS, Kersin KS, et al. Staged hernia repair preceded by gastric bypass for the treatment of morbidly obese patients with complex ventral hernias. *Hernia* 2008;12:465-9.
  55. Schlosser KA, Arnold MR, Kao AM, Augenstein VA, Heniford BT. Building a multidisciplinary hospital-based abdominal wall reconstruction program: nuts and bolts. *Plast Reconstr Surg* 2018;142:201S-8S.
  56. Motz BM, Schlosser KA, Heniford BT. Chemical Components Separation: Concepts, Evidence, and Outcomes. *Plast Reconstr Surg* 2108;142:58S-63S.
  57. Ueland W, Walsh-Blackmore S, Nisiewicz M, Davenport DL, Plymale MA, et al. The contribution of specific enhanced recovery after surgery (ERAS) protocol elements to reduced length of hospital stay after ventral hernia repair. *Surg Endosc* 2019; Epub ahead of print. doi: 10.1007/s00464-019-07233-8
  58. Harryman C, Plymale MA, Stearns E, Davenport DL, Chang W, et al. Enhanced value with implementation of an ERAS protocol for ventral hernia repair. *Surg Endosc* 2019; Epub ahead of print. doi: 10.1007/s00464-019-07166-2
  59. Stearns E, Plymale MA, Davenport DL, Totten C, Carmichael SP, et al. Early outcomes of an enhanced recovery protocol for open repair of ventral hernia. *Surg Endosc* 2018;32:2914-22.
  60. Joseph WJ, Cuccolo NG, Braon ME, Chow I, Beers EH. Frailty predicts morbidity, complication and mortality in patients undergoing complex abdominal wall reconstruction. *Hernia* 2020;24:235-43.
  61. Nilsson E, Haapaniemi S. Hernia registers and specialization. *Surg Clin North Am* 1998;78:1141-55.
  62. Iles JD. Specialisation in elective herniorrhaphy. *Lancet* 1965;1:751-5.
  63. Heniford BT, Walters AL, Lincourt AE, Novitsky YW, Hope WW, et al. Comparison of generic versus specific quality-of-life scales for mesh hernia repairs. *J Am Coll Surg* 2008;206:638-44.
  64. Belyansky I, Tsirlin VB, Klima DA, Walters AL, Lincourt AE, et al. Prospective, comparative study of postoperative quality of life in TEP, TAPP, and modified Lichtenstein repairs. *Ann Surg* 2011;254:709-14.
  65. Klima DA, Tsirlin VB, Belyansky I, Dacey KT, Lincourt AE, et al. Quality of life following component separation versus standard open ventral hernia repair for large hernias. *Surg Innov* 2014;21:147-54.
  66. Heniford BT, Lincourt AE, Walters AL, Colavita PD, Belyansky I, et al. Carolinas Comfort Scale as a measure of hernia repair quality of life: a reappraisal utilizing 3788 international patients. *Ann Surg* 2018;267:171-6.

Editorial

Open Access



# Introduction of special issue “Advances in Microsurgery for Upper and Lower Extremity Reconstruction and Limb Preservation”

Matthew L. Iorio

Division of Plastic and Reconstructive Surgery, University of Colorado Anschutz Medical Center, Aurora, CO 80045, USA.

**Correspondence to:** Dr. Matthew L. Iorio, Division of Plastic and Reconstructive Surgery, University of Colorado Anschutz Medical Center, Aurora, CO 80045, USA. E-mail: mattiorio@gmail.com

**How to cite this article:** Iorio ML. Introduction of special issue “Advances in Microsurgery for Upper and Lower Extremity Reconstruction and Limb Preservation”. *Plast Aesthet Res* 2020;7:22. <http://dx.doi.org/10.20517/2347-9264.2020.86>

**Received:** 17 Apr 2020 **Accepted:** 20 Apr 2020 **Published:** 29 Apr 2020

**Science Editor:** Raúl González-García **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

I am honored to present the special edition “Advances in Microsurgery for Upper and Lower Extremity Reconstruction and Limb Preservation” for *Plastic and Aesthetic Research*. Herein, world-class experts describe their techniques for functional reconstruction and rehabilitation in extremity injuries and limb salvage.

The goal of this edition was to provide a comprehensive reference for the extremity surgeon. These outstanding articles review not only individual techniques of flap coverage, such as chimeric and propeller flaps, but also the unique considerations for flap reconstruction timing, tendon reconstruction, techniques for perforator mapping and postoperative monitoring, and the importance of the plastic surgeon in mass casualty incidents. And as such, a significant improvement in the functional quotient can be seen through the use of vascularized bone grafting, composite allotransplantation, and functional muscle and nerve reconstructions.

Reconstructive surgeons are often asked for assistance to “close a wound” in patients with complex limb defects. This reductive approach belies a greater opportunity to enhance limb salvage through improving functional outcomes. Although it may stem from a lack of understanding of available options or from the mindset that complex reconstructions may have a lower chance of success, a truer definition of success should be restoring the patient to as close to the pre-injury state as possible. To fulfil our commitment to our patients, we must advocate for sophisticated and innovative reconstruction, demonstrating improved outcomes by return to functional baselines, ambulation, and decreased pain.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





Limb salvage is a multidisciplinary specialty, not only from the surgical perspective but also in the application of the reconstruction. Critical checkpoints include overall patient safety and trauma resuscitation, prioritization of salvage, and tailored options for reconstruction, all of which are paramount at a time when decision-making may be limited by a lack of resources as well as the sheer volume of patient needs. Reconstruction should proceed in an orderly and timely manner, not only from the initial stage but with contemplation of the secondary, tertiary, or even quaternary interventions that may be considered down the road. This concept is frequently seen in the setting of long bone fractures, which may require secondary bone grafting, external-fixator placement, and distraction osteogenesis. This edition attempts to integrate those concepts across extremity microsurgery, in cases such as combined tendon reconstruction, targeted muscle reinnervation, and functional muscle transfers.

Lastly, an area of care that can be overlooked at the detriment of the overall result concerns post-reconstructive orthotic assist devices. These are relevant both in choosing a stable, durable and appropriately shaped soft tissue reconstruction, but also in understanding the additional gains afforded by a well-designed prosthesis. With an understanding of these adaptive devices, a reconstruction can be better tailored to optimize outcomes, including a reference for various levels of amputation, as well as a guide for soft tissue tailoring to prevent fitment or wound durability complications.

Thanks to all of the authors, and also the reviewers and editorial staff from *Plastic and Aesthetic Research*, for making this an exceptional issue and contribution to the field of extremity microsurgery.

I hope that you find this special edition useful, and an active guide to extremity reconstruction.

## **DECLARATIONS**

### **Authors' contributions**

The author contributed solely to the article.

### **Availability of data and materials**

Not applicable.

### **Financial support and sponsorship**

None.

### **Conflicts of interest**

The author declared that there are no conflicts of interest.

### **Ethical approval and consent to participate**

Not applicable.

### **Consent for publication**

Not applicable.

### **Copyright**

© The Author(s) 2020.

Original Article

Open Access



# Exploring adherence to daytime compression in women with breast cancer related lymphedema: a multi-methods study

Mona Al Onazi<sup>1</sup>, Naomi Dolgoy<sup>1</sup>, Joanna Parkinson<sup>1</sup>, Margaret L. McNeely<sup>2</sup>

<sup>1</sup>Department of Physical Therapy, University of Alberta, Edmonton T6G 2G4, Canada.

<sup>2</sup>Department of Physical Therapy, University of Alberta & Cross Cancer Institute, Edmonton T6G 1Z2, Canada.

**Correspondence to:** Prof. Margaret L. McNeely, Department of Physical Therapy, University of Alberta & Cross Cancer Institute, 2-50 Corbett Hall, Edmonton T6G 2G4, Canada. E-mail: mmcneely@ualberta.ca

**How to cite this article:** Al Onazi M, Dolgoy N, Parkinson J, McNeely ML. Exploring adherence to daytime compression in women with breast cancer related lymphedema: a multi-methods study. *Plast Aesthet Res* 2020;7:23.  
<http://dx.doi.org/10.20517/2347-9264.2019.74>

**Received:** 16 Dec 2019 **First Decision:** 27 Mar 2020 **Revised:** 3 Apr 2020 **Accepted:** 15 Apr 2020 **Published:** 11 May 2020

**Science Editor:** Xiao Long **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

## Abstract

**Aim:** The objective of this follow-up study was to explore the barriers and facilitators to use of daytime compression among women with breast cancer related lymphedema who previously took part in a trial examining the efficacy of night compression.

**Methods:** We used a multi-methods approach involving a survey and subsequent focus group sessions. The survey questions were developed based on clinical experience and findings from the literature. Questions were framed to align with the Theoretical Domains Framework. For the focus group data, we applied an interpretive description qualitative methodology to understand participants' experiences and views on use of daytime compression. Qualitative findings were mapped to the Theoretical Domains Framework.

**Results:** Questionnaires were completed by 48 of 52 participants. Only 15 participants (31%) reported adhering to wearing the garment for greater than 12 h each day. Better adherence was positively associated with perceived control of lymphedema ( $r = 0.304$ ; 95%CI: 0.051-0.564 ;  $P = 0.021$ ). Survey findings suggest that participants have good knowledge about the rationale for, and the benefits of, wearing the compression sleeve. Twenty-three survey respondents took part in one of the five subsequent focus group sessions. Five key themes were identified representing the primary barriers to regular use of daytime compression: discomfort, negative emotions, interference with function, social situations and visibility, and use of alternative management strategies.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Conclusion:** The findings suggest less than optimal adherence to daytime compression sleeve use. Further research is needed to explore the relative benefit of daytime compression, optimal wear times, and implementable strategies to improve adherence.

**Keywords:** Lymphedema, breast cancer, compression therapy, adherence

## INTRODUCTION

Lymphedema, a significant swelling of the arm that occurs on the side of the breast cancer, is one of the more frequent complications following surgical interventions for breast cancer<sup>[1,2]</sup>. It is a lifelong condition that tends to worsen over time<sup>[1,3,4]</sup>. Recent data suggest that approximately 21% of women who undergo treatment for breast cancer are diagnosed with lymphedema<sup>[1]</sup>. Of these cases, approximately half will develop chronic progressive lymphedema, a more severe presentation of ongoing and complex swelling<sup>[2,5]</sup>.

At present, there are no known curative treatments, either surgical or pharmacological, for lymphedema<sup>[6,7]</sup>. Conservative treatments are prescribed to reduce and maintain limb size, restore function, reduce pain, and improve the appearance of the limb<sup>[6,8,9]</sup>. Decongestive lymphatic therapy, often prescribed to reduce the lymphedema volume, is an intensive treatment program usually delivered in a clinical or hospital setting<sup>[9]</sup>. This program is followed by a maintenance phase which aims to promote life-long control of lymphedema through use of self-management strategies<sup>[9,10]</sup>. Currently, daytime compression sleeves represent the standard of care and are the primary strategy in lymphedema maintenance<sup>[6]</sup>.

We conducted a trial examining the efficacy of the addition of nighttime compression to standard care, involving daytime use of a compression sleeve, on arm lymphedema volume (LYNC trial)<sup>[11]</sup>. The LYNC trial was a parallel three-arm, multicenter randomized fast-track trial involving three sites in Canada: (1) the Cross Cancer Institute in Edmonton; (2) Tom Baker Cancer Centre in Calgary; and (3) University of British Columbia in Vancouver<sup>[11]</sup>. One hundred twenty women enrolled in the trial and were randomly assigned to one of three groups: (1) standard care (daytime use of a compression sleeve alone); (2) standard care plus nighttime compression through self-administered or assisted compression bandaging; and (3) standard care plus nighttime compression through use of a nighttime compression system garment<sup>[11]</sup>. At the end of the randomized controlled trial portion of the study, participants in the two comparison groups were provided with a nighttime compression system garment and followed the night regimen for an additional three months<sup>[11]</sup>.

During the LYNC study, all participants were asked to follow standard care recommendations that involved wearing their daytime compression sleeve daily for a minimum of 12 h. We monitored adherence to use of daytime compression among participants and found that, while overall adherence (based on hours worn per week) was 81%, only 58% of participants adhered to the recommendations of seven days per week and 12 h per day. In contrast, mean adherence to nighttime compression during the trial was 100% both during the intervention and follow-up periods of the randomized controlled trial. As adherence to use of daytime compression is seen as a critical factor in long-term lymphedema control<sup>[12]</sup>, we aimed to explore the reasons participants reported less than optimal adherence to use of their daytime compression. Thus, the objective of this follow-up study was to explore the barriers and facilitators to use of a daytime compression sleeve.

## METHODS

### Study design

The study used a multi-methods approach involving quantitative and qualitative survey data. In this design, we conducted both a survey and five focus group sessions to collect information from participants on their perspectives and experiences with use of daytime compression.

### *Conceptual framework*

The survey questions were developed based on clinical experience of the researchers and findings from the literature around compression therapy adherence. Questions were then framed to align with the Theoretical Domains Framework (TDF). The TDF is a behavior change model used in the field of implementation science to inform healthcare practitioner behavior<sup>[13,14]</sup>. The TDF comprises 14 theoretical domains that are considered to influence behavior and behavioral change<sup>[15]</sup>. Used in conjunction, the TDF and the Behavior Change Wheel (BCW) link the identified determinants of behavior to appropriate behavior change techniques. At the center of a proposed BCW is a behavior system involving three essential conditions: capability, opportunity, and motivation (COM-B)<sup>[15]</sup>. These conditions interact to produce the behavior<sup>[16]</sup>. Capability is defined as the individual's psychological and physical capacity, which includes having the necessary knowledge and skills to perform the behavior<sup>[16]</sup>. Motivation is defined as the intellectual processes that energize and direct behavior, which includes goals, habits, emotional responses, and decision-making<sup>[16]</sup>. Opportunity is defined as the external influences that impact the individual, making possible or encouraging the occurrence of the behavior<sup>[16]</sup>.

Together, the TDF and BCW allow mapping of behavior change techniques to tailored implementation strategies<sup>[13]</sup>. Although originally developed for use with healthcare practitioners, the TDF is now commonly used to behavior change across patient populations<sup>[17]</sup>.

The survey consisted of 45 questions in six categories: demographic, health and lymphedema status, garment use, garment knowledge, number and cost of garments, and social and environmental factors. After development and initial testing for clarity and effectiveness with the research team and clinic staff, the survey was pilot tested with two women with breast cancer related lymphedema (BCRL) who were not part of the LYNC study [Supplement Table 1].

### *Qualitative methodology*

For the purpose of this study, we applied an interpretive description (ID) qualitative methodology to understand participants' experiences and perspectives on the use of daytime compression as a self-management strategy for lymphedema<sup>[18]</sup>. ID can be applied to qualitative inquiry, as a means to better understand individual health and illness, and to generate useful knowledge that can be used in clinical practice<sup>[18,19]</sup>. Further, ID used in focus groups offers a methodological approach to the discovery and creation of shared perspectives, rather than simply relying on individual reports<sup>[18]</sup>.

### **Participant sampling**

A convenience sample of 93 women who participated in the LYNC study within Alberta (Edmonton and Calgary) was screened for eligibility through the electronic medical records system.

Eligibility for the survey included: (1) prior participation and completion of the LYNC study; (2) current residence in Alberta, Canada; and (3) stable breast cancer medical status.

Eligible women were invited via email or phone to participate in the survey. Participants agreeing to the survey had the option to complete the survey online through REDCap<sup>[20,21]</sup> (a secure web application for building and managing online surveys) or to receive a paper copy through regular mail.

### **Data collection**

Findings from the surveys were used to develop the probing questions for the focus group sessions. The last question of the survey asked participants if they were willing to take part in a focus group session. Participants indicating interest were then contacted and booked for a session, either at the University of Alberta in Edmonton or at the Holy Cross Centre in Calgary. The focus group sessions took approximately 90 min each, and were simultaneously audio recorded and manually transcribed. The principal investigator

(MM) facilitated the focus group discussions at both locations, with a second researcher in attendance at each session in Edmonton (MAO) and Calgary (ND).

### Data analysis

The survey results were analyzed quantitatively using the means and standard deviations for continuous variables, and frequencies and percentages for nominal variables. We also examined the association between adherence to daytime compression and perceived control of lymphedema, overall and by lymphedema severity, using a Chi square correlation coefficient. All focus group tapes were audio recorded and transcribed verbatim by the research team. ID was used to guide the process of capturing patterns and themes about perceptions and perspectives related to the participants' adherence to daytime compression. Data analysis was based on a thematic analytic approach and followed the processes as described by Braun and Clarke<sup>[22]</sup>. The data were examined line by line by the researchers independently to identify patterns and key themes with sample quotations from the data. Codes for similar meanings and highlighted terms were developed, examined, and refined as necessary to identify the most expressive codes to represent the participants' voice. The researchers then discussed and reviewed the independently coded data together, until a consensus on themes was reached. Conceptual themes were inductively originated from the analysis, by first generating initial codes, followed by subthemes, and finally by generating an overarching theme<sup>[22]</sup>. An additional step was taken to map the qualitative data back to the TDF. Any responses or comments that did not answer the specific question were not included in the analysis.

### Ethical considerations

Ethical approval was obtained from the Health Research Ethics Board of Alberta: Cancer Committee for this additional follow-up component of the LYNC study. All participants provided amended informed consent. Each participant was coded by a study number to protect her identity.

## RESULTS

Questionnaires were completed by 48 of 52 participants who responded to our invitation to take part in the survey, for an overall completion rate of 92% [Figure 1]. Twenty-three of the survey respondents (48%) took part in one of the five subsequent focus group sessions. Four focus group sessions were held in Edmonton ( $n = 18$ ) and one was held in Calgary ( $n = 5$ ).

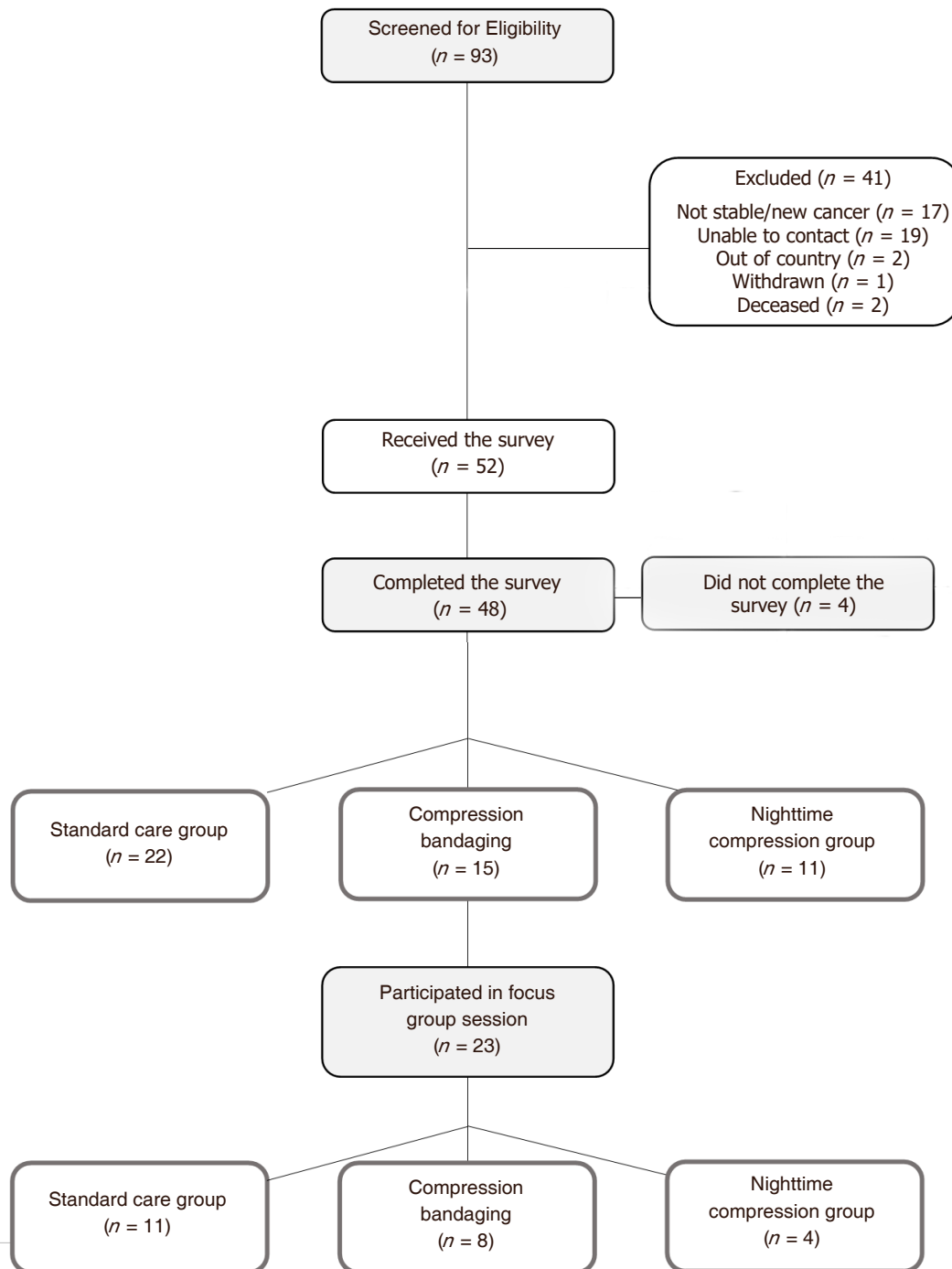
### Quantitative survey results

The mean age of participants was 65 years (range 39-82). The majority of participants (80%) described their general health as "good" or "very good". More than half of the participants reported a duration of lymphedema of greater than six years (58%), and that the extent of their swelling was "moderate" (69%). Most commonly, participants reported the status of their lymphedema as "well controlled" (38%) or "fluctuating" (44%), with 34% stating they were bothered by their lymphedema "moderately" or higher. Of the 12 participants (25%) reporting "slight/mild" swelling, only one (5%) stated she was bothered by her lymphedema more than "a little bit" [Table 1].

Twenty-eight participants (58%) reported using their sleeve daily, and 17 (35%) reported using the garment for > 12 h per day. When we examined adherence to both days and hours, only 15 participants (31%) reported adhering to wearing the garment > 12 h every day.

Survey findings suggest that the vast majority of participants (98%) have good knowledge about the rationale for, and the benefits of, wearing the compression sleeve [Table 2]. Forty-three participants (90%) reported that their compression sleeve helped them to manage their lymphedema. Most participants understood the different types (85%), compression levels (77%), and recommended number of hours to





**Figure 1.** Flowchart of recruitment

wear the sleeve (83%). In addition, the majority of participants reported they had the skills, confidence, and ability to don the compression sleeve [Table 2].

The main identified factors related to non-adherence were weather, social settings, and interference with function. Twenty-three participants (48%) reported that their ability to wear the sleeve was affected by weather, with 21 (44%) reporting wearing their sleeve less during summer season due to discomfort related to heat and humidity. Twenty-four participants (50%) reported not wearing the compression sleeve when

**Table 1. Summary of participant characteristics (n = 48)**

Age	Mean (range)	65 years (39-82)
Employment, n (%)	Retired	28 (58%)
	Sick leave/disability	1 (2%)
	Part-time	3 (6%)
	Full-time	14 (29%)
	Homemaker	2 (4%)
Marital status, n (%)	Married or common-law	30 (64%)
	Single	2 (4%)
	Divorced	9 (19%)
	Widowed	6 (13%)
Current cancer treatments, n (%)	Hormone therapy	13 (28%)
	None	33 (72%)
General health, n (%)	Excellent	4 (8%)
	Very good	19 (40%)
	Good	19 (40%)
	Fair	6 (12%)
Lymphedema duration, n (%)	4-5 years	20 (42%)
	6-10 years	19 (40%)
	> 10 years	9 (19%)
Lymphedema severity, n (%)	Slight-mild swelling	12 (25%)
	Moderate swelling	33 (69%)
	Severe swelling	1 (2%)
	Very severe	2 (4%)
Lymphedema status, n (%)	Well-controlled, stable	18 (38%)
	Improving	7 (15%)
	Fluctuating: sometimes better, sometimes worse	21 (44%)
	Worsening	2 (4%)
Bothered by lymphedema, n (%)	Not at all	8 (17%)
	A Little bit	19 (40%)
	Moderately	12 (25%)
	Quite a bit	6 (13%)
	Extremely	3 (6%)

attending specific social settings and 13 (27%) reported hiding the sleeve when in public settings due to unwanted attention or questions. Fourteen participants (29%) reported that their sleeve interfered with their work or daily routine [Table 3].

#### *Adherence and perceived control of lymphedema*

A total of 28 participants (58%) reported wearing their sleeve every day, and, of those, 21 (76%) reported their lymphedema was “well controlled or improving” ( $P = 0.01$ ). When we compared daily adherence of > 12 h daily to non-adherence, there was no significant difference in self-perceived control of lymphedema ( $P > 0.05$ ). However, when we examined daily adherence at a level of > 9 h daily, of the 25 participants who reported adherence at > 9 h, 17 (68%) reported their lymphedema was “well controlled” or “improving” ( $r = 0.304$ ; 95%CI: 0.051-0.564;  $P = 0.021$ ). The strength of the relationship increased when we limited the analysis to only those participants ( $n = 33$ ) with moderate lymphedema [Table 4].

When we explored the data based on severity of lymphedema, of the 12 participants with slight or mild swelling, 11 reported their lymphedema was well controlled or improving; however, only 6 (50%) reported wearing their sleeve daily for > 9 h. In contrast, of the 36 participants with moderate-to-severe lymphedema, 14 (39%) participants reported their lymphedema was well controlled or improving, with 11 of the 14 reporting wearing their sleeve daily for > 9 h ( $P < 0.047$ ) [Table 5].

#### **Qualitative findings**

Based on the findings of survey, the following probing questions were developed for the subsequent focus group sessions, to help us to better understand the participants’ perspectives on wearing a sleeve, when

**Table 2. Reported compression garment data**

<b>Garment use</b>		
Garment adherence	7 days/week	28 (58%)
Days per week	6 days/week	7 (15%)
	4-5 days/week	6 (13%)
	1-3 days/week	4 (8%)
	0 days/week	3 (6%)
Garment adherence	4 h or fewer	7 (15%)
Hours per day	5-8 h	9 (19%)
	9-12 h	15 (31%)
	≥ 12 h	17 (35%)
Garment adherence	≥ 12 h	15 (31%)
7 days/week		
Compression sleeve interfere with daily routine	Yes	14 (29%)
	No	34 (71%)
Wear the sleeve for house work/activities/exercise	Yes	42 (88%)
	No	6 (12%)
<b>Garment knowledge</b>		
Topics discussed with therapist	The compression garment types	41 (85%)
	The compression level or degree	37 (77%)
	Number of hours per day to wear the garment	40 (83%)
	No discussion	3 (6%)
Compression sleeve helps to manage lymphedema	Yes	43 (90%)
	No	5 (10%)
*Understand the reason for wearing the compression sleeve every day, % (SD)	VAS (0-100)	88% (16)
*Have a good knowledge about the compression sleeve benefits, % (SD)	VAS (0-100)	86% (15)
Have personal benefits from wearing the garment	Yes	43 (90%)
	No	5 (10.4%)
Confident in putting on the compression sleeve properly	Yes	47 (97.9%)
	No	1 (2.1%)

\*Visual analog scale (VAS): 0: not at all; 100: very much. SD: standard deviation

**Table 3. Reported social and environmental factors**

<b>Social and environmental factors</b>		
The weather affects the compression sleeve adherence	Yes	23 (47.9%)
	No	25 (52.1%)
Wear it less during the following seasons	Spring	4 (8.3%)
	Summer	21 (43.8%)
	Fall	1 (2.1%)
	Winter	1 (2.1%)
	Wear it the same throughout the year	26 (54.2%)
Wear the sleeve at home	Yes	39 (81.3%)
	No	9 (18.8%)
Wear the sleeve in public	Yes	42 (87.5%)
	No	6 (12.5%)
Hide the sleeve in public	Yes	13 (27.1%)
	No	35 (72.9%)
Not wearing the sleeve during some social settings	Yes	24 (50.0%)
	No	24 (50.0%)
Have supportive family and friends on wearing compression sleeve	Yes	47 (97.9%)
	No	1 (2.1%)
*Have issues putting the garment on, % (SD)	VAS (0-100)	16% (24)
*Need help with sleeve donning, % (SD)	VAS (0-100)	16% (28)

\*Visual analog scale (VAS): 0: not at all, 100: very much . SD: standard deviation

**Table 4. Association between adherence and perceived control of lymphedema overall**

Adherence	Well-controlled or improving	Fluctuating or worsening	Total
Daily > 9 h	17	8	25
< 7 days or < 9 h	8	15	23
Total	25	23	48

$r = 0.304$ ; 95%CI: 0.051-0.564;  $P = 0.021$

**Table 5. Association between adherence and perceived control for those with moderate to severe lymphedema**

Lymphedema severity	Adherence	Well-controlled or improving	Fluctuating or worsening	Total
Moderate	Daily >9 h	11	9	20
	< 7 days or < 9 h	3	13	16
	Total	14	22	36

$r = 0.396$ ; 95%CI: 0.043-0.745;  $P = 0.047$

and why they do not wear the sleeve in specific circumstances (e.g., social situations and weather), and the impact of wearing the sleeve on daily function:

1. What are the benefits for you with wearing the sleeve?
2. Does anything make it easier or harder for you to wear a sleeve for 12 hours a day?
3. How do you feel about wearing your compression sleeve?
4. What is your experience/perspective on times when you don't wear your sleeve?
- 4.a. Do you have strategies to manage your lymphedema in these situations?

Sample supporting quotations are provided in each section to illustrate participants' perspectives.

#### *Benefits of wearing the garment*

Across all focus group sessions, the primary identified benefit of wearing the sleeve was to reduce or control the lymphedema. Other reported benefits included symptom management (e.g., reduced pain/discomfort, tension, and heaviness in limb), protection of the skin (e.g., reduced risk of damaging the skin), preventing/reducing episodes of cellulitis, and keeping the shape/contour of the limb.

- "I am pretty good about wearing the sleeve, because I know it manages the swelling."

- "I know I need to wear my sleeve, so I do."

#### *Facilitators to regular use of the sleeve*

Facilitators to regular use of daytime compression included using adaptive strategies (e.g., using older sleeves for gardening or dirty tasks), having more garment options (e.g., colors to match outfits and lower compression garment options for exercise), and having the money or medical insurance to help cover costs of extra garments (e.g., less washing and a clean garment for each work day). The majority of participants reported that wearing their garment was a habit and that they had accepted the need to wear it.

- "It is part of my dress every day."

- "I never worry about social situations, it is who I am; never think about it."

- "I do get asked a lot by children, sometimes adults, I explain simply. My granddaughter who is 18 months keeps me honest - she notices if I haven't got it on."

- "Nice to dress it up and have fun with it. If I have to wear it, I want it to be a fashion statement!"

#### *Barriers to regular use of the sleeve*

Five key themes were identified representing the primary barriers to regular use of the compression sleeve or reasons for non-use: discomfort, negative emotions, interference with function, social situations and visibility, and alternative management strategies.

*Theme: discomfort*

Discomfort was reported as a result of a number of factors including: (1) poor fit of the garment; (2) heat and humidity/weather; and (3) poor tolerance to compression. Issues related to the fit of the garments mainly included fit at the hand (e.g., irritation of thumb and web spaces), wrist (e.g., space between glove and sleeve and overlap of compression), elbow (e.g., nerve compression and chafing in elbow crease), or at the upper most aspect of arm (e.g., band too tight, top of sleeve sliding down, and blistering due to silicon band). Participants also reported that fit tended to worsen over the course of the day and when the sleeve was older.

- "My arm is full, and circulation at elbow can feel cut off if the arm is bent for prolonged periods of time."
- "I don't think I get the benefits out of it as I would if it fit well."
- "My sleeve tends to slide down, and I am constantly adjusting it."

Heat was a common factor for removing the sleeve. Participants mostly reported weather and temperature as contributors to heat-related discomfort; however, some participants reported increased heat due to hot flashes or from activity and exercise.

- "In the summer, it is way too hot to wear a sleeve and glove."
- "It's too hot, I can't even get it on in warm weather as I am too sweaty."
- "When I have a hot flash, I want to take the sleeve off."

Increased discomfort due to progressive tension/tightness in the limb was also reported as a primary reason for earlier removal of the sleeve prior to the 12-h recommended time.

- "At the end of my work day, it feels too restrictive and uncomfortable, so I take it off."

*Theme: negative emotions*

A minority of participants expressed negative feelings associated with wearing the sleeve daily. The expressed feelings included words such as "hate", "guilt", and "fear"; participants reported the following concerns regarding negative emotions towards the garments:

- "I hate it. I am tired of the reminder that it is always there, and no end in sight for getting better."
- "Life changing, ruins my life to wear a sleeve, I can't do the things I want to do with a sleeve and glove."
- "When I don't wear it, I feel guilty."
- "I fear the lymphedema will get worse."

Many participants reported burdens related to wearing the sleeve daily, including laundering their garments, the need for ongoing fitting appointments, and having to get garment prescriptions renewed.

- "I get tired of the regimen, I need a break."
- "I am tired of proving I still need a sleeve, this is a lifelong condition."
- "I still need a prescription each year for insurance, this is frustrating."

Participants requiring custom compression sleeves and gloves expressed frustration with fit, issues with returning poorly fitted garments, and wait times for garments; participants expressed the following concerns with their garments:

- "Fittings are hit and miss, especially garment length."
- "When it comes wrong, I don't bother sending it back - too much of a hassle."
- "Long wait for custom garment, then it comes but does not fit well."

*Theme: interference with function*

The primary finding in regards to interference with function related to hand function and concerns over hygiene, especially when using a glove or gauntlet. With respect to function, participants reported the following concerns:

- "I have difficulty manipulating objects, doing crafts, and with writing due to the glove, so I remove it."



- "I live on a farm, and I spend a lot of time taking it on and off so it will not get wet or dirty."
- "I need to feel the food with my fingers, so I can't wear the glove in the kitchen."
- "The glove gets wet with meal preparation, sleeve is uncomfortable with the glove off, so I remove both."
- "The glove is cumbersome and interferes with cooking and hand washing."

With respect to maintaining cleanliness of the garments, participants reported the following concerns with managing hygiene:

- "I work as a surgical nurse and cannot wear my garment during surgery as I cannot scrub and stay sterile, and wear the sleeve."
- "I need to wash my hands at work, so I have to remove the glove a lot."
- "I worry about germs. Is the glove carrying germs?"

#### *Theme: social situations and visibility*

When we explored reasons for non-use of the sleeve for social events or special occasions, participants reported issues with appearance and with the garments drawing unwanted attention or questions.

##### ● Appearance:

- "When I go out for a special evening event, and when wearing an evening outfit, I will go without the sleeve."
- "I won't wear it for weddings or formal events, as it does not look good with my outfit"
- "I won't wear it for a photo session, doesn't look good."
- "Clothes don't fit well when I have it on."

##### ● Drawing unwanted attention:

- "Lady with the sleeve."
- "I am always conscious of it because I have people asking me if I have been burned or why I wear it."
- "It draws attention to the arm and awkward questions."
- "I like fall and winter more because I can wear long sleeve shirts and nobody asks me anything."

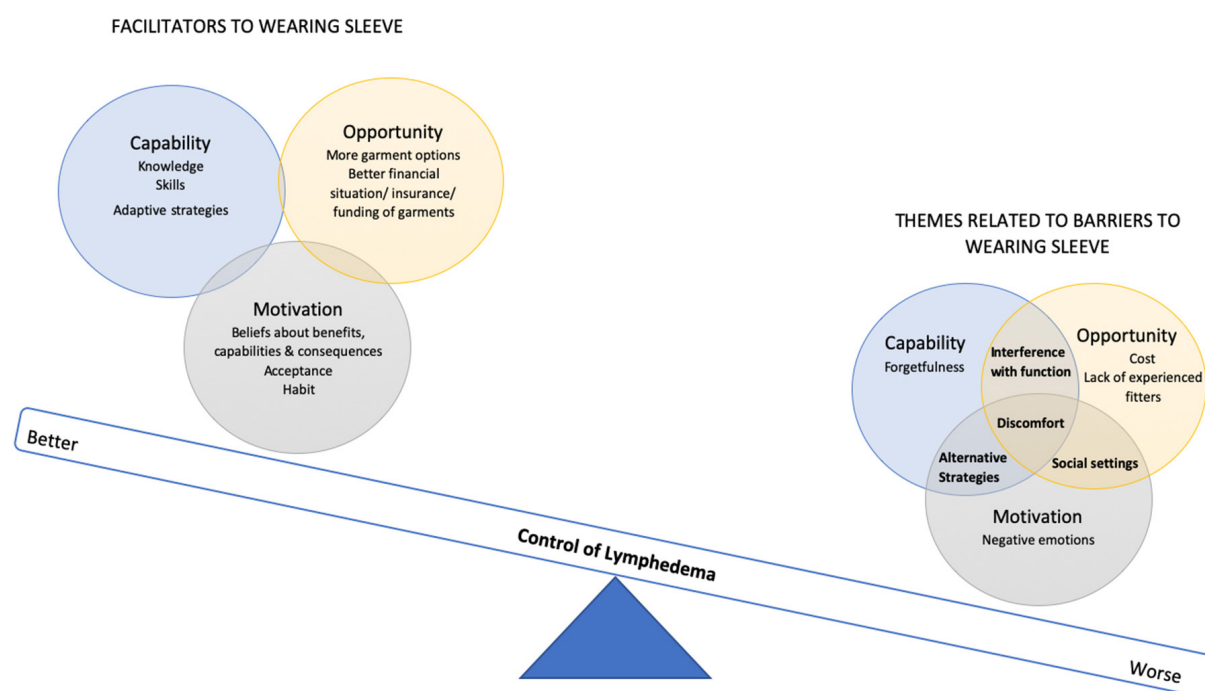
#### *Theme: alternative management strategies*

Many of the participants reported the use of alternative strategies that helped them to control their lymphedema and allowed them to "get away" with reducing daytime compression sleeve wear. Nighttime compression, through bandaging or a night garment, was the main strategy used as an alternative to wearing daytime compression. Other strategies included managing body weight, exercise, and nutritional intake (such as avoiding alcohol and salt).

- "My night system controls my lymphedema, so I don't need to wear a daytime sleeve anymore."
- "Even minor weight loss seems to help me control the lymphedema better."
- "Exercise helps me a lot."
- "Drinking alcohol makes the swelling worse, I know I will need to wear my garment more after a social event."
- "Salt definitely makes the swelling worse, especially if I eat out."

#### **Data synthesis: mapping of facilitators and key themes to the TDF**

The findings of the qualitative study were combined with survey data and mapped to the TDF data to allow for the identification of potential strategies to enhance adherence to daytime compression. We found that the main themes could not be categorized into a single component of the COM-B model. Clearly, relationships existed among the components of opportunity, capability, and motivation, while some barriers influenced motivation. For example, not wearing the sleeve when attending social events was related to visibility of the garment with dress clothes representing social influences (opportunity), while the unwanted



**Figure 2.** Survey and qualitative findings mapped to the capability, opportunity, and motivation behaviour change wheel (COM-B)

attention was related to emotion (motivation). “Discomfort” crossed components of capability (i.e., lack of adaptive strategies) and opportunity (e.g., lack of experienced fitters and environmental factors/weather) leading to issues with motivation (i.e., negative emotions). Interference with function appeared to primarily involve the lack of opportunity in relation to proper fit of the garment, and the need for garments that both facilitate functional/work activities and are hygienic. We noticed that interference with function also appeared to be influenced by a lack of adaptive strategies (capability) [Figure 2].

## DISCUSSION

A primary finding of this study was that adherence to daytime compression was less than optimal. At present, compression garments are the mainstay of lymphedema self-management<sup>[10]</sup>, and are generally recommended for use during all waking hours<sup>[23,24]</sup>. Poor adherence to BCRL self-care practices has been associated with increased arm volume and progression of BCRL to more advanced stages<sup>[12]</sup>, supporting the importance of good adherence. A prior research study examining adherence to self-care strategies had similar findings, reporting that only 39% of their study participants adhered to greater than 75% of recommended compression sleeve wear time across a one-year follow-up period<sup>[25]</sup>. While overall adherence was poor among participants in our study, those participants who reported daily adherence of > 9 h also reported better perceived-control of their lymphedema, suggesting a positive medium effect from use of daytime compression. Based on our findings, however, only 9.2% of the total variation in perceived control can be explained by adherence to use of daytime compression. Thus, 91% of the variation is related to other potential factors including the use of other maintenance strategies identified by the participants.

### Capability

The results of this study suggest that strict adherence to use > 12 h per day, while recommended, may not be necessary to achieve control of lymphedema, especially in the case of those with mild lymphedema. Other self-management practices reported by many participants in this study were used to replace the need for strict adherence to daytime compression, by supplementing wear schedules with nighttime

compression. This is not surprising given that all participants had previously taken part in the LYNC study and had been provided with a nighttime compression garment. In a previous cohort study, adherence to daytime compression and use of nighttime compression bandaging four nights a week was shown to result in better control of lymphedema over the longer term as compared to daytime compression alone<sup>[12]</sup>.

Other strategies reported by participants included using lower compression sleeves for activities and exercise, and older sleeves for housework and gardening. In addition, participants reported use of lifestyle strategies such as exercise, weight management, and proper nutrition to improve overall control of their lymphedema<sup>[26]</sup>. As lymphedema is a chronic condition, tailored approaches involving combinations of self-management strategies may allow women more flexibility in terms of daytime compression wear time.

### Opportunity

The findings of this study suggest the need for healthcare providers and patients to work with industry partners to develop options to address barriers to garment wear. For example, innovation is needed to explore options for garments that facilitate activity participation, such as more breathable garment fabrics to address discomfort related to heat; waterproof and more hygienic options for gloves; and garments that match skin tones to reduce visibility of the sleeve. There is also a clear need for increased training and more experience for garment fitters and/or devices that can improve the accuracy of the fitting process. While 78% of participants in this study reported that cost was not a barrier to replacing sleeves, for some, cost did limit the number of sleeves purchased each year. Participants with the financial resources and/or insurance coverage reported purchasing additional garments as a means to reduce burden (e.g., more garments to reduce the need for daily washing), improve comfort (e.g., different compression levels, sizes, or fabrics to accommodate fluctuations in lymphedema volume), and have more fashionable options (e.g., different colors to match outfits).

### Motivation

The study findings suggest that efforts to support and enhance motivation to improve adherence to daytime compression is likely critical to long-term management of lymphedema. Although the vast majority of participants reported benefits from wearing the sleeve and negative consequences when they did not wear the sleeve, these incentives did not consistently influence adherence. Barriers such as discomfort, interference with function, and visibility of the garment were significant, and they were often reported as reasons for removal or non-wear of the sleeve. Besides efforts to address these barriers, healthcare provider approaches to increasing motivation could include developing wear-schedules and individual goals, planning strategies for times of non-wear, building in strategic “breaks”, providing regular feedback on lymphedema status, and encouraging successful performance<sup>[27]</sup>.

Interventions to address psychosocial issues and negative emotions associated with daytime sleeve wear may include: (1) in person or virtual lymphedema support groups for sharing of issues and strategies; and (2) providing access to financial and psychosocial services<sup>[27]</sup>.

### Limitations

There are a number of limitations to the research described in this paper. First, the study involved a convenience sample of women who previously took part in the LYNC trial. Thus, participants in this research may have been more motivated and better informed due to their prior involvement in a randomized controlled trial, and may not be a representative sample of women with BCRL. Second, lymphedema severity and perceived control were self-reported and based on participants’ perception; thus, findings may not be an accurate representation of the status or control of their lymphedema. Third, the survey did not include questions on use of nighttime compression; however, in the focus group sessions, 20 of the 23 participants reported continued use of their night garment as a self-management

strategy. Fourth, judgement when interpreting qualitative research is inevitably biased by the researchers' perspective. Although there may be different ways to interpret the data, we used a known framework and methodology to address this issue and to help us understand and better represent the findings. Fifth, very little research exists examining adherence to daytime compression, thus we were limited in our ability to make comparisons to findings of other studies.

In conclusion, the findings of this study suggest that, among past-participants of the LYNC trial, adherence to daytime compression sleeve use is less than optimal. While participants reported having good knowledge on the benefits of compression and the skills associated with use of a sleeve, numerous barriers to wear exist. The key themes related to barriers/non-use included discomfort, negative emotions, interference with function, social situations and visibility, and the use of alternative strategies. Further research is needed to explore the relative benefit of daytime compression and optimal wear times, as well as implementable strategies to improve adherence to daytime compression.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Al Onazi M, Dolgoy N, Parkinson J, McNeely ML

Performed data acquisition, as well as provided administrative, technical, and material support: Al Onazi M, Dolgoy N, Parkinson J, McNeely ML

### Availability of data and materials

Data supporting the findings of the study will be deposited in a data repository at the University of Alberta.

### Financial support and sponsorship

This work was supported by an Alberta Cancer Foundation Investigator Initiated Trials Grant and through funding from the Canadian Institutes of Health Research.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Ethical approval was obtained from the Health Research Ethics Board of Alberta: Cancer Committee for this additional follow-up component of the LYNC study. All participants provided amended informed consent.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. DiSipio T, Rye S, Newman B, Hayes S. Incidence of unilateral arm lymphoedema after breast cancer: a systematic review and meta-analysis. *Lancet Oncol* 2013;14:500-15.
2. Hayes SC, Janda M, Cornish B, Battistutta D, Newman B. Lymphedema after breast cancer: incidence, risk factors, and effect on upper body function. *J Clin Oncol* 2008;26:3536-42.
3. McWayne J, Heiney SP. Psychologic and social sequelae of secondary lymphedema: a review. *Cancer* 2005;104:457-66.
4. Ostby PL, Armer JM. Complexities of adherence and post-cancer lymphedema management. *J Pers Med* 2015;5:370-88.
5. Paskett ED, Naughton MJ, McCoy TP, Case LD, Abbott JM. The epidemiology of arm and hand swelling in premenopausal breast cancer

- survivors. *Cancer Epidemiol Biomarkers Prev* 2007;16:775-82.
6. Moffatt C. International consensus: best practice for the management of lymphoedema. International Consensus London MEP Ltd 2006;3-52.
  7. Chang CJ, Cormier JN. Lymphedema interventions: exercise, surgery, and compression devices. *Semin Oncol Nurs* 2013;29:28-40.
  8. Armer JM, Hulett JM, Bernas M, Ostby P, Stewart BR, et al. Best practice guidelines in assessment, risk reduction, management, and surveillance for post-breast cancer lymphedema. *Curr Breast Cancer Rep* 2013;5:134-44.
  9. Lasinski BB, McKillip Thrift K, Squire D, Austin MK, et al. A systematic review of the evidence for complete decongestive therapy in the treatment of lymphedema from 2004 to 2011. *PM R* 2012;4:580-601.
  10. Ridner SH, Fu MR, Wanchai A, Stewart BR, Armer JM, et al. Self-management of lymphedema: a systematic review of the literature from 2004 to 2011. *Nurs Res* 2012;61:291-9.
  11. McNeely ML, Campbell KL, Webster M, Kuusk U, Tracey K, et al. Efficacy of night-time compression for breast cancer related lymphedema (LYNC): protocol for a multi-centre, randomized controlled efficacy trial. *BMC Cancer* 2016;16:601.
  12. Vignes S, Porcher R, Arrault M, Dupuy A. Factors influencing breast cancer-related lymphedema volume after intensive decongestive physiotherapy. *Support Care Cancer* 2011;19:935-40.
  13. Cane J, O'Connor D, Michie S. Validation of the theoretical domains framework for use in behaviour change and implementation research. *Implement Sci* 2012;7:37.
  14. Francis JJ, O'Connor D, Curran J. Theories of behaviour change synthesised into a set of theoretical groupings: introducing a thematic series on the theoretical domains framework. *Implement Sci* 2012;7:35.
  15. Michie S, Johnston M, Francis J, Hardeman W, Eccles M. From theory to intervention: mapping theoretically derived behavioural determinants to behaviour change techniques. *Applied Psychol* 2008;57:660-80.
  16. Michie S, van Stralen MM, West R. The behaviour change wheel: a new method for characterising and designing behaviour change interventions. *Implement Sci* 2011;6:42.
  17. Atkins L, Francis J, Islam R, O'Connor D, Patey A, et al. A guide to using the Theoretical Domains Framework of behaviour change to investigate implementation problems. *Implement Sci* 2017;12:77.
  18. Thorne S. Interpretive description: qualitative research for applied practice. New York: Routledge; 2016.
  19. Thorne S, Kirkham SR, MacDonald-Emes J. Interpretive description: a noncategorical qualitative alternative for developing nursing knowledge. *Res Nurs Health* 1997;20:169-77.
  20. Harris PA, Taylor R, Minor BL, Elliott V, Fernandez M, et al.; REDCap Consortium. The REDCap consortium: Building an international community of software platform partners. *J Biomed Inform* 2019;95:103208.
  21. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, et al. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform* 2009;42:377-81.
  22. Braun V, Clarke V. Using thematic analysis in psychology. *Qualitative Res Psychol* 2006;3:77-101.
  23. Moseley AL, Carati CJ, Piller NB. A systematic review of common conservative therapies for arm lymphoedema secondary to breast cancer treatment. *Ann Oncol* 2007;18:639-46.
  24. Harris SR, Schmitz KH, Campbell KL, McNeely ML. Clinical practice guidelines for breast cancer rehabilitation: syntheses of guideline recommendations and qualitative appraisals. *Cancer* 2012;118:2312-24.
  25. Brown JC, Cheville AL, Tchou JC, Harris SR, Schmitz KH. Prescription and adherence to lymphedema self-care modalities among women with breast cancer-related lymphedema. *Support Care Cancer* 2014;22:135-43.
  26. Stuiver MM, Ten Tusscher MR, McNeely ML. Which are the best conservative interventions for lymphoedema after breast cancer surgery? *BMJ* 2017;357:j2330.
  27. Fu MR, Ridner SH, Hu SH, Stewart BR, Cormier JN, et al. Psychosocial impact of lymphedema: a systematic review of literature from 2004 to 2011. *Psychooncology* 2013;22:1466-84.



Review

Open Access



# Dorsal hand reconstruction with radial artery perforator-based adipofascial flap

Sho Yamakawa, Kenji Hayashida

Division of Plastic and Reconstructive Surgery, Shimane University Hospital, Izumo, Shimane 693-8501, Japan.

**Correspondence to:** Dr. Sho Yamakawa, Division of Plastic and Reconstructive Surgery, Shimane University Hospital, 89-1 Enyacho, Izumo, Shimane 693-8501, Japan. E-mail: yamakawashoright@yahoo.co.jp

**How to cite this article:** Yamakawa S, Hayashida K. Dorsal hand reconstruction with radial artery perforator-based adipofascial flap. *Plast Aesthet Res* 2020;7:24. <http://dx.doi.org/10.20517/2347-9264.2020.20>

**Received:** 7 Feb 2020 **First Decision:** 21 Apr 2020 **Revised:** 25 Apr 2020 **Accepted:** 28 Apr 2020 **Published:** 11 May 2020

**Science Editor:** Alessandro Thione **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

## Abstract

Reconstruction of dorsal hand soft tissue defects after severe injury is challenging for surgeons. Depending on the degree of defect, extensor tendon reconstruction may also be necessary. Various reconstruction methods are commonly performed to cover dorsal hand defects, such as skin grafting and distant, free, or local flaps. Among them, free vascularized flap transplantation is an ideal procedure because the major vessels that feed the local flap may have been damaged, and the affected limb can be reconstructed using a flow-through method. Although free flap surgery has advanced, few surgeons can choose this option due to its technical difficulty and uncertainty. On the other hand, distant flaps have been commonly used for the reconstruction of dorsal hand defects, and local flaps, such as reverse forearm flaps and retrograde posterior interosseous flaps, do not require microvascular anastomosis. However, they have some problems; distant flaps require at least two surgeries, reverse forearm flaps sacrifice major vessels and leave a scar at the donor site, and retrograde posterior interosseous flaps require meticulous dissection of the vascular pedicle. The radial artery perforator-based adipofascial flap is a versatile flap that is safe and easy to elevate without sacrificing the radial artery. In addition, elevating it as an adipofascial flap enables surgeons to avoid an unacceptable donor scar. We present two cases, demonstrating the usefulness of this pedicled perforator flap.

**Keywords:** Perforator, dorsal hand reconstruction, adipofascial flap, hand replantation, color Doppler ultrasonography



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



## INTRODUCTION

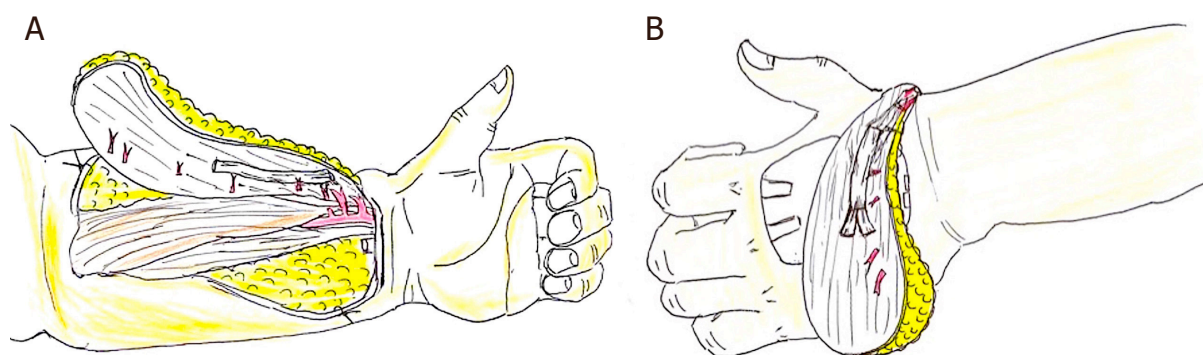
Soft tissue defects of the dorsal hand are commonly caused by relatively high-energy trauma such as industrial accidents. In such cases, free vascularized flap transplantation is the ideal procedure because the major vessels in the zone of injury that may have been damaged, and the vascular supply of the flap and affected limb can be reconstructed using a flow-through method<sup>[1]</sup>. Although free flap surgery has advanced, not all surgeons can select this complicated procedure. Recently, several surgeons have reported local flap procedures for the reconstruction of dorsal hand defects<sup>[2,3]</sup>. Among them, the reverse radial forearm flap is a common method for the treatment of dorsal hand injuries<sup>[4]</sup>. This flap is reliable in regards to vascular supply; however, the main vessels of the forearm and hand must be sacrificed. Thus, this flap cannot be used, if Allen's test shows an incomplete palmar arch. Moreover, unacceptable donor site morbidity is a major drawback of this flap. The posterior interosseous flap is also an effective option<sup>[5,6]</sup>. Although this flap enables covering the dorsal hand defect with well-vascularized tissue without sacrificing major vessels, it requires surgeons to perform fine and burdensome procedures. The radial artery perforator based adipofascial flap is a versatile flap that is safe and easy to elevate without sacrificing the radial artery<sup>[7,8]</sup>. In addition, elevating it as an adipofascial flap enables surgeons to avoid an unacceptable donor scar. We present two case reports demonstrating the usefulness of this pedicled perforator flap for dorsal hand reconstruction.

## ANATOMY AND PREOPERATIVE EVALUATION

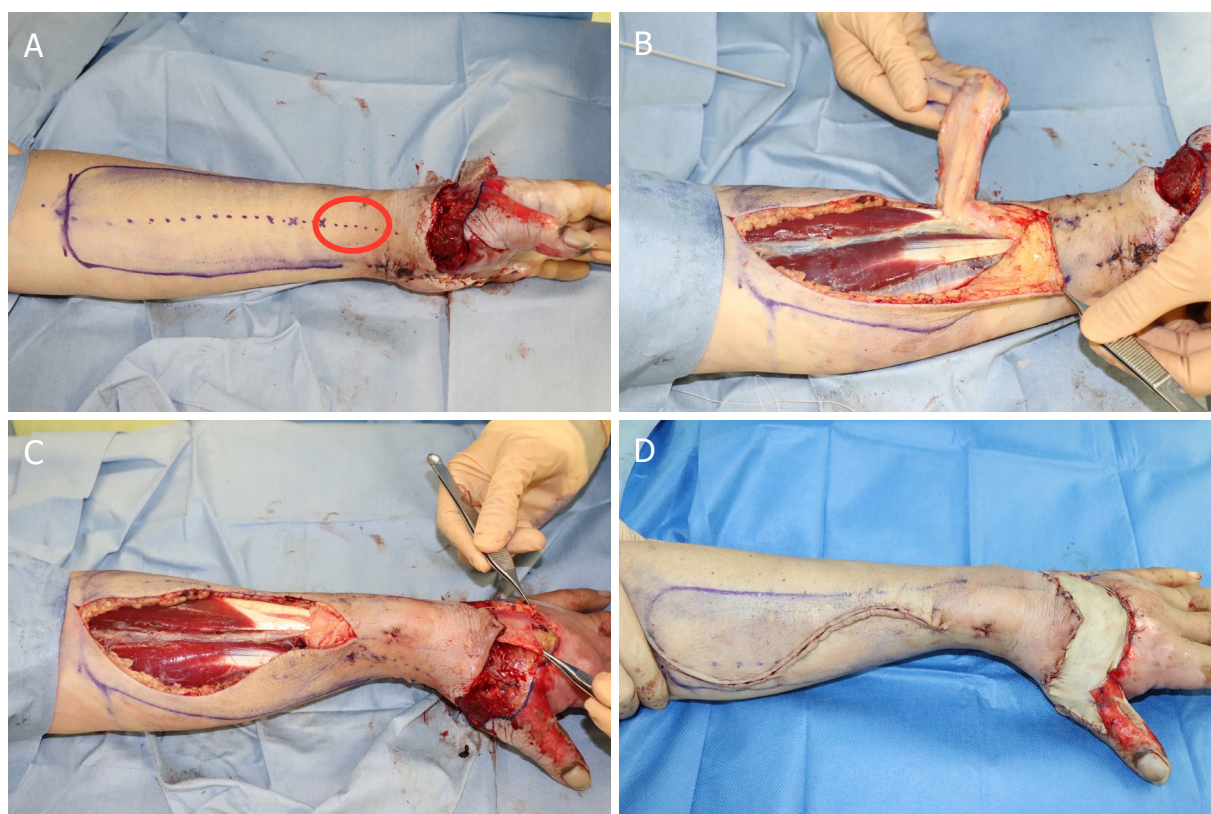
Before flap elevation, contrast-enhanced computed tomography and/or color Doppler ultrasonography of the affected forearm were performed to evaluate the blood circulation of radial vessels. Using color Doppler ultrasonography, Onode *et al.*<sup>[9]</sup> reported that an average of six fasciocutaneous perforators arising from the distal radial vessels can be observed within 15 cm proximal to the distal wrist crease. There are two or more clinically significant ( $> 0.5$  mm) cutaneous perforators at the pivot point within 2 cm proximal to the radial styloid<sup>[8]</sup>. The adipofascial flap on the lateral aspect of the distal forearm is raised proximal to distal, including the lateral antebrachial cutaneous nerve and the cephalic vein. Although a number of surgeons have reported using this flap, there is no consensus regarding the maximum safe longitudinal flap size. In our opinion, it can be the length of the forearm, because as Taylor *et al.*<sup>[10]</sup> reported, designing the flap in the longitudinal direction along the cutaneous nerve and the cutaneous vein, blood circulation of a perforasome composed of multiple perforators connected to each other by a true anastomosis can be stabilized. Therefore, including the lateral antebrachial cutaneous nerve and the cephalic vein within the flap enables the distal perforator-based adipofascial flap to be applied safely [Figure 1A and B].

## SURGICAL PROCEDURE OF RADIAL ARTERY PERFORATOR-BASED ADIPOFASCIAL FLAP TRANSFER

1. Design a rectangular adipofascial flap along the radial artery sufficient to cover the defect. An S-shaped skin incision is favorable in consideration of the convenience of fascial flap dissection and direct closure of the skin [Figure 2A].
2. Dissect the subcutaneous plane sufficiently deep to avoid skin necrosis, leaving the dorsal forearm cephalic vein on the adipofascial flap.
3. After incising the proximal edge of the adipofascial flap, dissect the sub-fascial flap from the proximal to distal direction. Several perforators arising from radial vessels can be found in the distal one-third of the forearm. Next, elevate the flap through the intertendinous septum in the middle of the flexor carpi radialis, abductor pollicis longus, and brachioradialis tendons, and enter the fascia of the forearm. Under direct observation, at least one perforator entering the flap must be preserved around the styloid, which is a pivot point [Figure 2B].



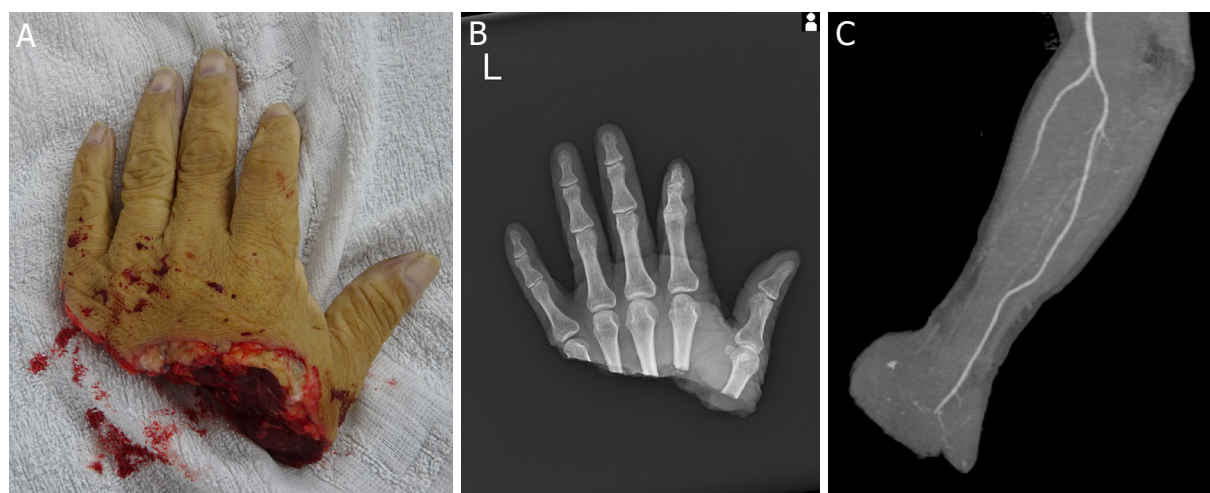
**Figure 1.** A, B: illustration of radial artery perforator-based adipofascial flap elevation, including connection of both perforators to the subdermal vascular network in the proximal and distal ends of the forearm. This flap can be elevated and transferred to the defect of the dorsal hand safely



**Figure 2.** Procedure of radial artery perforator-based adipofascial flap elevation (From Case 1 in this article). A: the radial forearm adipofascial flap was designed along the radial artery. Red circle indicates pivot port; B: the adipofascial flap was elevated, preserving perforators at the distal end of the flap; C: once elevated, the flap was turned over or rotated to reach the dorsal defect; D: the donor site was closed and a skin graft was applied over the flap

4. Transfer the flap through the subcutaneous tunnel to the dorsal hand defect [Figure 2C].
5. Resurface the transferred fascial flap on the dorsal hand with a split-thickness skin graft [Figure 2D]. Elevate the affected arm and bandage with slight pressure to avoid postoperative congestion.





**Figure 3.** A 43-year-old male smoker with left hand amputation. A: amputated left hand; B: on X-ray, the left hand was completely cut at the metacarpal level; C: contrast-enhanced computed tomography showed poor blood circulation of the radial artery in the distal forearm

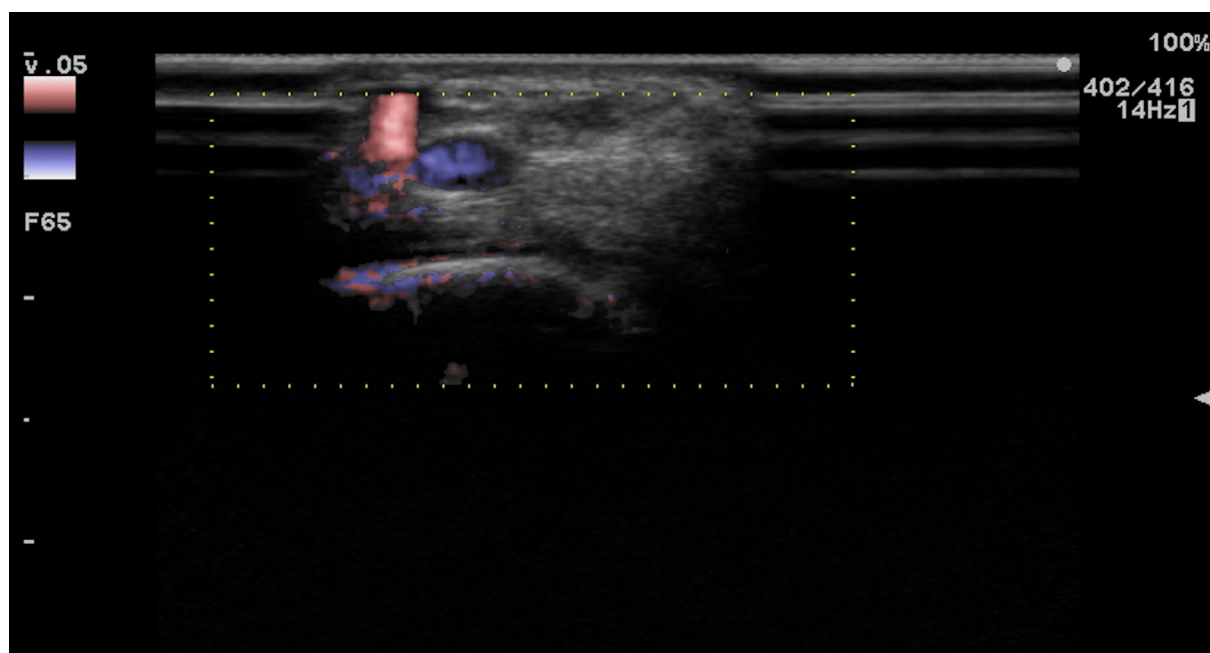
## CASE REPORTS

### Case 1: reconstruction after hand replantation

A 43-year-old man had his hand amputated. He accidentally cut his non-dominant left hand with an electric saw while cutting wood [Figure 3A]. His left hand was amputated at the metacarpal level. X-ray examination and contrast-enhanced computed tomography of the affected limb were performed [Figure 3B and C]. The surfaces of both parts were crushed and severely damaged. Hand replantation surgery was performed under general anesthesia. After bone fixation and tendon repair, microvascular anastomosis was performed. The ulnar side of the palmar arterial was repaired, but not the radial side because the flow through the radial artery was insufficient. Venous repair was performed by vein grafting into three dorsal hand cutaneous veins. The dorsal hand defect after debridement of crushed skin was covered by artificial dermis. The amputated hand survived. Seventeen days after hand replantation surgery, the second operation for reconstruction of the dorsal hand tissue defect was performed. As contrast-enhanced computed tomography after hand replantation surgery revealed an incomplete palmar arch, the combination of a radial artery perforator adipofascial flap and split-thickness skin graft was selected. The radial artery perforators were identified using color Doppler ultrasonography and demarcated on the skin [Figure 4]. There was a 4-cm × 7-cm defect over the dorsal hand with exposure of extensor digitorum tendons after debridement of necrotized tissue. A lazy S-shaped longitudinal incision on the forearm was designed [Figure 5A]. An 18-cm × 5-cm radial artery perforator-based adipofascial flap was elevated. As a vascular pedicle, the distal radial artery fasciocutaneous perforator was left with the soft tissue around the styloid process [Figure 5B]. The flap was turned over and transferred to the defect. A skin graft was immediately placed over the flap. The donor site of the flap was closed [Figure 5C]. The postoperative course was uneventful. The flap and skin graft took completely. Half a year after surgery, the skin of the reconstructed dorsal hand was thin and pliable [Figure 6A and B]. In addition, there was no restriction in the mobility of the wrist or forearm [Figure 6C and D]. However, the thumb-index finger web space was narrow due to poor thumb bone healing caused by poor blood supply. A free flap transfer to the thumb-index finger web space is planned in the next surgery.

### Case 2: functional reconstruction of index finger extension

A 62-year-old man accidentally injured his non-dominant left hand while shaving wood. Skin and soft tissue defects were observed, in addition to exposure of the second metacarpal bone head on the dorsal side

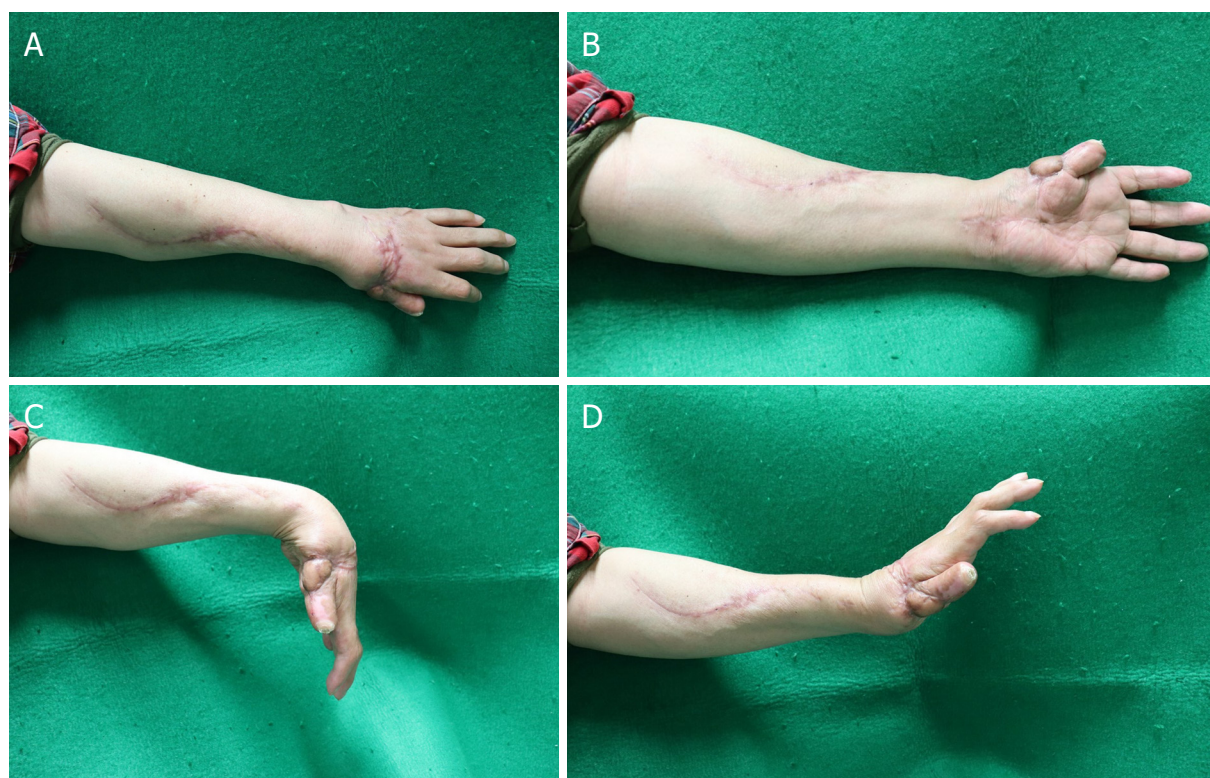


**Figure 4.** Preoperative color Doppler ultrasonography showed a perforator arising from the radial artery. The perforator was 1.8 mm in diameter. Most of the fasciocutaneous perforators were within 2 cm proximal to the styloid



**Figure 5.** A 43-year-old man 17 days after hand replantation surgery. A: a soft tissue defect of the dorsal hand still remains with exposure of bones and tendons; B: a distally based adipofascial perforator flap was raised from proximally to distally; C: a skin graft was applied over the flap and the donor site was closed





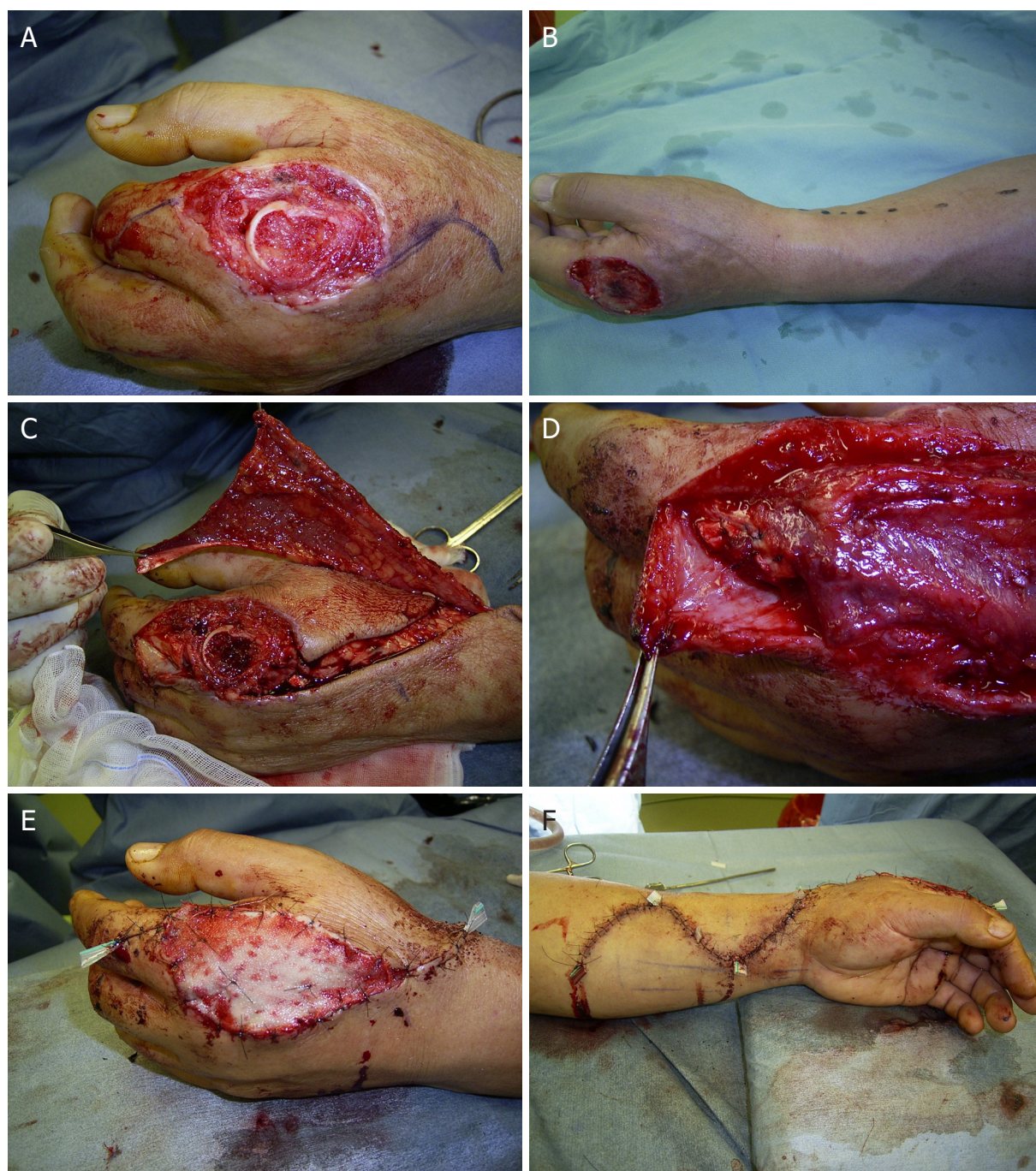
**Figure 6.** A 43-year-old man 6 months after dorsal hand reconstructive surgery. A, B: the reconstructed skin of the dorsal hand was thin and soft and the donor site was inconspicuous; C, D: the forearm had no adhesion and little restriction of the wrist

[Figure 7A]. Emergency reconstructive surgery was performed. The radial artery perforators were identified using color Doppler ultrasonography. Following marked water lavage and debridement of necrotized soft tissue of the dorsal hand, there was a 6-cm × 3-cm defect over the dorsal radial hand. [Figure 7B]. A 10-cm × 5-cm distally radial artery perforator-based adipofascial flap was elevated. The palmaris longus tendon was included in the adipofascial flap to reconstruct the extension function of the index finger [Figure 7C]. As a vascular pedicle, the distal radial artery fasciocutaneous perforator was left with the soft tissue around the styloid process. The flap was transferred to the defect and the tendon was sutured with the extensor indicis muscle tendon using the interlacing suture technique [Figure 7D]. The tension strength of the tendon suture was adjusted in a functional position. The exposed metacarpophalangeal joint was simply covered with the adipofascial flap. A split-thickness skin graft from the thigh was placed over the flap and the donor of the flap was closed [Figure 7E and F]. After 3 weeks, the skin graft survived and rehabilitation using a dynamic splint was started. Ten months after surgery, index finger joint movement was slightly restricted. However, there was no problem in daily life and the color matching of the skin graft was good [Figure 8A-C].

## DISCUSSION

Reconstruction of dorsal hand tissue defects after hand injury is challenging for surgeons because of the exposure of blood vessels, nerves, tendons, and bones. Thus, skin grafts are not suitable for use in reconstruction in the case of severe injuries. Furthermore, the palmar arch may not be reconstructed after hand reconstruction, as in our case. In such cases, vascular insufficiency of the limb and flap may develop using retrograde-flow pedicle flaps<sup>[11]</sup>. Therefore, flap options that do not require retrograde blood flow are needed. In case 1, the radial artery perforator-based adipofascial flap was selected for reconstruction of a dorsal hand defect after hand replantation. When soft tissue of the dorsal hand is crushed and extensor

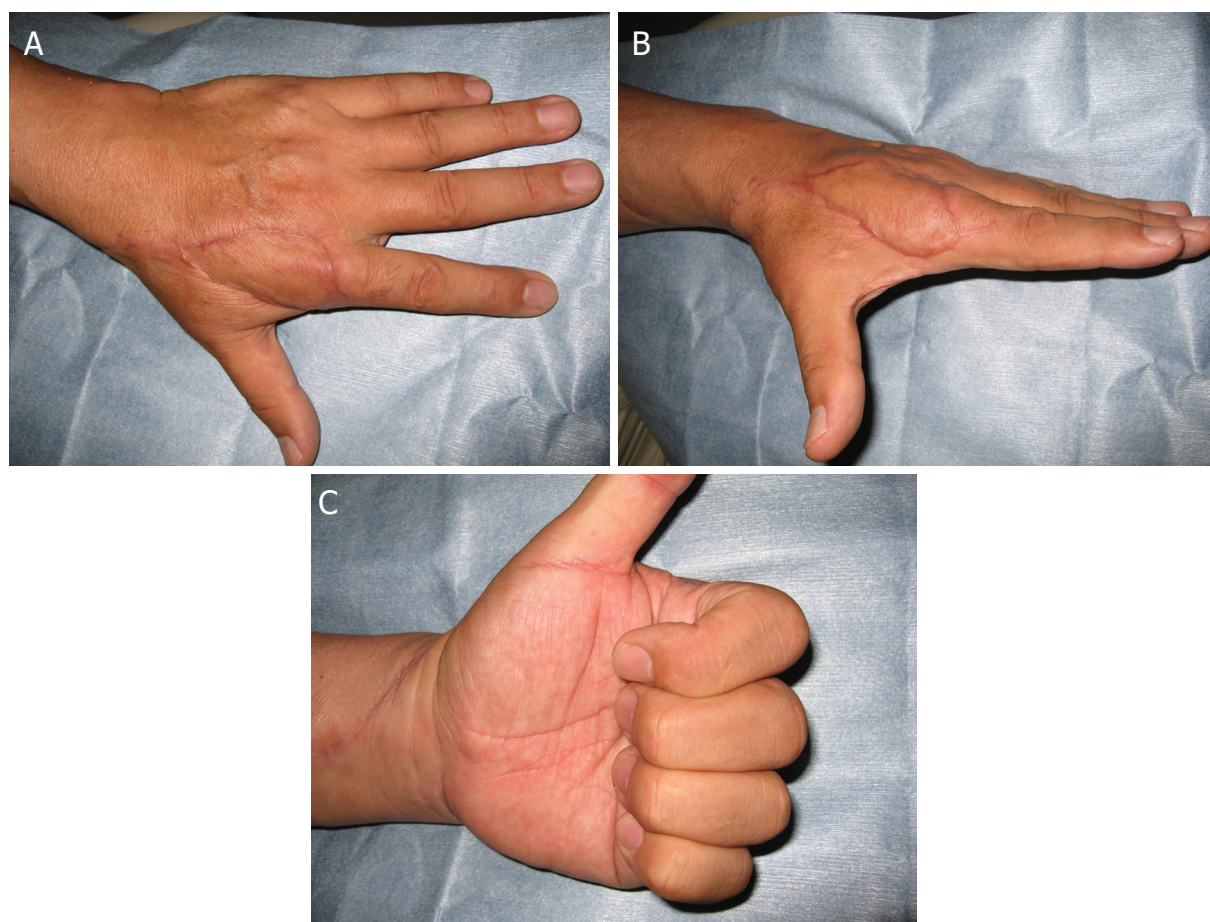




**Figure 7.** A 62-year-old man with skin and extensor tendon avulsion on the dorsum of his index finger. A: the index MP joint was exposed; B: design of the forearm adipofascial flap along the radial artery; C: raising of the forearm adipofascial flap, including the palmaris longus tendon; D: interlacing suturing of the extensor digitorum tendon and palmaris longus tendon; E: split-thickness skin grafting over the adipofascial flap; F: the flap donor defect was closed

tendons or bones are exposed or lost, soft tissue reconstruction using a flap with good circulation is required to resurface the defect. We were able to reconstruct the defects of blood vessels, nerves, and tendon by the adipofascial flap with forearm tissue. In case 2, the radial artery perforator adipofascial flap with the palmaris longus tendon was selected for reconstruction of the dorsal hand defect and the defect of the extensor indicis muscle tendon.





**Figure 8.** A 62-year-old man 10 months after reconstructive surgery for dorsal hand skin and extensor tendon defects. A: the color matching was good; B: extension of the index finger was close to full; C: all index finger joints have slightly limited flexion

Currently, distant, free, or local flaps are generally used to cover dorsal hand defects [Table 1]. Distant flaps, including abdominal, groin, and intercostalis flaps, are simple and good options for large defects<sup>[12-14]</sup>. However, they require at least two surgeries and prolonged recovery periods. Although blood supply of these flaps is reliable, the risks of elbow or shoulder contracture are higher than other options due to the prolonged recovery period. In addition, it is difficult to reconstruct thin and pliable skin.

Free flaps, including anterolateral thigh flaps, lateral arm flaps, and scapular flaps, enable one-step reconstruction<sup>[15]</sup>. Moreover, donor site morbidity can be minimized, and the extensor digitorum tendon and its gliding surface can be reconstructed as required. Muneuchi *et al.*<sup>[16]</sup> reported that a free anterolateral thigh fasciocutaneous flap was useful to fill the dead space of a dorsal hand defect after resection of the bursa, and the flap also aided in reconstructing a two-layer gliding surface of the extensor tendons. However, as the arteries and veins are usually damaged, anastomosis of vessels is problematic.

Local flaps, including the retrograde posterior interosseous flaps and retrograde forearm flaps, have good color and texture matching for dorsal hand reconstruction<sup>[17]</sup>. However, retrograde posterior interosseous flaps require meticulous dissection and the posterior interosseous artery in the middle of the forearm varies. Furthermore, the presence of distal communication between the posterior interosseous artery and the anterior interosseous artery must be confirmed<sup>[17]</sup>. If communication between the two vessels is unsatisfactory, this flap cannot be used for reconstruction. Moreover, retrograde posterior interosseous flaps have been reported to have relatively high complication rates such as flap necrosis<sup>[18-20]</sup>.

**Table 1. Flap options for reconstruction of dorsal hand defects**

Flap options	Advantages	Disadvantages	Representative flaps
Distant flap	Technically simple procedure High reliability of blood supply	Relatively longer down-time High risk of joint contracture Requires at least two surgeries Inability to reconstruct tendons Bulky	Abdominal flap Groin flap
Free flap	Reconstruction in one surgery Low donor site morbidity and risk of joint contracture Tendon reconstruction is possible	Technically complex	Free anterolateral thigh flap Free peroneal flap Free groin flap
Local flap	Good color and texture matching Requires no microvascular anastomosis	Requires retrograde blood flow High risk of donor site morbidity when a large flap is harvested	Retrograde posterior interosseous flap Retrograde forearm flap

**Table 2. Advantages and disadvantages of representative local flaps and the radial artery perforator-based adipofascial flap**

Local flaps	Advantages	Disadvantages
Retrograde posterior interosseous flap	Good color and texture matching Low risk of donor site morbidity	Requires meticulous dissection Relatively high complication rate Limitation of flap size
Retrograde forearm flap	Technically easier than the retrograde posterior interosseous flap Tendon reconstruction is possible	Requires sacrifice of the main artery High risk of donor site dysfunction
Radial artery perforator-based adipofascial flap	Retrograde blood flow is not needed Sacrificing main artery is not required Donor site morbidities are minimal Possible to reconstruct thin dorsal hand by skin grafting Tendon reconstruction is possible Simple and short surgery	Temporary pain due to fascial traction Skin graft is needed

Taghnia *et al.*<sup>[3]</sup> recommended a retrograde radial forearm adipofascial flap to avoid functional problems caused by adhesion at the donor site. Although this flap is technically easier to use than the retrograde posterior interosseous flap, it has the disadvantage of sacrificing the main artery. In certain cases, such as Allen test-negative, this flap is not applicable because it requires retrograde blood flow.

In 1989, Koshima *et al.*<sup>[21]</sup> reported inferior epigastric artery skin flaps without rectus abdominis muscle and called them perforator flaps. Since then, many perforator flaps have been developed. The radial artery perforator-based flap is one, which does not require retrograde blood flow and can be applied to hand reconstruction without sacrificing major vessels<sup>[22,23]</sup>. Donor site problems can be minimized by using this flap as an adipofascial flap<sup>[22-24]</sup>. In addition, the thin and pliable dorsal hand skin can be reconstructed by performing skin grafting over the adipofascial flap. The extensor digitorum tendon can be reconstructed by including the tendon, such as the palmaris longus or brachioradialis tendon, in the adipofascial flap as described in Case 2<sup>[23,25]</sup>. Furthermore, this flap has a great advantage in that the procedure is simple and can be completed in a short time<sup>[23,24,26]</sup>. Although the patients may complain about pain due to fascial traction, it disappeared in approximately one month in our cases. The advantages and disadvantages of representative local flaps and radial artery perforator-based adipofascial flaps are summarized in Table 2.

## CONCLUSION

The radial artery perforator-based adipofascial flap is an excellent functional reconstructive option for complex dorsal hand defects with minimal donor site morbidity.

## DECLARATIONS

### Authors' contributions

Contributed to data acquisition and also provided administrative, technical, and material support: Yamakawa S, Hayashida K

Reviewed the manuscript for content and grammar/spelling mistakes: Yamakawa S, Hayashida K

**Availability of data and materials**

Not applicable.

**Financial support and sponsorship**

None.

**Conflicts of interest**

All authors declared that there are no conflicts of interest.

**Ethical approval and consent to participate**

Written informed consent was obtained from both subjects to participate in this case report. A copy of the written consent is available for review upon request.

**Consent for publication**

Written informed consent was obtained from both subjects for publication of this case report and accompanying images. A copy of the written consent is available for review upon request.

**Copyright**

© The Author(s) 2020.

**REFERENCES**

1. Zhang G, Su H, Ju J, Li X, Fu Y, et al. Reconstruction of dorsal and palmar defects of hand with anterolateral thigh flaps from one donor site. *J Plast Reconstr Aesthet Surg* 2019;72:1917-22.
2. Medalie DA. Perforator-based forearm and hand adipofascial flaps for the coverage of difficult dorsal hand wounds. *Ann Plast Surg* 2002;48:477-83.
3. Taghinia AH, Carty M, Upton J. Fascial flaps for hand reconstruction. *J Hand Surg Am* 2010;35:1351-5.
4. Doğan T, Gürünlüoğlu R, Imer B, Numanoglu A. The distally based forearm island flap in hand reconstruction. *Plast Reconstr Surg* 1999;104:1581-2.
5. Suzuki S, Iwamoto T, Koshima I. Adipofascial turnover perforator flap for dorsal hand reconstruction based on both the posterior interosseous artery and radial artery. *J Hand Surg Eur Vol* 2012;37:178-80.
6. El-Sabbagh AH, Zeina AA, El-Hadidy AM, El-Din AB. Reversed posterior interosseous flap: safe and easy method for hand reconstruction. *J Hand Microsurg* 2011;3:66-72.
7. Sham E, Masia JA, Reddy TJ. Vascular analysis of radial artery perforator flaps. *Ann Maxillofac Surg* 2018;8:66-72.
8. Saint-Cyr M, Mujadizic M, Wong C, Hafez D, Lajoie AS, et al. The radial artery pedicle perforator flap: vascular analysis and clinical implications. *Plast Reconstr Surg* 2010;125:1469-78.
9. Onode E, Takamatsu K, Shintani K, Yokoi T, Uemura T, et al. Anatomical origins of radial artery perforators evaluated using color Doppler ultrasonography. *J Reconstr Microsurg* 2016;32:594-8.
10. Taylor GI, Chubb DP, Ashton MW. True and 'choke' anastomoses between perforator angiosomes: part i. anatomical location. *Plast Reconstr Surg* 2013;132:1447-56.
11. Sananpanich K, Tu YK, Kraissarin J, Chalidapong P. Reconstruction of limb soft-tissue defects: using pedicle perforator flaps with preservation of major vessels, a report of 45 cases. *Injury* 2008;39:55-66.
12. Nazerani S, Motamedi MH, Nazerani T, Bidarmaghz B. Treatment of traumatic degloving injuries of the fingers and hand: introducing the "compartmented abdominal flap". *Tech Hand Up Extrem Surg* 2011;15:151-5.
13. McGregor IA, Jackson IT. The groin flap. *Br J Plast Surg* 1972;25:3-16.
14. Gao JH, Hyakusoku H, Inoue S, Aoki R, Kanno K, et al. Usefulness of narrow pedicled intercostal cutaneous perforator flap for coverage of the burned hand. *Burns* 1994;20:65-70.
15. Griffin M, Hindocha S, Malahias M, Saleh M, Juma A. Flap decisions and options in soft tissue coverage of the upper limb. *Open Orthop J* 2014;8:409-14.
16. Muneuchi G, Suzuki S, Ito O, Kawazoe T. Free anterolateral thigh fasciocutaneous flap with a fat/fascia extension for reconstruction of tendon gliding surface in severe bursitis of the dorsal hand. *Ann Plast Surg* 2002;49:312-6.
17. Liu DX, Wang H, Li XD, Du SX. Three kinds of forearm flaps for hand skin defects: experience of 65 cases. *Arch Orthop Trauma Surg* 2011;131:675-80.
18. Büchler U, Frey HP. Retrograde posterior interosseous flap. *J Hand Surg Am* 1991;16:283-92.
19. Lu LJ, Gong X, Lu XM, Wang KL. The reverse posterior interosseous flap and its composite flap: experience with 201 flaps. *J Plast Reconstr Aesthet Surg* 2007;60:876-82.



20. Akinci M, Ay S, Kamiloglu S, Erçetin O. The reverse posterior interosseous flap: a solution for flap necrosis based on a review of 87 cases. *J Plast Reconstr Aesthet Surg* 2006;59:148-52.
21. Koshima I, Soeda S. Inferior epigastric artery skin flaps without rectus abdominis muscle. *Br J Plast Surg* 1989;42:645-8.
22. Weinzwieg N, Chen L, Chen ZW. The distally based radial forearm fasciosubcutaneous flap with preservation of the radial artery: an anatomic and clinical approach. *Plast Reconstr Surg* 1994;94:675-84.
23. Chang SM, Hou CL, Zhang F, Lineaweaver WC, Chen ZW, et al. Distally based radial forearm flap with preservation of the radial artery: anatomic, experimental, and clinical studies. *Microsurgery* 2003;23:328-37.
24. Koshima I, Moriguchi T, Etoh H, Tsuda K, Tanaka H. The radial artery perforator-based adipofascial flap for dorsal hand coverage. *Ann Plast Surg* 1995;35:474-9.
25. Appleton SE, Morris SF. Anatomy and physiology of perforator flaps of the upper limb. *Hand Clin* 2014;30:123-35.
26. Samson D, Power DM. The adipofascial radial artery perforator flap: a versatile reconstructive option in upper limb surgery. *Hand Surg* 2015;20:266-72.

Original Article

Open Access



# Endoscopic assisted facial rejuvenation: a 35 year personal journey

Oscar M. Ramirez

Department Plastic Surgery Cleveland Clinic Florida, Adjunct Clinical Faculty, Cleveland Clinic, FL 33331, USA.

**Correspondence to:** Dr. Oscar M. Ramirez, Ramirez Plastic Surgery, 19495 Biscayne Blvd, Ste. 200, Aventura, FL 33180, USA.  
E-mail: ramirezmdps@gmail.com

**How to cite this article:** Ramirez OM. Endoscopic assisted facial rejuvenation: a 35 year personal journey. *Plast Aesthet Res* 2020;7:25. <http://dx.doi.org/10.20517/2347-9264.2019.78>

**Received:** 26 Dec 2019 **First Decision:** 23 Mar 2020 **Revised:** 10 Apr 2020 **Accepted:** 7 May 2020 **Published:** 23 May 2020

**Science Editor:** Kai O. Kaye, John Yousif **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

## Abstract

**Aim:** Traditional facelift techniques rely on pulling. They approach the superficial or intermediate layers where the facial nerves and muscles are located, increasing the risk of facial nerve injury. They approach the central oval from the periphery and produce unnatural vectors of pull and aesthetic results. Alternative techniques that work on the subperiosteal plane using endoscopic techniques are described. Modern concepts of volume augmentation, beautification and rejuvenation of the facial expression are an inherent part of such techniques, or can be easily integrated.

**Methods:** The central oval is approached via four small scalp incisions and additional intraoral, upper gingivo-buccal incisions. The interconnected frontal subperiosteal, temporal subfascial and midface subperiosteal areas are lifted, imbricated and suspended sequentially. The brow/forehead is suspended to the skull using cortical screws. The midface and lower periorbital area are suspended to the fascia of the temporal muscle. The buccal fat pad is used to enhance the ogee line of the midface. Other three-dimensional volumetric maneuvers can easily be applied. In this setting, upper and lower lid blepharoplasties become more straightforward, skin only procedures. Actinic or nicotine damaged skin can be treated with lasers, peels or fluidified fat grafting in the same setting. The excess skin on the lower face and neck can be redraped with standard cervicofacial techniques. Deep subplatysmal cervicoplasty can be done concomitantly, or at another time to complete comprehensive rejuvenation.

**Results:** The procedures described herein have been performed in 824 patients with excellent aesthetic results and low complication rate. The average rate of rejuvenation was 18 years.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Conclusion:** These combination techniques are called Biplanar Endoscopic Assisted Mask and Triplanar Endoscopic Assisted Mask facial rejuvenation. They are advanced techniques of facial rejuvenation that provide comprehensive, natural, long lasting results.

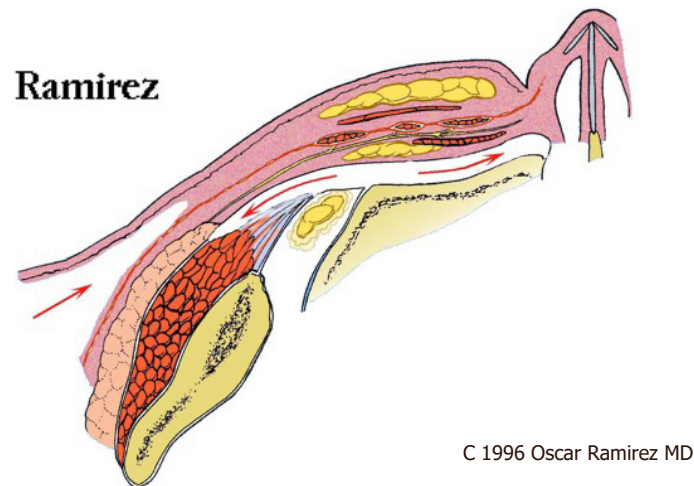
**Keywords:** Endoscopic face lift, videoendoscopy, facial rejuvenation, high definition face, picograft, ogee face, midface

## INTRODUCTION

The procedures, concepts and principles described in this article were developed over a 35 year period, beginning during my Plastic Surgery residency at the University of Pittsburgh. The driving force behind the innovations described in this article were the unnatural, in vogue facelift results of that era.

Traditionally, the aging face was approached using techniques that pulled and stretched the facial soft tissues. The face was approached in a superficial plane, tightening the skin and SMAS (superficial muscular aponeurotic system) only<sup>[1-3]</sup>. Over time, face lift techniques beneath the SMAS evolved, including the deep plane facelift or composite facelift<sup>[4,5]</sup>. Initially, some surgeons considered this technique unsafe, and were hesitant to go deep to SMAS due to the proximity of facial nerve branches<sup>[6]</sup>. Moreover, the degree of facial edema was considered more marked using intermediate layer techniques. Around the same time, Paul Tessier described and popularized the subperiosteal approach<sup>[7-12]</sup>. I first described my five experience into the subperiosteal technique at the 1989 Biannual Congress of the International Society for Aesthetic Plastic Surgery in Zurich, Switzerland, and the 1990 American Society for Aesthetic Plastic Surgery Annual Congress in Chicago Illinois<sup>[13]</sup>. Another paradigm shift that influenced my thinking was the endoscopic approach to the forehead pioneered by Luis Vasconez<sup>[14]</sup>. This was first presented at the 1992 annual meeting of the American Society of Plastic and Reconstructive Surgeons, in Washington DC. I quickly adopted and modified Vasconez's forehead rejuvenation technique<sup>[14]</sup>. Noticing the advantages of the endoforehead compared to the traditional coronal approach I extended the application of the endoscopic approach to total facial rejuvenation<sup>[15-18]</sup>. It became clear that the subperiosteal plane was better suited to endoscopic techniques including secondary rhytidectomies<sup>[19]</sup>. It also made it safer and easier to add supplementary techniques. Those are described below<sup>[20-24]</sup> [Figure 1]. Along the way Adrien Aiache and I discovered the suborbicularis fat that I coined SOOF (sub-orbicularis oculi fat)<sup>[25]</sup>. It was an excellent structure for filling the tear trough and to lift and imbricate the cheek. More recent research regarding the innervation of the lower eyelid orbicularis has also been relevant to the endo-midface, and the preservation of function of this muscle was another added benefit of this approach<sup>[26]</sup>. The most important side effects of introducing these new techniques were that surgeons were compelled to compare these with the older techniques. In the process we started to focus more critically on the anatomy and aesthetics of the face<sup>[27,28]</sup>. We began thinking more about the benefits of volume preservation and restoration in contrast to the pulling maneuvers of traditional methods<sup>[21-24,28,29]</sup>. This also created a new landscape for developing new minimally invasive techniques and non-invasive techniques, including the use of fillers and neuromodulators as temporary alternatives to surgical approaches<sup>[30,31]</sup>.

Following the realization that loss of volume was an important component of the aging process many surgeons and dermatologists started over filling faces creating an unnatural aesthetic<sup>[32]</sup>. In my opinion we need to swing the pendulum back and treat all features of facial aging in a more balanced approach. Moreover, a comprehensive approach addressing all thirds of the face gives a more natural result than when surgery is performed in a segmental fashion. An endoscopic approach to the face can address the three thirds of the face in a balanced fashion. If the endoscopic approach is insufficient to address all of the components of the aging face, other main or ancillary procedures can be easily integrated without burning any bridges.



**Figure 1.** The combination of subperiosteal dissection in the central oval and subcutaneous dissection in the periphery of the face is safest. The intermediate layers where the nerves and the muscles are located are not safe

The endoscopic subperiosteal approach to the aging face can address the forehead/brow, the midface and the lower face around the mandible, i.e., mentopexy. Soft tissue endoscopic cervicoplasty can also be done in younger patients that do not require excisional approaches. In patients over 50 years of age the endoscopic approach becomes the foundation of the rejuvenation and the excess skin of the upper/lower eyelids, lower face and neck can be removed using standard access incisions. In this setting standard techniques become complementary procedures for a total facial rejuvenation. Likewise, facial implants, fat grafting and laser resurfacing can be easily integrated in the endoscopic subperiosteal facial rejuvenation.

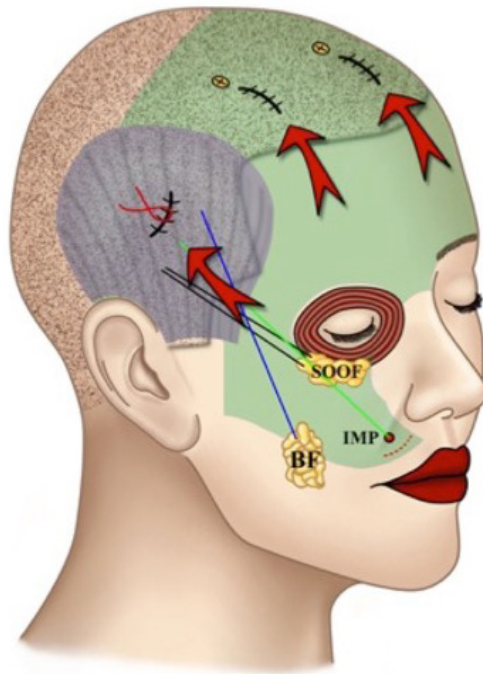
Using these advanced rejuvenation techniques you will achieve the following goals: (1) volume restoration; (2) embellishing; (3) revitalization of facial countenance; (4) lifting of ptotic tissues; (5) tightening of the skin envelope; and (6) volume subtraction, particularly in the neck.

## METHODS

### Central oval rejuvenation

#### *Endoforehead*

Forehead rejuvenation is approached via four scalp incisions, each measuring approximately 1.5-2 cm. Two symmetrical paramedian incisions are also made in the frontal scalp, about 2 cm from the midline. A final 1.5-2 cm incision is made in the temporal scalp, at the end of a line from the alar implantation, passing the lateral canthus, and finishing 2-3 cm inside the temporal hairline. Using triangulation techniques for both the endoscope and the periosteal elevators or endoscopic manipulators, a complete subperiosteal dissection of the frontal bone can be achieved (Ramirez Endoscopic Instruments, Marina Medical. Davie, Florida). This is connected with dissection beneath the tempoparietal fascia. Connection between frontal and temporal areas of dissection is done across the temporal line of fusion, coming from lateral to medial, and not the other way around. The frontal dissection stops when the supraorbital (SON) and supratrochlear (STN) nerves and the associated corrugator muscles are identified. The retaining ligaments of the brow are also elevated off the superolateral orbital rim. Temporal dissection stops at the upper limits of the zygomatic arch. The fat pad that surrounds the temporal nerve are elevated off the deep plane of dissection, ensuring protection of the facial nerve. The sentinel vein and the sensory zygomaticotemporal nerves are preserved. Centrally, 80% of the corrugator muscles are resected using special muscle biters (Marina Medical. Davie, Florida), ensuring preservation of the SON and STN. The procerus is then transected in a horizontal orientation at the level of the nasoglabellar angle. The periosteum of the frontal bone is cut



**Figure 2.** The three-point fixation of the endomidface. Each suture has a specific effect. The combined effect creates a beautiful Ogee line. SOOF: sub-orbicularis oculi fat; BF: Bichat's fat; IMP: inferior Malar Periosteum

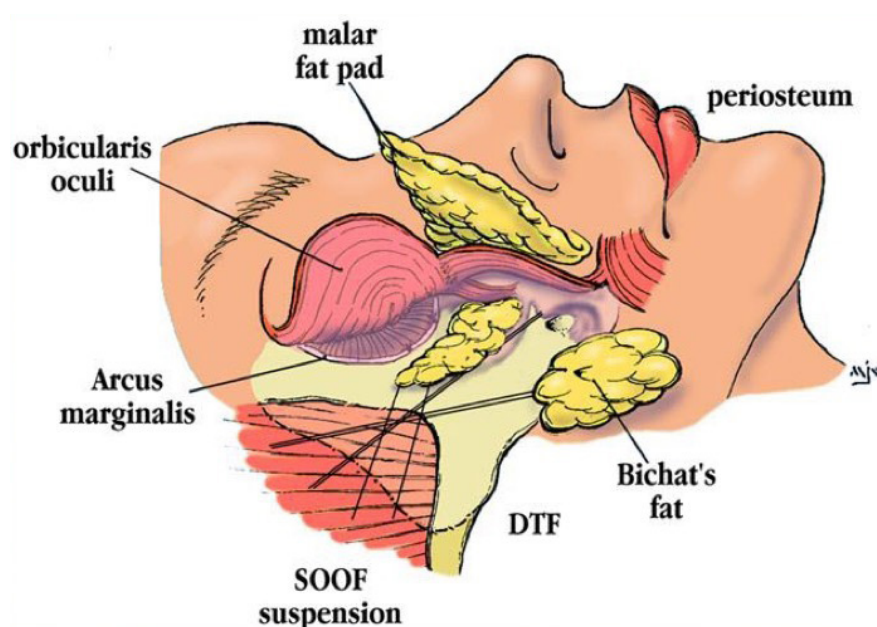
from one tail of the brow to the other about 5-10 mm above the arcus marginalis (a variation of the original technique) using “double curved down” Ramirez’s endoscopic scissors (Marina Medical. Davie, Florida). A single small butterfly drain is then placed at the level of the glabella. Fixation of the frontotemporal scalp is done after the midface dissection and fixation is complete. The vector of pull is in the superomedial direction.

### *Endomidface*

The endomidface technique that I proposed many years back accesses the midface using a 2 cm endoforehead-temporal slit incision and a Caldwell-Luck type incision<sup>[22-24,28,29,33]</sup>. Both incisions are interconnected across the zygoma, therefore avoiding a trans-blepharoplasty approach<sup>[34]</sup>.

The intraoral incision is done vertically at the level of first or second premolar. The subperiosteal dissection of the maxillary and inferior portions of the malar bones was initially done with the Aufricht lighted retractor. The endoscope was introduced to dissect the orbital rim and the rest of the malar bone. Laterally the fascia of the masseter muscle is elevated along the line that continues from the inferior border of the zygoma towards the middle third of the zygomatic arch. This elevates some of the retaining masseteric ligaments and the preparotideal SMAS. Using the temporal and intraoral incisions the pockets created are connected across the zygomatic arch, preserving the infraorbital nerve. The next step is to lift the midface and create volume. Both are accomplished using three structures: (1) Bichat's fat pad; (2) suborbicularis oculi fat (SOOF); and (3) modiolus. Each of these elements is manipulated and suspended using polydioxanone (PDS) 3-0 and 4-0 sutures. Elevation of the Bichat's fat pad elevation over the zygoma augments the convexity of the upper midface, and at the same time creates a concavity of the lower midface. With one maneuver the ogee line of the midface can be recreated [Figures 2 and 3]<sup>[29,33]</sup>. The SOOF overlaps the deep tissues of the midface and effaced any tear trough deformity [Figure 4]<sup>[17-25]</sup>. The modiolus lifts the corner of the mouth [Figure 5]<sup>[22-24,33]</sup>. The Bichat's fat pad suture is piggybacked to the SOOF suture. The SOOF and modiolus sutures, are then anchored to the temporal fascia using the adjustable Peruvian



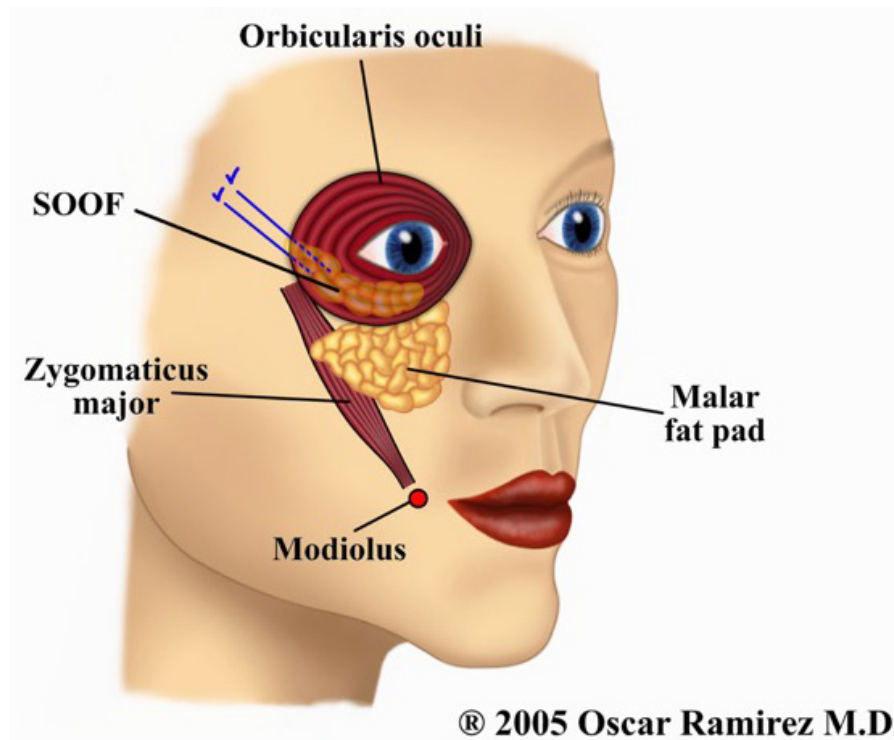


**Figure 3.** The SOOF suspension lifts the entire cheek and effaces the tear trough deformity. The addition of the Bichat's fat pad accentuates the Ogee line of the face. SOOF: sub-orbicularis oculi fat; DTF: deep temporal fascia



**Figure 4.** The Ogee line is the curvilinear line of beauty and youth

fisherman's knot<sup>[35]</sup>. This suture controls the proper tension for volume control and desired elevation. A 2 mm butterfly drain is placed in the midface and fixated to the temporal scalp. The temporoparietal fascia is lifted and anchored to the temporal fascia with 3-0 PDS sutures. This provides additional remodeling of the upper cheek and periorbital region. The intraoral incision is closed with 4-0 chromic catgut sutures. The frontal scalp is suspended with two self-stabilizing Ramirez endoforehead screws (Dupuy Synthes, Warsaw, Indiana). These were applied percutaneously in the frontal scalp away from the access incisions.



**Figure 5.** The subperiosteal repositioning of the orbicularis oculi and the origin of the muscles inserted in the modiolus and the specific suture applied to the area near the modiolus will lift the corner of the mouth. All of these will rejuvenate facial expression. SOOF: sub-orbicularis oculi fat

#### *Upper blepharoplasty*

Endoforehead lifts the brow and eliminates a small amount of excess skin from the upper eyelid. Greater skin excess will still require an additional blepharoplasty. However, the amount of skin resection was far less than if this was done in isolation. Ptosis of the brow creates apparent or real excess skin in the upper eyelid area. The apparent excess is reversed with the endoforehead. This will make the need for upper blepharoplasty less likely.

#### *Lower blepharoplasty*

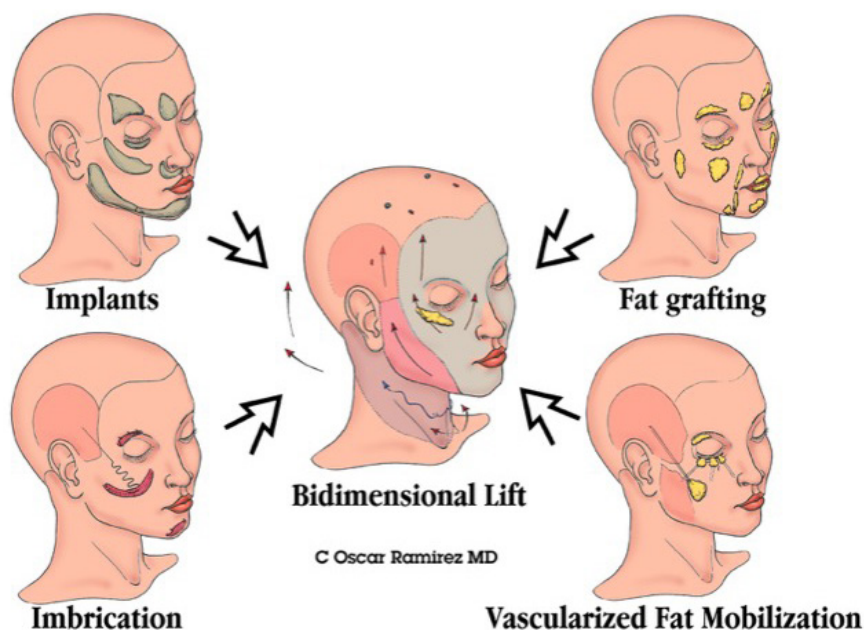
When combined with endomidface, lower blepharoplasty becomes more straight forward procedure. Lateral orbicularis is suspended using a 4-0 nylon suture to the temporal fascia, using a skin only resection. This is done after endomidface fixation. Midface lift has the additional benefit of filling the tear trough, and blending it better with the infraorbital fat. I do not remove any intraorbital fat except under unusual indications, i.e., globular eyes, excessive and protruding lower eyelid fat pads.

#### **Advanced objectives of facial rejuvenation**

The steps of endoscopic central oval rejuvenation (endoforehead-endomidface) and blepharoplasty described above were all performed in patients below 50 years of age. This was the cornerstone over which other techniques were added to provide a more comprehensive rejuvenation in the older cohort of patients. The techniques described above may by themselves attained some of the objectives outlined below. However, other techniques are needed to obtain the following objectives:

#### *Volume enhancement*

Volume augmentation of the face was obtained using one of the following methods: (1) facial implants; (2) imbrication techniques; (3) vascularized fat mobilization; and (4) fat grafting. A representative illustration summarizes these methods [Figure 6].



**Figure 6.** Additional methods of volumetric enhancement that can be applied to the pureendoscopic central oval facial rejuvenation or the biplanar technique (First published in study<sup>[22]</sup>)

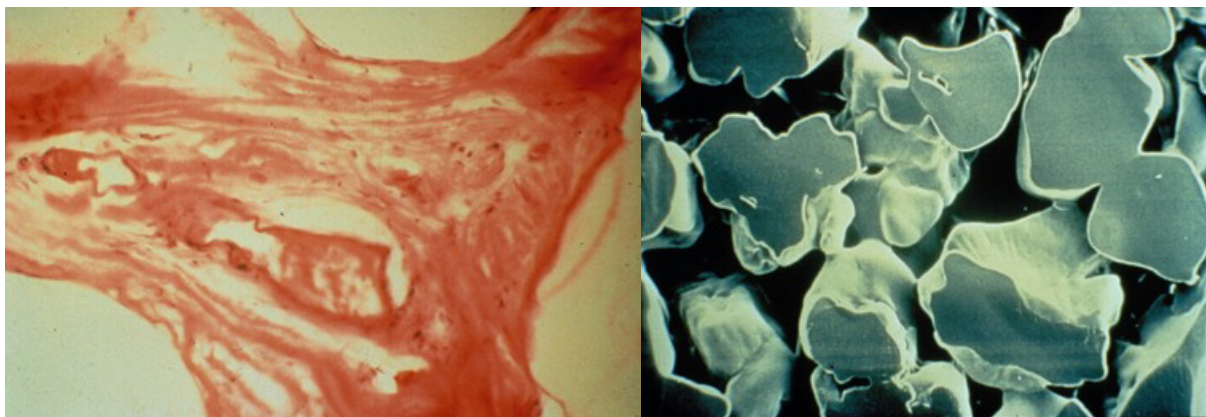
(1) Facial implants. In a previous publication I stated that facial beauty is “bone deep”<sup>[36]</sup>. To enhance the facial skeleton my preferred choice of implant material is the porous polypropylene [Medpor (Stryker. Portage, Michigan) Su-Por (Poriferous. Newman, Georgia)]. These implants were found to have a different biological behavior to silicone implants and do not erode bone. The soft tissues grew into the porous channels of the implant providing vascularization and a secondary method of fixation. The in-grow of vessels into the implant makes it less susceptible to infection and in cases where infection did develop, antibiotics were able to penetrate the implant [Figure 7]. The implants are less prone to capsule formation, and therefore they do not undergo implant shifting due to contracture. Overall, they behave more like bone than any other implant I have employed in my practice. The porous implants were placed following wide dissection, and although this was initially seen as a disadvantage, one must consider this technique as a soft tissue remodeling and reposition. The porous implants are fixed with screws, which do not allow the implant to migrate or move around. I designed implants for each of the different areas of the face [Figure 8]. These implants have given me figuratively and literally an extra dimension to my facial rejuvenation practice.

(2) Imbrication techniques. Subperiosteal dissection is also a great method to lift the composite tissues. Applying sutures to the mid or lower boundaries of the dissected areas they were imbricated and suspended to a higher position. The distance of the lower and higher points of imbrication is decreased while the antero-posterior projection is increased. Examples are browpexy, SOOF lift and mentopexy.

(3) Vascularized fat mobilization. Subperiosteal access also allows manipulation of some of the sagging deep scyssarcosis fat for reshaping. The upper eyelid fat pads are placed over the upper orbital rim augmented brow projection. The lower eyelid fat pads are placed over the lower orbital rim to filled in the tear trough region. The herniated Bichat’s fat pad is used to create the Ogee line of the midface.

(4) Fat grafting. With subperiosteal endoscopic techniques there is no associated delamination of the anatomical planes, therefore fat can be injected anywhere from dermis to periosteum. Fat is injected with 1cc Luer-Lock syringes attached to the “Ramirez Super-Luer-Lock micro-cannulas” (Tulip Medical, San





**Figure 7.** The ingrowth of tissue in the omni-dimensional porous structure of the porouspolypropylene implants



**Figure 8.** Some of the facial implants designed to provide support and enhance volume in the face

Diego CA). Fat grafting corrected residual asymmetries and erased dermal creases particularly in the nasolabial folds and glabellar lines. An average of 30 cc of fat is sufficient for the entire face, however I use more fat for gaunt faces, or when the imbrication of the Bichat's fat pad elevation did not provide enough volume. Lately I have incorporated stem cell-rich fat grafting obtained with the Diode 1210 laser. This fat is devoid of fibers and can be injected into the dermal/subdermal plane with 1 cc syringes and 23-gauge needles without the need for centrifugation or filtration. We call it Picograft<sup>TM</sup> or Picofat<sup>TM</sup><sup>[37,38]</sup>. This provided an excellent alternative for skin damaged by sun exposure or smoking, improving skin quality on several fronts: pigmentation, elasticity, and fine wrinkles *etc.*<sup>[37,38]</sup>.

### *Beautification*

Rejuvenation is also a beautification procedure. The three-dimensional enhancement brings back the curves and projection of a youthful and beautiful face. This is different to the stretched look of traditional facelifts. The creation of the ogees of the face is a powerful maneuver to obtain simultaneous beautification and rejuvenation. Other attributes of a beautiful face are angularity and facets, provided by facial implants, in addition to replacing the bony support lost during aging.

### *Rejuvenation of facial expression*

The subperiosteal repositioning of the point of origin of muscles of facial expression in the entire face gives a generalized happy expression in repose and a gentle smile without effort. These changes arose due to the elevation of the modiolus, lifting of the corner of the mouth. The orbicularis oculi muscles are rotated upwards. The dynamics of the perioral muscles changed when mentopexy or chin implants are integrated during surgery. The origin of the lip depressors and of the lip elevator (Mentalis muscle) are detached and advanced upwards. These steps allow the modiolus to be elevated even further and the lower orbicularis oris to be relaxed, diminishing hypertrophy secondary to orbicularis straining. This allows the lower lip to pout easily. The chin dimples secondary to mentalis straining also disappear creating a youthful, happy look. In my view rejuvenation of facial expression is the ultimate paradigm shift in facial rejuvenation<sup>[36,39]</sup>.

### *Repositioning of sagging tissues*

Although deflation of tissues has been emphasized as the most important component of aging, sagging of facial tissues is just as important as deflation. The Endotemporo-midface, that includes a periorbitoplasty and a Bichat's fat pad repositioning, elevates most of the sagging structures of the central oval of the face. The effectiveness of midface elevation decreases as the distance from cheek to jawline increased. Therefore, correction of jowls requires additional maneuvers. This is particularly relevant for patients over 50 years of age, who require the addition of traditional cervicofacial lift to remove the excess skin of the lower face and jawline.

My cervicofacial lift includes skin undermining of the neck from side to side across the midline. The platysma with or without a digastric corset is advanced towards the midline and the skin in the opposite direction. Treatment of the submental crease and the marionette lines, requires skin separation from the platysma, allowing unrestricted opposing vectors of pull during repair and closure. I open the deep subplatysmal space in approximately 30% of my patients. This is done to resect deep fat, treat the enlarged digastric muscles and the enlarged salivary glands. Digastric corset, or shaving, is done for thick digastric muscles. Ptotic and or enlarged submaxillary salivary glands are treated with partial resection or suspension<sup>[40,41]</sup>.

Patients with poor chin and/or mandibular support usually age worse than those that have good support. Moreover, aging produces atrophy of the skeletal support. This is addressed with chin and gonial angle implants. Implants make remodeling of the lower face and neck easier and provide superior aesthetic results. Rejuvenation with implants appear to be more durable. Enhancement of the entire mandible is done using specially designed implants that I named the "Mandibular Matrix Implant System" [Figures 9 and 10]<sup>[42]</sup>.

### *Rejuvenation of the skin envelope*

A comprehensive approach also requires skin excision. After improving the foundation with volume augmentation, with any or all of the strategies described, skin excision is more a re-draping maneuver followed by a tensionless closure. This avoids tension bands on the face that give a typical windswept look. The dissected tissues are robust, allowing use of laser resurfacing when required. I also use stem cell rich-fat graft in the intermediate and subdermal plane without fear of vascular compromise of the skin.





**Figure 9.** The effect of the Mandibular Matrix System to enhance the lower face, neck and jaw line

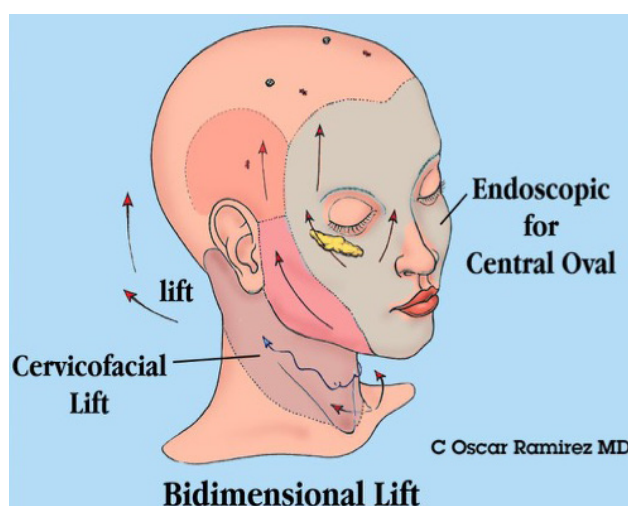


**Figure 10.** Lateral view of the same patient

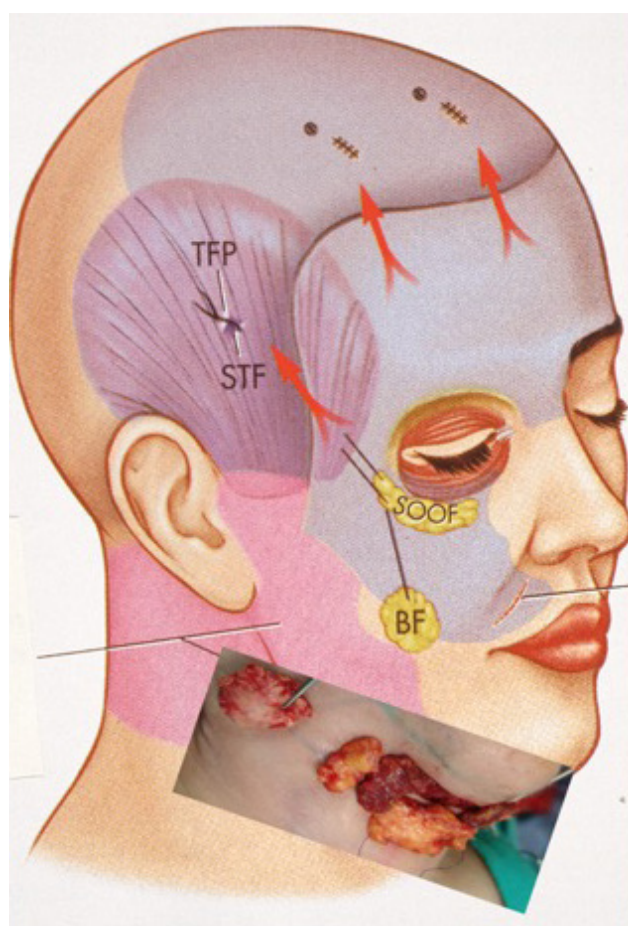
#### *Subtractive maneuvers particularly on the neck*

Not everything is augmentation in facial rejuvenation. The jowls and the neck require subtraction. Jowl fat and supra-platysma fat are removed using a small liposuction cannula, preferably under direct visualization. The deep subplatysmal fat is removed only under direct vision. The danger of liposuction in the deep compartment is bleeding, nerve injury or salivary fistula. Its fibrous consistency also makes suction difficult. Fat removal is in a planimetric fashion that includes extension around salivary gland. Isolated fat excision in between the digastric muscles is not advisable as it can lead to cobra neck deformity. The digastric muscles are tangentially shaved or advanced medially to reduce bulk. If salivary glands are found to be enlarged excision can be performed.

The combination of central oval endoscopic with peripheral excisional cervicofacial lift is called BEAM (Biplanar Endoscopic Assisted Mask) facial rejuvenation [Figure 11]. The combination of BEAM facial rejuvenation with Deep Subplatysmal Cervicoplasty is called TEAM (Triplanar Endoscopic Assisted Mask) facial rejuvenation [Figure 12].



**Figure 11.** The Biplanar EndoscopicAssisted Mask facial rejuvenation: central oval endoscopic subperiosteal and lowerneck and face subcutaneous open dissection



**Figure 12.** If you add the sub-platysma surgery to the neck, the procedure is called Triplanar Endoscopic Assisted Mask (First published in study<sup>[36]</sup>). SOOF: sub-orbicularis oculi fat; BF: Bichat's fat; STF: superficial temporal Fascia; TFP: temporal Fascia Proper

## RESULTS

I have performed 824 total facial rejuvenation procedures over a 35-year period. In a random sample of 100 of these cases, independent observers and patient's rated the average number of years of rejuvenation to be 18 (range 11-25 years). This group had a minimum of two years follow-up. Patient satisfaction was excellent in 80%, moderate in 18% and substandard in 2%.

Refinements in endoscopic techniques over time have resulted in globally low complication rates. Temporary frontal neuropraxia in endoforehead cases was approximately four percent, with function returning in all cases between one and three months. I have had one case of permanent unilateral frontal nerve injury. This was a secondary case after a previous coronal approach. Likewise, neuropraxia of the zygomatic or buccal nerve branches were uncommon, affecting less than two percent of patients. More common were neuropraxia or musculopraxia of isolated muscles of the face, particularly the levator labii superioris (4%). They were also temporary, recovering by two months on average. Mentopexy, chin and mandibular implants, and deep cervicoplasty all had a combined rate of marginal mandibular neuropraxia of less than three percent. All of these were temporary and likely related to edema or traction. Localized alopecia at the site of scalp access was highly dependent on surgical technique. Silastic port protectors decreased hair follicle damage. Occasionally, patients experienced telogen effluvium, with two cases of near total scalp effluvium that completely resolved after several months. They were related to systemic and localized stresses. Infection was also rare and occurred in less than one percent of cases.

To prevent infection due to bacterial contamination from saliva I use Chlorhexidine gluconate 0.12% oral rinse twice a day pre-operatively. Intra-operatively I clean the intraoral mucosa with Betadine solution and a diluted solution of Betadine was used in the dissection cavity, applied in neurosurgical sponge pads. I also leave a 2 mm drain in the cavity with the drain brought out thru a ministab incision in the temporal scalp. If an abscess occurs post-operatively drainage, irrigation and antibiotics are sufficient to manage this complication. I lost some of the lifting effect following removal of the internal suspension sutures. None required reoperations.

Bleeding complications were rare in this endoscopic cohort. One patient developed a moderate volume post-operative hematoma that required drainage but did not require blood transfusion. A few other cases developed minor localized hematomas that were treated by simple aspiration. The overall hematoma rate is less than 1%.

## DISCUSSION

In the last 30 years many advances have been made in the treatment of the aging face. Previously the central oval of the face has been more elusive. The subperiosteal endoscopic approach works beautifully for forehead and midface. The trans-blepharoplasty approach is associated with a high rate of eyelid malposition, but using the endoscopic technique, avoiding eyelid incisions, is almost devoid of this complication<sup>[23]</sup>. Additionally, rates of neuropraxia are less common than those reported in the intermediate layer techniques. The plane of work in sub-SMAS techniques is where the mimetic muscles and nerves are located. Therefore, neuropraxia is common to all sub-SMAS techniques. The subperiosteal plane is deep to these structures. Nevertheless, there are two areas that are still at particular risk: the frontal nerve as it crosses the zygomatic arch and the zygomatic and buccal nerves as they cross over the masseter tendon. The infraorbital nerves can also be injured by blind dissection or excessive traction. Proper technique can significantly reduce these complications. My personal rate of nerve injury on the midface, forehead and mandible is around 2%, highlighting that this procedure can be performed safely and consistently over time.



**Figure 13.** The endoscopic approach to the central oval in younger patients is called Facial Beautification. Frontal view of a 36 years old female patient



**Figure 14.** Three quarter view of the same patient. She had endoforehead, endomidface, eyelid ptosis repair and fat grafting to several areas of the face

Endoscopic rejuvenation of the central oval of the face is the cornerstone of facial rejuvenation at any age (young, old, older). These techniques in isolation can provide the objectives of modern facial rejuvenation previously outlined. The central face approach alone is done in patients between early 20's to late 40's. In the very young group we use the same lifting, imbricating and volumetric approach used in older patients. This is done to correct the congenitally prone sagging of the central oval soft tissues. Because lifting or rejuvenation may have negative connotations in this cohort of patients, we call it “facial beautification”, because in principle that is what is accomplished [Figures 13 and 14]. One of the advantages of the deeper approach is that you can manipulate the tissues to obtain volume. The brow lift is a remodeling procedure





**Figure 15.** Observe the beautification of the periorbital area. Patient had endoforehead, endomidface and concomitant laser resurfacing of the face. No upper or lower blepharoplasties were required



**Figure 16.** Another patient with endoforehead, endomidface. No upper blepharoplasty was done. Lower blepharoplasty required skin only excision

more than lift. Volume augmentation takes up the anterior-posterior dimension more than the vertical dimension. The forehead lift also relaxes the frontal muscle. Because of the vertical elevation of soft tissues in the midface, it effaces the tear trough deformity. In cases that you need more building material the composite tissues due to the deep dissection allows you inject fat in the intermediate and or superficial planes, Bichat's fat pad can be mobilized over the lower malar area or implants can be introduced to correct different areas (malar, para-nasal, orbital rim). Perhaps these are the most significant differences to other techniques described. In this technique the lower eyelid and midface are treated as one aesthetic unit. It simplifies lower blepharoplasty because you provide support from below and the central SOOF fills-in the tear-





**Figure 17.** Frontal view of a 48-year-old patient with prior standard facelift three years earlier. Still looks sad, the cheeks are sagging, there is hyperactivity of the forehead, and eyelid ptosis (left picture). The post-operative view on the right are two years later observe the rejuvenation of "facial expression"



**Figure 18.** Tilted-down view of the same patient. Before and after views

trough. In that scenario lower eyelid surgery becomes a skin-only operation. Likewise, endoforehead makes upper blepharoplasty unnecessary, or minimizes the amount of skin excision required [Figures 15 and 16]. The case examples [Figures 17-19] demonstrate the exquisite results that can be obtained. Creation of the ogee, blending of lower eyelid-midface interface, natural contour and fullness of the lower eyelids and rejuvenation of the facial expression are features that you do not see consistently with other techniques. The reposition of the facial muscle mask explains changes in facial countenance [Figures 20-22]. Treatment of the lower face and neck can be easily incorporated into the surgical plan. They can be done simultaneously, or if time constraints exist a month later.

After pure endoscopic procedures patients can resume work after about 2-3 weeks. The addition of standard facelift or deep subplatysmal cervicoplasty will increase the amount of swelling and extend the recovery period to about 4-6 weeks. Subtle, subclinical swelling can take up to 6 months to subside. Despite this, the degree of rejuvenation that can be obtained (many times as much as 25 years difference) makes the procedure appealing to those patients that are willing to spend the time and resources for long lasting results and paradoxical "very natural results" [Figures 23-29].

In conclusion, endoscopic techniques of the central oval of the face are the cornerstone of rejuvenation for patients of any age. In my view, addressing the central oval of the face is what makes the major difference.



**Figure 19.** Same patient in three quarter view. Before and after. She underwent endo-forehead, endomidface, eyelid and ptosis repair, lip lift, skin only lowerblepharoplasty and revision of facelift scars



**Figure 20.** Front vies of a 50-year-old patient, before and after. Patient with history of three prior facial procedures. One standard facelift, one endoscopic midface lift and one redo of midface lift and one fat grafting session to several areas of the face. Patient underwent Biplanar Endoscopic Assisted Mask Facial Rejuvenation. It is not only volumetric restoration or lifting, it is the highest level of rejuvenation: rejuvenation of the facial expression



**Figure 21.** The overlay drawings show the artistic conception of the changes on the muscles of facial expression



**Figure 22.** The three-quarter view illustrates these changes better



**Figure 23.** Before and after of a 52-year-old patient. Frontal view. Patient had endoforehead, endomidface, cervicofaciallift and deep subplatysmal cervicoplasty (Triplanar Endoscopic Assisted Mask facial rejuvenation)



**Figure 24.** Lateral views of the same patient. Before and after. The neck was addressed by removing deep fat, digastric muscle tangential shaving, and superficial salivary gland excision





**Figure 25.** Frontal views, before and after of a 56-year-old edentulous patient with two previous standard face lifts and an anterior hairline brow lift



**Figure 26.** Lateral view of the same patient. Before and after. Observe that she lost the sideburns. The lack of mandibular support is evident. Patient had shortening of the forehead, endomidface, and cervico-facial lift, deep subplatysmal cervicoplasty with fat removal, digastric shaving and partial salivary gland excision. A mandibular matrix implant system was also used. The side burn was recreated with a scalp flap. A lip lift was performed. Eyelid ptosis repair done. Skin only lower lid blephoroplasties were also done



**Figure 27.** Frontal views of a 54-year-old patient with most of the changes of aging. The comparative before and after photographs demonstrates the significant improvement that can be obtained with a comprehensive facial rejuvenation done using correct planes, vectors of pull, volumetric changes and other ancillary procedures



**Figure 28.** Three quarter view of the same patient. She underwent Triplanar Endoscopic Assisted Mask facial rejuvenation: endoforehead, endomidface, cervicofacial lift, deep subplatysmal cervicoplasty (deep fat removal, digastric shaving and partial salivary gland excision). She also had geniomandibular porous polypropylene implant. No upper blepharoplasty was done. Lower -skin only- blepharoplasty was performed (First published in study<sup>[22]</sup>)



**Figure 29.** Lateral view of the same patient. Before and after

Patients up to 49 years of age will require only endoscopic techniques. Older patients will require biplanar (BEAM) or Triplanar (TEAM) techniques. Peripheral to the central oval the sagging jowls and excess skin of lower face and neck is approached with a thick-skin rhytidectomy. Problems of the deep neck are approached using a submental incision<sup>[40,41]</sup>. Methods of three-dimensional enhancement can be incorporated easily into any area of the face with any combination of the techniques described above. Damaged skin can be treated with lasers or stem cell-rich fat grafting<sup>[18,20,37,38]</sup>. Despite the comprehensive and seemingly aggressive approach the results show few telltale signs of an operated look and has long-term, durable results.



## DECLARATIONS

### Authors' contributions

The author contributed solely to the article.

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

Consultant for Marina Medical. Davie, Florida.

Consultant for "Villani Spongilla Matrix". Newport Beach, California.

Consultant for Poriferous (Facial Implants). Newnan, Georgia.

Speaker Bureau & Technical Support DMC.

Founder & Co-owner of "Laser Stem Cell Technologies LLC".

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

A written informed consent for publication has been obtained from patients.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Skoog T. Useful Techniques in Face-lifting. Presented at the meeting of the American Association of Plastic Surgeons; San Francisco, Calif. April 1969.
2. Lemmon, Mark L. (Mark Leonard). Color atlas of SMAS rhytidectomy. Thieme Medical, G. Thieme Verlag, 1993.
3. Mitz V, Peyronie M. The superficial Musculo-Aponeurotic System (SMAS) in the parotid and cheek area. *Plast Reconstr Surg* 1976;58:80-8.
4. Hamra ST. The deep-plane rhytidectomy. *Plast Reconstr Surg* 1990;86:53-61; discussion 62-3.
5. Hamra ST. Composite rhytidectomy. *Plast Reconstr Surg* 1992;90:1-13.
6. Baker DC. Deep dissection rhytidectomy: a plea for caution. *Plast Reconstr Surg* 1994;93:1498-9.
7. Tessier P. Le lifting facial sous-perioste. *Ann Chir Plast Esthet* 1989;34:193-7. (in French)
8. Psillakis JM, Rumley TO, Camargos A. Subperiosteal approach as an improved concept for correction of the aging face. *Plast Reconstr Surg* 1988;82:383-94.
9. Ramirez OM, Maillard GF, Musolas A. The extended subperiosteal face lift: a definitive soft-tissue remodeling for facial rejuvenation. *Plast Reconstr Surg* 1991;88:227-36; discussion 237-8.
10. Ramirez OM. The subperiosteal rhytidectomy: the third-generation face-lift. *Ann Plast Surg* 1992;28:218-32; discussion 233-4.
11. Krastinova D. Lifting facial sous-perioste. *Ann Chir Plast Esthet* 1989;34:199-211.
12. Santana PSM. Metodologia craneomaxilofacial en Ritidoplastias. *Cirugia Plastica Ibero-Latino Americana* 1984;10:321.
13. Ramirez OM. Extended Subperiosteal Facelift. Presented at the Xth Biannual Congress of the International Society of Aesthetic Plastic Surgery. Zurich, Switzerland. September 1989.
14. Vasconez LO, Core GB, Gamboa-Bobadilla M, Guzman G, Askren C, et al. Endoscopic techniques in coronal brow lifting. *Plast Reconstr Surg* 1994;94:788-93.
15. Ramirez OM. Anchor subperiosteal forehead lift: from open to endoscopic. *Plast Reconstr Surg* 2001;107:868-71.
16. Ramirez OM. Endoscopic techniques in facial rejuvenation. An overview: Part I. *Aesthetic Plast Surg* 1994;18:141-7.
17. Ramirez OM. Endoscopic full facelift. *Aesthetic Plast Surg* 1994;18:363-71.
18. Ramirez OM, Pozner JN. Subperiosteal minimally invasive laser endoscopic rhytidectomy: the SMILE facelift. *Aesthetic Plast Surg* 1996;20:463-70.
19. Ramirez OM, Pozner JN. Subperiosteal endoscopic techniques in secondary rhytidectomy. *Aesthet Surg J* 1997;17:22-6.

20. Ramirez OM, Pozner JN. Laser resurfacing as an adjunct to endo-forehead lift and biplanar facelift. *Ann Plast Surg* 1997;38:315-22.
21. Ramirez OM, Pozner JN. High-tech facelift. *Aesthetic Plast Surg* 1998;22:318-28.
22. Ramirez OM. Full face rejuvenation in three dimensions: a “face-lifting” for the new millennium. *Aesthetic Plast Surg* 2001;25:152-64.
23. Ramirez OM. Three-dimensional endoscopic midface enhancement: a personal quest for the ideal cheek rejuvenation. *Plast Reconstr Surg* 2002;109:329-40.
24. Ramirez OM. The central oval of the face: tridimensional endoscopic rejuvenation. *Facial Plast Surg* 2000;16:283-98.
25. Aiche AE, Ramirez OM. The suborbicularis oculi fat (SOOF): an anatomical and clinical study. *Plast Reconstr Surg* 1995;95:37-42.
26. Ramirez OM, Santamarina R. Spatial orientation of motor innervation to the lower orbicularis oculi muscle. *Aesthetic Surg J* 2000;107:13.
27. Knize DM. An anatomically based study of the mechanism of brow ptosis. *Plast Reconstr Surg* 1996;97:1321-33.
28. Ramirez OM. Fourth generation subperiosteal approach to the midface: the tridimensional functional cheek lift. *Aesthetic Surg J* 1998;8:133-5.
29. Ramirez OM. Buccal fat pad pedicle flap for midface augmentation. *Ann Plast Surg* 1999;43:109-18.
30. Carruthers A, Carruthers J. Aesthetic indications for botulinum toxin injections. *Plast Reconstr Surg* 1995;95:427-8.
31. Narins RS, Brandt F, Leyden J, Lorenc ZP, Rubin M, et al. A randomized, double-blind, multicenter comparison of the efficacy and tolerability of Restylane versus Zyplast for the correction of nasolabial folds. *Dermatol Surg* 2003;29:588-95.
32. Ramirez OM. What is wrong with aesthetic approach to facial volumization: a judgement to the hollywood legacy. Presented at the 54th Brazilian Congress of Plastic Surgery. Florianopolis, SC Brazil. November 15-18, 2017.
33. Ramirez OM, Volpe CR. Double ogee facial rejuvenation. In: Panfilov DE, editor. *Aesthetic surgery of the facial mosaic*. Berlin: Springer-Verlag; 2007. pp. 288-99.
34. Hurwitz DJ, Raskin EM. Reducing eyelid retraction following subperiosteal face-lift. *Aesthet Surg J* 1997;17:149-56.
35. Ramirez OM, Tezel E, Ersoy B. The Peruvian fisherman’s knot: a new, simple, and versatile self-locking sliding knot. *Ann Plast Surg* 2009;62:114-7.
36. Ramirez OM. Treating the aging face: “high definition-high tech” comprehensive facial rejuvenation. *MKG-Chirug* 2019;12:68-77.
37. Ramirez OM, Centurion P. Super rich stem cell-fat grafting procured by a novel laser technology: a paradigm shift in aesthetic surgery and regenerative medicine. Presented at the 2018 Bi-Annual ISAPS Congress. November, 2018, Miami, Florida.
38. Ramirez OM, Centurion P. Super Rich Stem Cell-Fat Grafting Procured by a Novel Laser Technology: A Paradigm Shift in Aesthetic Surgery and Regenerative Medicine. Abstract at the Annual Meeting of the American Society for Aesthetic Plastic Surgery. New Orleans, LA. May, 2019.
39. Ramirez OM. Rejuvenation of the Facial Expression: The Ultimate Paradigm Shift in Facial Surgery. Presented at the International Symposium, “All About Face 2019”, Curitiba, Brazil, August 2019.
40. Ramirez OM. Advanced considerations determining procedure selection in cervicoplasty. Part two: surgery. *Clin Plast Surg* 2008;35:691-709.
41. Ramirez OM. Multidimensional evaluation and surgical approaches to neck rejuvenation. *Clin Plast Surg* 2014;41:97-105.
42. Ramirez OM. Mandibular matrix implant system: a method to restore skeletal support to the lower face. *Plast Reconstr Surg* 2000;106:176-89.

Original Article

Open Access



# Cryopreserved fat: our clinical experience and applications

Masanori Ohashi

The Clinic Tokyo, Minato-ku, Tokyo 106-0031, Japan.

**Correspondence to:** Dr. Masanori Ohashi, The Clinic Tokyo, Nishiazabu 3-16-23 Minato-ku, Tokyo 106-0031, Japan.  
E-mail: ohashi@theclinic.jp

**How to cite this article:** Ohashi M. Cryopreserved fat: our clinical experience and applications. *Plast Aesthet Res* 2020;7:26.  
<http://dx.doi.org/10.20517/2347-9264.2020.15>

**Received:** 29 Jan 2020 **First Decision:** 25 Mar 2020 **Revised:** 27 Apr 2020 **Accepted:** 7 May 2020 **Published:** 23 May 2020

**Science Editor:** Jian-Xing Song **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

## Abstract

**Aim:** Cryopreservation of fat is an effective method for repeat fat grafting, but there are few reports about the clinical use of cryopreserved fat. The aim of this study was to determine the effectiveness and safety of cryopreserved fat for clinical use.

**Methods:** Between Aug 2015 and Dec 2018, we investigated 590 patients who underwent fat harvesting at our clinic. The harvested fat was cryopreserved at a temperature of -196 °C at a cell processing center and injections were performed in our clinic.

**Results:** Of the 590 patients studied, 216 (312 cases) have undergone fat injections so far. Volume augmentations using harvested fat, such as facial and breast augmentations, were performed on 180 patients. For 84 patients, harvested fat was utilized only for revitalization/fertilization purposes, such as to improve skin condition. There were no severe complications in any patients. However, volume maintenance was rarely observed. Skin rejuvenation effects were comparable to that in cases using fresh fat.

**Conclusion:** The clinical use of cryopreserved fat is thought to be safe and effective.

**Keywords:** Cryopreserved fat, frozen fat, fat grafting, skin rejuvenation



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



## INTRODUCTION

Fat grafting is used worldwide for volume augmentation. Recently, it has become clear that fat grafting has a regenerative effect (revitalization/fertilization) which leads to rejuvenation<sup>[1-6]</sup>.

However, one of the biggest problems of fat grafting is the unpredictable retention rate for volume augmentation. Additionally, skin rejuvenation effects vary<sup>[7]</sup>. Injections must often be repeated to achieve the targeted results<sup>[7-10]</sup>.

Another problem associated with fat grafting is the painful process of harvesting fat, which is also time-consuming for both patients and doctors. Accordingly, it would be advantageous to both patients and plastic/aesthetic surgeons if fat could be harvested during one procedure and cryopreserved for later use.

The cryopreservation method has been researched for a long time, and many doctors conclude that the use of cryopreserved fat is useful and safe when appropriate methods are used<sup>[11-13]</sup>. However, most of these studies are experimental, and documented clinical use of such fat is scarce<sup>[14,15]</sup>.

The aim of this study was to determine the safety and benefits of the clinical use of cryopreserved fat.

## METHODS

### Patients and methods

Patients: [Table 1](#).

From Aug 2015 to Dec 2018, we harvested fat from 490 patients and sent it to a cell processing center (CPC) (CellSource Co., Ltd., Tokyo, Japan) for cryopreservation at -196 °C.

### Flowchart of harvesting, cryopreserving, and repeat injection

At our clinic, we harvest patients' fat for same-day use. The residual fat is sent to the CPC. The CPC (CellSource Co.) cryopreserves the fat using their own cryoprotective agent at -196 °C. The company will then send back the thawed fat to our clinic for repeat injections [[Figure 1](#)].

### Anesthesia

Usually, we use a combination of local anesthesia with tumescent technique and intravenous anesthesia.

### Harvest in the clinic

When harvesting the patients' fat, we use a tumescent technique (20 mL of 8.4% sodium hydrogen carbonate, 1 mL of epinephrine, and 50 mL of 1.0% lidocaine per 1000 mL of saline) with suction pressure of less than 1 atm.

### Choice of donor site

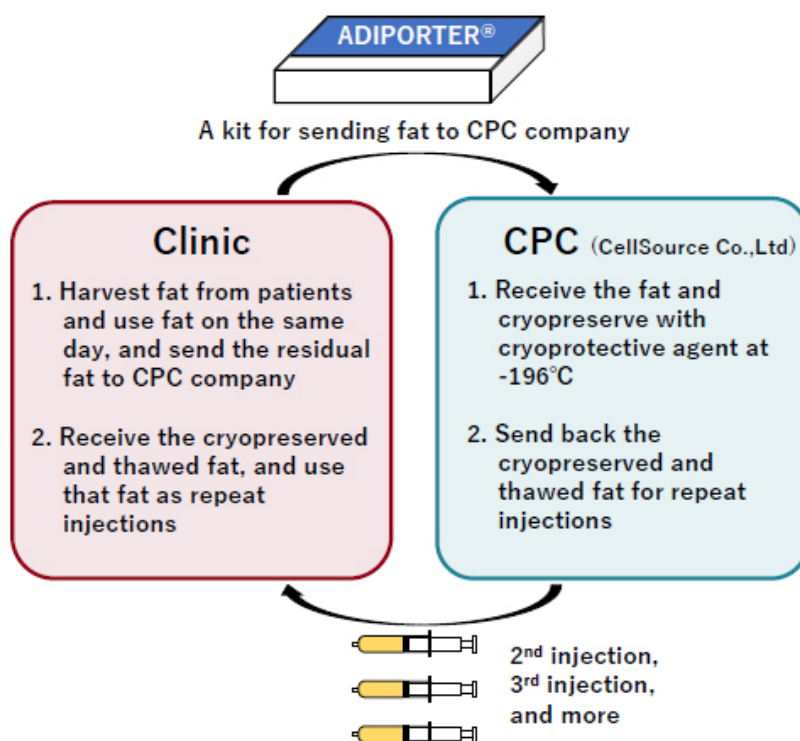
Fat is typically harvested from the thighs, lower abdomen, and flanks (so-called LFD: localized fat deposit)<sup>[17]</sup>, because these areas bleed the least and are the easiest sites from which to harvest fat.

### First injection at the clinic

We usually inject the fat on the same day it is harvested. Fat grafting is used not only for volume augmentation but also for regenerative effects such as skin rejuvenation. When we use the fat for volume augmentation, we apply the so-called "Coleman technique", and for skin rejuvenation, we use the nanofat or squeezed fat technique<sup>[5,6]</sup>.

**Table 1. Characteristics of patients who had their fat cryopreserved ( $n = 490$ )**

Duration	Aug 2015 - Dec 2018
Sex	Male 26 Female 464
Age (years)	19-81 ( $40.9 \pm 11.6$ )
Height (cm)	142-188 ( $160.0 \pm 5.8$ )
Weight (kg)	37.6-90.7 ( $52.1 \pm 8.0$ )
Body mass index ( $\text{kg}/\text{m}^2$ )	15.0-33.5 ( $20.3 \pm 2.5$ )

**Figure 1.** Flowchart of cryopreservation and fat grafting. This figure is used with permission from Ohashi published in *Clin Plast Surg*<sup>[16]</sup>

The main injection sites for volume augmentation are the breasts and the face (such as the forehead and cheeks). For skin rejuvenation, the main injection sites are the lower eyelids, the area surrounding the lips, and areas with signs of facial aging, e.g., wrinkles. Sometimes fat grafting is used for scar treatment such as in cases of double eyelids, liposuction revision, or contracture release in percutaneous aponeurotomy (so-called rigotomy).

### How to send the fat

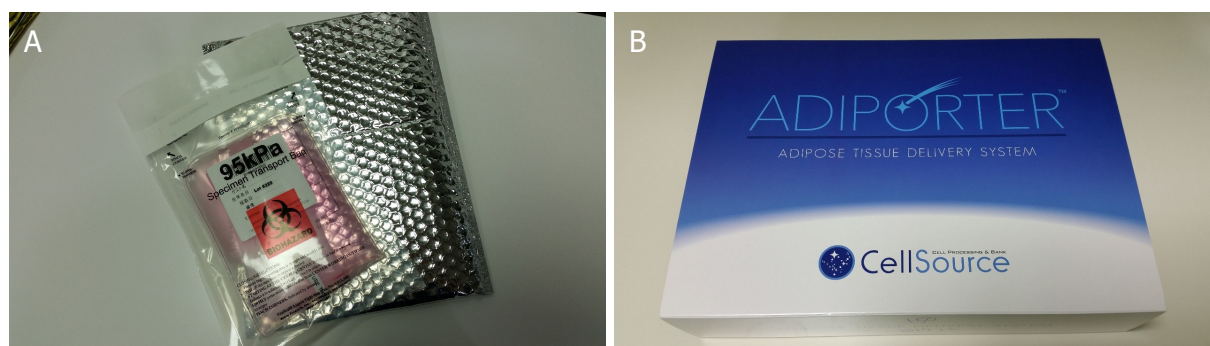
After fat is utilized on the day of harvest, the remaining fat is collected in a bag (FB-bag: CellSource Co.) which is then packed in an ADIORTER box (CellSource Co., Ltd., Tokyo, Japan). After packing, the bag is sent to the CPC at CellSource Co. in a refrigerated state (below  $10^{\circ}\text{C}$ ) [Figure 2].

### Cryopreservation and storage of fat

The process of cryopreservation and storage at  $-196^{\circ}\text{C}$  was performed in the CPC at CellSource Co.

The details of this process are confidential matters for the company. Roughly speaking, delivered fat is washed using Ringer's lactate solution. The fat is mixed with a cryoprotectant and divided into 4- to 5-mL aliquots in syringes. These syringes are gradually frozen at a controlled rate until the temperature reaches





**Figure 2.** Kit for transportation. A: FB-bag (CellSource Co., Ltd., Tokyo, Japan), which contains an adipose tissue transport medium; B: Adiporter (CellSource Co., Ltd., Tokyo, Japan), which is the box in which the fat is sent to the cell processing center company in a refrigerated state (below 10 °C). This figure is used with permission from Ohashi *et al.*<sup>[18]</sup> published in *Plast Reconstr Surg Glob Open*



**Figure 3.** Thawed cryopreserved fat. This photograph was taken at our clinic. The material looks like fresh fat, and there is almost no oil in those syringes. This figure is used with permission from Ohashi *et al.*<sup>[18]</sup> published in *Plast Reconstr Surg Glob Open*

-80 °C. They are then transferred to liquid nitrogen and stored at -196 °C.

Using this process, we can store many syringes of patients' fat after single harvesting sessions.

### Return of fat

When we want to use cryopreserved fat, we use an Internet web-ordering service from the CellSource CPC to place an order. After the CPC receives the order, the patient's cryopreserved fat is thawed rapidly (37 °C) and the cryoprotectant liquid is washed out. The CPC sends back that fat to our clinic in a refrigerated state (below 10 °C) [Figure 3].

### Injection of thawed cryopreserved fat

The thawed fat must be injected within 48 hours of receiving it. We follow the “Coleman technique” for volume augmentation, and use the “nanofat (emulsified fat) technique” for skin rejuvenation. The fat is usually used at multiple injection sites. For example, the first injection of cryopreserved fat may be used for breast augmentation and the second injection for forehead volume augmentation and/or scar treatment. We may use the cryopreserved fat many times if the supply lasts. Therefore, we can inject each patients many times easily.

**Table 2. Characteristics of patients who received their cryopreserved fat (n = 216)**

Duration	Aug 2015 - Dec 2018
Sex	Male 14 Female 202
Age (years)	19-79 (40.8 ± 11.5)
Height (cm)	147.5-188 (160.0 ± 5.9)
Weight (kg)	38.5-90.7 (52.1 ± 8.1)
Body mass index (kg/m <sup>2</sup> )	15.9-31.6 (20.3 ± 2.5)

**Table 3. Number of patients with different injection times using cryopreserved fat from one harvesting**

1 time	216 patients
2 times	59 patients
3 times	25 patients
4 times	10 patients
5 times	1 patient
6 times	1 patient

**Table 4. The ways cryopreserved fat was used**

Volume augmentation only - 169 cases	
Facial rejuvenation	143 cases
Body augmentation	26 cases
Revitalization/fertilization only - 98 cases	
Improve skin condition only	11 cases
Scar only	55 cases
Improve skin condition and for scar	32 cases
Volume augmentation + revitalization/fertilization	87 cases
(Treatment for scar & fibrous tissue with/without PALF)	
Face (Injury scar, revision of liposuction, acne scar)	53 cases
Revision of liposuction (thigh, abdomen)	19 cases
Revision of SIEF	5 cases
Incision scar (IMF, nipple, axillar)	10 cases

SIEF: simultaneous implant exchange with fat; PALF: percutaneous aponeurotomy and lipofilling

### Follow-up of patients

We followed-up each patient after at one month and 3-6 months after the first injection using fresh fat, and also at one month and 3-6 months after repeat injections using thawed cryopreserved fat.

### Comparison of stromal vascular fraction

We compared the amount of stromal vascular fraction (SVF) in fresh fat before sending it to the CPC with the SVF of thawed cryopreserved fat. SVF was digested by collagenase (Wako Pure Chemical, Osaka, Japan). Cell count and viability of SVF were performed by KUNA-STEAM Automated Fluorescence Cell Counter (Logos Biosystems, South Korea).

## RESULTS

Of the 490 patients who underwent fat harvesting in our clinic, 216 patients (312 cases) received fat grafting with cryopreserved fat. The characteristics of those patients are shown in Table 2. The number of patients who received a varying number of injections from one harvesting session is shown in Table 3. The ways of using cryopreserved fat are shown in Table 4. The injected volume of cryopreserved fat was from 0.2 to 24.0 mL for the face and from 4.0 to 100.0 mL for the body.

There were no severe complications in any patients. Mild complications occurred in 5 patients (2.3%), all of which were temporary pigmentation [Table 5].

**Table 5. Complication**

Severe or moderate complication*	0 patients (0%)
Mild complication**	5 patients (1.9%)

\*For example, infection and fat necrosis; \*\*only temporary pigmentations (inflammatory pigmentation and scars from needling)

**Table 6. Volume change of fat after cryopreservation**

	Not centrifuged	Centrifuged
Sent volume	169 mL $\pm$ 72.7 mL	143 mL $\pm$ 67.9 mL
Returned volume	59 mL $\pm$ 29.2 mL	76 mL $\pm$ 43.9 mL
% volume	34.4% $\pm$ 5.5%	51.3% $\pm$ 9.8%

Not centrifuged: only gravity; centrifuged: usually 700 - 1200 g, 3 min. Table 6 is used with permission from Ohashi *et al.*<sup>[18]</sup> published in *Plast Reconstr Surg Glob Open*

A comparison of sent and returned fat volumes showed that fat volume decreased 34.4% if it was not centrifuged before being sent, and decreased 51.3% if centrifuged before sending [Table 6].

SVF in fresh fat was  $7.1 \times 10^5$ /mL (before sending) and  $14.8 \times 10^5$ /mL in thawed cryopreserved fat (returned fat) ( $n = 5$ ). These amounts reflect the amounts of SVF/mL that were concentrated from cryopreserved fat as compared to fresh fat.

### We show some cases below

#### Case 1: facial rejuvenation using cryopreserved fat [Figure 4]

A 46 y.o. patient disliked her bony face and wanted to look younger. She did not want to undergo painful harvesting many times. Therefore, we planned serial injections after one harvesting session followed by the cryopreservation of her fat. She received facial rejuvenation surgery (first operation) involving fat grafting to her forehead (20.0 mL), cheeks (8.0 mL each), and lips (1.5 mL each) with thread lift (Silhouette Soft; Sinclair Pharma, London, UK) using the bidirectional floating method for her sagging cheeks, and her residual fat was sent for cryopreservation. After her first operation, she received two more fat grafting procedures using her cryopreserved fat within one year (three and six months after her first operation). She looked younger and healthy after these operations.

#### Case 2: facial rejuvenation using cryopreserved fat [Figure 5]

A 47 y.o. patient hated her bony forehead and complained of looking older than her real age. Her first operation involved facial fat grafting to her forehead (22.0 mL), malar area (3.0 mL each), cheeks (5 mL each), upper and lower eyelids (1.0 mL each, and 1.5 mL each, respectively) and chin (2.3 mL) with lower orbital fat removal. After her first operation, she received fat grafting using her cryopreserved fat two more times within two years (6 and 18 months after first operation). Postoperative photographs [Figure 5] are one year after her last fat grafting procedure using cryopreserved fat (3.5 years after her first injection), where she appears more youthful than she did prior to her first operation.

#### Case 3: repeat rigotomy (needling) using cryopreserved fat [Figure 6]

A 41 y.o. patient had undergone breast implant removal and simultaneous fat grafting (SIEF)<sup>[19,20]</sup>. However, three months after the operation, her right breast became deformed due to capsule contracture. Therefore, we performed a rigotomy (percutaneous aponeurotomy) with fat grafting using fresh fat followed by serial injections using cryopreserved residual fat. After receiving five total rigotomies with fat grafting (two with fresh fat, three with cryopreserved fat), her breasts developed a nearly natural appearance.

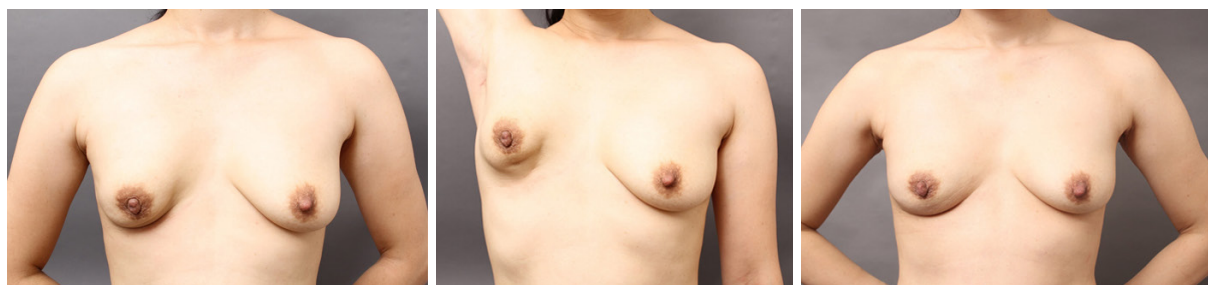


**Figure 4.** case 1: facial rejuvenation using her cryopreserved fat. A: front view B: diagonal view. (left) Preoperative. (right) 6 months after second cryopreserved fat grafting. This figure is used with permission from Ohashi *et al.*<sup>[18]</sup> published in *Plast Reconstr Surg Glob*



**Figure 5.** Case 2: facial rejuvenation using her cryopreserved fat. A: front view B: diagonal view. (left) Preoperative. (right) 3.5 years after her first injection. This figure is used with permission from Ohashi published in *Clin Plast Surg*<sup>[16]</sup>





**Figure 6.** Csae 3: repeat rigotomy (needling) using cryopreserved fat. (left) after 3 months postoperative follow-up. Her right side residual capsule was greatly shrunken; (middle) her appearance when she holds up her right arm; (right) after receiving a total of five rigotomies with fat grafting (two with fresh fat, three with cryopreserved fat). This figure is used with permission from Ohashi *et al.*<sup>[18]</sup> published in *Plast Reconstr Surg Glob Open*



**Figure 7.** Case 4: hand rejuvenation using residual fat. (left) Before operation. (right) After injection of cryopreserved fat, veins covered with fat and unremarkable. This figure is used with permission from Ohashi *et al.*<sup>[18]</sup> published in *Plast Reconstr Surg Glob Open*

#### Case 4: hand rejuvenation using residual fat [Figure 7]

A 65 y.o. woman received fat grafting for breast augmentation and requested her cryopreserved residual fat be used as well. Four months after the first operation, she received fat grafting in her hands (16.0 mL for each hand) for hand rejuvenation without any additional harvesting needed.

## DISCUSSION

Fat grafting is a major procedure for volume augmentation of areas such as the breasts, buttocks, and face. It has become clear that there are other merits of fat grafting including but not limited to skin rejuvenation, improve of fibrous scars, burn relief, alleviation of scleroderma symptoms, and healing of radiation damage. This is due to the revitalization/fertilization and regenerative effects of fat<sup>[4,7,21,22]</sup>.

However, one of the biggest demerits of fat grafting is the unpredictable nature of results involving varying maintenance rates for volume augmentation and the unpredictable degree of revitalization/fertilization and regeneration effects.

Due to varying effects, we often administer repeat injections to achieve satisfactory results. But repeat harvesting of fresh fat has detrimental impacts on patients with regard to pain and high costs. Therefore, it is in the interest to both aesthetic/plastic surgeons and patients to be able to preserve and use cryopreserved fat.

There are concerns regarding the viability of cryopreserved fat<sup>[23,24]</sup>. Many authors have recently suggested that slow cooling and fast thawing with cryoprotective agents may improve the viability of stored fat to a degree comparable to that of fresh fat<sup>[25-27]</sup>.



Improper cryopreservation techniques may compromise fat viability; however, with adequate cryopreservation techniques such as slow cooling and fast thawing, preservation at -196 °C (below -85 °C) and the addition of cryoprotective agents foster high fat viability<sup>[27-29]</sup>.

We trust the CPC (CellSource Co. in Tokyo) to use proper cryopreservation techniques. Our responsibilities, therefore, only include packaging and sending residual fat. To use the cryopreserved fat, we then recall the fat [refer to [Figure 1](#)]. This ordering system allows small clinics to use cryopreserved fat without needing high-cost cell processing facilities on site. This makes it easier for clinics to start using cryopreserved fat safely while ensuring the stable quality of the fat. Having specialists conduct cell processing also benefits patients by preserving the quality of their fat for repeat injections.

Regarding volume and SVF count of cryopreserved fat, our results showed that the volume of fat decreased after cryopreservation; however, the amount of SVF per mL increased. This may be attributed to the fact that SVF (and adipose-derived stromal cells) is stronger than normal adipocytes in response to stress from factors such as ischemia, mechanical damage (during transportation), and cryopreservation<sup>[6]</sup>. According to our results, cryopreserved fat is very useful for rejuvenation and fertilization/revitalization.

Lastly, our cases indicated that cryopreserved fat has comparable viability as fresh fat; however, further studies are needed to compare the retention rate and regeneration effects of fresh and cryopreserved fat in a pathological study.

In summary, we did not experience any severe complications in any of our 216 patients over three and half years. Our results indicated that fat grafting with cryopreserved fat closely mimics that with fresh fat, making cryopreserved fat a safe and useful source for repeat fat grafting injections.

## **DECLARATIONS**

### **Acknowledgments**

We thank Hidato Kaneshima, MD, PhD, Satoshi Tsunoda, and Syunsuke Tazumi from CellSource Co., Ltd. for providing methods for cryopreservation and thawing.

### **Authors' contributions**

The author contributed solely to the article.

### **Availability of data and materials**

Not applicable.

### **Financial support and sponsorship**

None.

### **Conflicts of interest**

The author declared that there are no conflicts of interest.

### **Ethical approval and consent to participate**

In Japan, the Regenerative Medicine Safety Act came into effect as of November 25, 2014 under an institutional framework for promoting the implementation of regenerative medicine. This act, which covers clinical research and private practice, stipulates three risk-dependent standards and the procedures for notification of plans for regenerative medicine as well as the standards of cell culture and processing facilities and the licensing procedures to ensure the safety of regenerative medicine.

## Consent for publication

The author obtained consent for publication from all patients we show in this article.

## Copyright

© The Author(s) 2020.

## REFERENCES

- Coleman SR. Structural fat grafts: the ideal filler? *Clin Plast Surg* 2001;28:111-9.
- Coleman SR. Structural fat grafting: more than a permanent filler. *Plast Reconstr Surg* 2006;118:108S-20S.
- Khouri RK, Rigotti G, Cardoso E, Khouri RK Jr, Biggs TM. Megavolume autologous fat transfer: part I. Theory and principles. *Plast Reconstr Surg* 2014;133:550-7.
- Khouri RK, Smit JM, Cardoso E, Pallua N, Lantieri L, et al. Percutaneous aponeurotomy and lipofilling: A regenerative alternative to flap reconstruction? *Plast Reconstr Surg* 2013;132:1280-90.
- Tonnard P, Verpaele A, Peeters G, Hamdi M, Cornelissen M, et al. Nanofat grafting: basic research and clinical applications. *Plast Reconstr Surg* 2013;132:1017-26.
- Mashiko T, Wu SH, Feng J, Kanayama K, Kinoshita K, et al. Mechanical micronization of lipoaspirates: Squeeze and emulsification techniques. *Plast Reconstr Surg* 2017;139:79-90.
- Sautereau N, Dumas A, Truillet R, Jouve E, Magalon J, et al. Efficacy of autologous microfat graft on facial handicap in systemic sclerosis patients. *Plast Reconstr Surg Glob Open* 2016;4:e660.
- Losken A, Pinell XA, Sikoro K, Yezhelyev MV, Anderson E, et al. Autologous fat grafting in secondary breast reconstruction. *Ann Plast Surg* 2011;66:518-22.
- Gatti JE. Permanent lip augmentation with serial fat grafting. *Ann Plast Surg* 1999;42:376-80.
- Kim HY, Jung BK, Lew DH, Lee DW. Autologous fat graft in the reconstructed breast: Fat absorption rate and safety based on sonographic identification. *Arch Plast Surg* 2014;41:740-7.
- Pu LLQ, Coleman SR, Cui X, Ferguson REH Jr, Vasconez HC. Cryopreservation of autologous fat grafts harvested with the Coleman technique. *Ann Plast Surg* 2010;64:333-7.
- Gir P, Brown SA, Oni G, Kashefi N, Mojallal A, et al. Fat grafting: evidence-based review on autologous fat harvesting, processing, reinjection, and storage. *Plast Reconstr Surg* 2012;130:249-58.
- Pu LLQ, Cui X, Fink BF, Gao D, Vasconez HC. Adipose aspirates as a source for human processed lipoaspirate cells after optimal cryopreservation. *Plast Reconstr Surg* 2006;117:1845-50.
- Ibrahiem SMS, Farouk A, Salem IM. Facial rejuvenation: serial fat graft transfer. *Alexandria J Med* 2016;52:371-6.
- Butterwick KJ, Bevin AA, Iyer S. Fat transplantation using fresh versus frozen fat: a side-by-side two-hand comparison pilot study. *Dermatol Surg* 2006;32:640-4.
- Ohashi M. Fat grafting for facial rejuvenation with cryopreserved fat grafts. *Clin Plast Surg* 2020;47:63-71.
- Geissler PJ, Davis K, Roostaeian J, Unger J, Huang J, et al. Improving fat transfer viability: the role of aging, body mass index, and harvest site. *Plast Reconstr Surg* 2014;134:227-32.
- Ohashi M, Chiba A, Nakai H. Serial injections of cryopreserved fat at -196 °C for tissue rejuvenation, scar treatment, and volume augmentation. *Plast Reconstr Surg Glob Open* 2018;6:e1742.
- Del Vecchio DA. "SIEF" - simultaneous implant exchange with fat: a new option in revision breast implant surgery. *Plast Reconstr Surg* 2012;130:1187.
- Ohashi M, Yamakawa M, Chiba A, Nagano H, Nakai H. Our experience with 131 cases of simultaneous breast implant exchange with fat (SIEF). *Plast Reconstr Surg Glob Open* 2016;4:e691.
- Khouri RK Jr, Khouri RK. Current clinical applications of fat grafting. *Plast Reconstr Surg* 2017;140:466e-86e.
- Rigotti G, Marchi A, Galiè M, Baroni G, Benati D, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. *Plast Reconstr Surg* 2007;119:1409-22.
- Pu LLQ, Cui X, Fink BF, Cibull ML, Gao D. Long-term preservation of adipose aspirates after conventional lipoplasty. *Aesthet Surg J* 2004;24:536-41.
- Wolter TP, von Heimburg D, Stoffels I, Groeger A, Pallua N. Cryopreservation of mature human adipocytes: In vitro measurement of viability. *Ann Plast Surg* 2005;55:408-13.
- Jeon IK, Lee H, Shin JY, Oh SH. Cryopreserved autologous fat injections as a filler agent for facial augmentation: are they still safe? *Yosei Med J* 2014;55:280-1.
- MacRae JW, Tholpady SS, Ogle RC, Morgan RF. Ex vivo fat graft preservation: effects and implications of cryopreservation. *Ann Plast Surg* 2004;52:281-2.
- Pu LL. Cryopreservation of adipose tissue. *Organogenesis* 2009;5:138-42.
- Hwang SM, Lee JS, Kim HD, Jung YH, Kim HI. Comparison of the viability of cryopreserved fat tissue in accordance with the thawing temperature. *Arch Plast Surg* 2015;42:143-9.
- Shu Z, Gao D, Pu LLQ. Update on cryopreservation of adipose tissue and adipose-derived stem cells. *Clin Plast Surg* 2015;42:209-18.

Case Report

Open Access



# Role of limited access dressing in achieving improved aesthetic results during resurfacing of wounds

Pramod Kumar

Department of Plastic surgery, King Fahad Central Hospital, Jazan 82666, Saudi Arabia.

**Correspondence to:** Dr. Pramod Kumar, Department of Plastic Surgery, King Fahad Central Hospital, Jazan 82666, Saudi Arabia.  
E-mail: pkumar86@hotmail.com

**How to cite this article:** Kumar P. Role of limited access dressing in achieving improved aesthetic results during resurfacing of wounds. *Plast Aesthet Res* 2020;7:27. <http://dx.doi.org/10.20517/2347-9264.2020.07>

**Received:** 11 Jan 2020 **First Decision:** 14 Apr 2020 **Revised:** 18 Apr 2020 **Accepted:** 6 May 2020 **Published:** 27 May 2020

**Science Editor:** Raúl González-García **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

With refinement and better understanding of Plastic Surgery, there is increasing expectation of aesthetic outcomes after resurfacing of wounds. The major problems in resurfacing procedures are tissue bulk, donor site issues, excessive scarring and distal edema due to damaged lymphatics and veins after flap harvest from adjacent areas in the extremities. Ultra-conservative debridement simplifies reconstruction by reducing the need for flaps and improves the chances of skin graft take through limited access dressing, which can improve the final aesthetic result following reconstruction. In this paper, we describe three representative cases treated under limited access dressing.

**Keywords:** Aesthetic reconstruction, limited access dressing, LAD

## INTRODUCTION

If reconstructive surgery restores a defect to a normal looking appearance, aesthetic surgery then surpasses normal. Aesthetic surgery is fascinating because it improves the appearance and makes it pleasing to the observer's eye. After resurfacing procedures, commonly observed problems include a bulky reconstructed part<sup>[1,2]</sup>, scarring and/or defects over the donor and recipient sites, and distal edema in the affected extremities. Reconstructive surgeons have achieved reasonable aesthetic results in reconstruction by utilizing super-thin flaps, reducing donor site scarring by harvesting small islanded flaps, avoiding skin grafting by using rotational/Limberg flaps *etc.* Hence, in recent years the emphasis has switched towards improving the appearance, texture and better color match in reconstruction.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Limited Access Dressing (LAD)<sup>[3,4]</sup> is a combination of moist wound healing and negative pressure dressing. Being a dressing technique, it has its own limitations in improving aesthetic appearance. LAD helps in aesthetic reconstruction by simplifying reconstruction with minimal donor area problems and reducing complications due to infection, scarring *etc.* This is achieved in the following ways:

#### **Ultra conservative debridement**

LAD delivers ultraconservative debridement to conserve viable tissue maximally. It does so through autolytic debridement by tissue enzymes and mechanical debridement by negative pressure and LAD wash. Due to the leech effect, it is possible to keep the wound slough for longer periods with reduced or no risk of SIRS/sepsis. During this waiting period, the living cells proliferate and tissue enzymes separate the attachment of slough to the living tissues. Hence, under LAD, all living tissue is preserved while dead tissue becomes separated. In contrast, when surgery is performed without LAD, much live tissue is removed and depending on the area operated on, if bones or tendons are exposed, reconstruction becomes more difficult with relatively more scarring and poorer aesthetic results.

**Debridement and LAD:** LAD is applied after thorough mechanical wash and surgical removal of dead tissue. After about 1 week (the actual time required is determined by the appearance of soft and relatively loose slough), if deemed necessary, the wound is debrided again. During debridement, slough is removed easily with minimal blood loss. In cases with compound, comminuted fractures, bone pieces should be preserved, as much as possible, to increase the chances of survival while the wound undergoes LAD.

#### **Minimal scarring**

It has been claimed that occlusive dressings promote rapid wound healing by preventing dehydration and scab formation, facilitating debridement, minimizing the chances of inflammation and infection, reducing pain, increasing the rate of epithelialization, and thus, diminishing scarring.

#### **Less complications after major reconstruction**

After flap cover, intermittent pressure reduces the chances of hematoma formation and venous drainage is improved, thereby reducing the chances of flap failure. It also reduces the chances of infection and inflammation, which in turn, reduces scarring.

#### **Simpler reconstructive procedure**

When treated under LAD, majority of cases can avoid complex reconstruction and split thickness skin grafting (SSG) is usually sufficient. Also, skin graft take is 95%-100% and this helps to reduce the chances of a bulky reconstruction and outcome.

#### **Minimal donor deformity**

As the majority of cases can be covered by SSGs, the expected donor area deformity is thus minimal when compared to flaps. Treatment of the donor area under LAD further reduces the chances of donor site complications.

#### **Less chance of distal edema**

In lower extremity reconstruction, when more than half the circumference is damaged or avulsed and only the flap donor area is intact, further disruption of lymphatics and veins in the flap donor site, if distally based flaps are used, may lead to pedal edema distally. The use of SSGs instead of flaps after LAD reduces or avoids further disruption of the drainage system, which reduces the risk of developing such edema.

#### **Avoiding amputation/major amputation/limb salvage**

On several occasions, it has been possible to avoid limb amputation by using LAD. If aesthetic outcome is the only concern, avoiding major amputation would be an aesthetic gain.

**Table 1. Advantages and disadvantages of limited access dressing in achieving aesthetic results**

Advantages <sup>[3-7]</sup>	Disadvantages <sup>[3]</sup>
<ol style="list-style-type: none"> <li>1. Ultraconservative debridement</li> <li>2. Minimal scarring</li> <li>3. Reduces flap failure, wound inflammation and infection</li> <li>4. Simpler reconstructive procedure required (graft vs. flap)</li> <li>5. Minimal donor site deformity</li> <li>6. Reduces chances of distal lymphedema</li> <li>7. Increases chances of limb salvage</li> </ol>	<ol style="list-style-type: none"> <li>1. More time required for treatment if not assisted by surgery</li> <li>2. Malodor of occlusive dressing requires limited access dressing wash</li> <li>3. Risk of pressure necrosis, especially in ischemic limbs</li> <li>4. Hemorrhage</li> </ol>

## Disadvantages

### *Time taken*

Preparation of the wound bed through LAD is faster than other dressing methods, especially if combined with surgical treatment. In cases of gangrene, if there was no surgical debridement, the wound may take 1-1 and 1/2 months.

### *Odor*

The malodor of occlusive dressings is usually taken care of by LAD wash and change of soaked dressings at the site of inadequate sealing with leaks.

### *Pressure necrosis*

Tight bandaging at the site where the tube emerges between the skin surface and the LAD bag, or a tight LAD bag may lead to pressure necrosis of the wound. Our improved LAD design has been effective in preventing such complications.

### *Hemorrhage*

The chances of hemorrhage can be reduced by achieving complete hemostasis prior to application of LAD, adjusting negative pressure, and placing the suction tubing in the folds of plastic, similar to that of a mesentery.

The advantages and disadvantages of LAD are summarized in [Table 1](#).

## CASE REPORT

### Case 1

This is a case of a brachial artery injury in an 18 year old boy that was reconstructed in the emergent setting with a Gortex implant by the vascular surgeon. He underwent LAD immediately after repair [[Figure 1](#)]. After 20 days of LAD, the implant was covered by granulation tissue from adjacent muscle and soft tissue. On day 28, wound resurfacing was achieved by SSG and the patient was discharged on day 38 with 100% graft take. At 5 months' follow-up, there were no issues with the SSG.

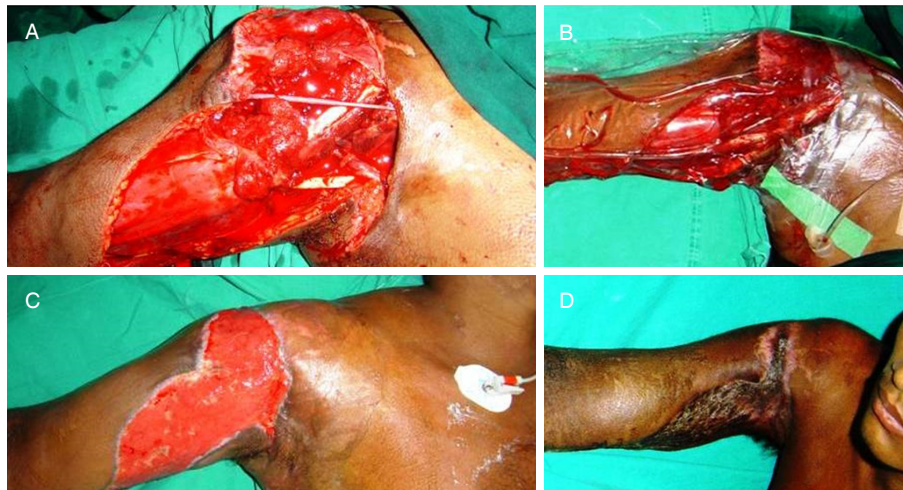
### Case 2

A 26 year old female was referred from General Surgery for wound coverage following debridement for necrotizing fascitis with exposure of the lateral aspect of the leg, knee, and lateral side of the thigh with exposed biceps femoris tendon [[Figure 2](#)]. The proximal part of the wound was closed primarily and the rest were treated under LAD. 12 days later, the patient underwent SSG under LAD. After another 10 days, the LAD was removed with 100% graft take and the patient was discharged. At 6 months' follow up, the skin graft was aesthetically acceptable and the affected limb did not develop distal pedal edema.

### Case 3

A 32 year old male was admitted with trauma to the right knee following a road traffic accident. Examination revealed a 7 cm × 4 cm wound over the extensor aspect of the knee joint with exposure of the lower half of





**Figure 1.** Photographs of case 1 showing: A: exposed Gortex vascular implant; B: limited access dressing covering the wound; C: granulation covering implant after 20 days; D: 3 months after resurfacing by skin graft



**Figure 2.** Photographs of case 2 showing: A: extensive wound following debridement for necrotizing fascitis; B: wound preparation under limited access dressing; C: final result after resurfacing without development of distal edema

the patella, upper part of the patellar ligament, and subpatellar fat pad [Figure 3]. After thorough cleaning and minimal wound debridement, the defect was partially closed with sutures as complete closure was not possible, due to insertion of a tube drain and tubular LAD along with application of a posterior plaster of Paris slab. The LAD was changed after 10 days and final wound closure was achieved under slight tension. After 20 days, the patient was discharged with linear healing of the wound at the suture site [Figure 3C]. After 1 month, the patient was advised for physiotherapy. At 3 months' follow-up, the wound had healed well with mild hypertrophy and full range of motion of the knee.

## DISCUSSION

### Case 1

Major options available in this case were pedicled flaps including the latissimus dorsi flap, subscapular flap and lateral thoracic flap<sup>[8]</sup>. These flaps provide relative bulk to the recipient site compared to a SSG and come with donor site related problems such as compromised function and scarring. By using LAD, it was possible to achieve granulation over the Gortex implant and later, definitive coverage with a SSG in 28 days



**Figure 3.** Photographs of case 3 showing: A: post-traumatic defect over knee; B: limited access dressing applied over the wound (see text for detail); C: 20 days post-limited access dressing result; D: result after 3 months

with a better and less bulky aesthetic result. At centers with microsurgical capabilities, free flap surgery can be expected to provide a better cosmetic result in a shorter time. But in the absence of such facilities and expertise, LAD is more reliable and a better alternative for a better aesthetic result. Also, the patient required physiotherapy in the post-operative period. There was minimal wound contracture after 6 months and no corrective surgery was required.

### Case 2

In this case, ultraconservative debridement<sup>[4]</sup> under LAD avoided further tissue loss and intermittent negative pressure with moist healing promoted granulation tissue formation over the wound bed and exposed tendon. Generally, skin flaps from the remaining half of the circumference of the affected limb may cause damage to the remaining lymphatics and result in intractable lymphedema. Successful resurfacing with SSG provided an acceptable result without distal edema and limb contracture.

### Case 3

Common flaps available for coverage of the exposed patella and patellar tendon are the medial gastrocnemius, extended myocutaneous or fasciocutaneous flap<sup>[9]</sup>. All these flaps cause significant donor site defects, which could easily be avoided by using LAD. In the present case, the reconstruction result was not bulky and did not have any donor defect or distal edema. The exposed patella and patellar tendon were covered by granulation tissue under the moist environment of LAD. Approximating sutures were applied to bring the skin edges together and the gap in between epithelialized.

The above cases suggest that LAD may be a useful tool in achieving a higher level of aesthetic outcomes in the resurfacing of extensive or difficult to treat wounds by avoiding bulky flaps, significant donor site defects, and distal edema due to compromised drainage from harvesting large regional flaps.

## DECLARATIONS

### Authors' contributions

The author contributed solely to the article.

### Availability of data and materials

Not applicable.

**Financial support and sponsorship**

None.

**Conflicts of interest**

The author declared that there are no conflicts of interest.

**Ethical approval and consent to participate**

Informed consent to participate in the study was obtained from the patients.

**Consent for publication**

A written informed consent for publication was obtained from the patients.

**Copyright**

© The Author(s) 2020.

**REFERENCES**

1. Kim TG, Choi MK. Secondary contouring of flaps. *Arch Plast Surg* 2018;45:319-24.
2. Wei FC, Mardini S. Flap and reconstructive surgery. 2nd ed. Amsterdam: Elsevier; 2019.
3. Kumar P. Exploiting potency of negative pressure in wound dressing using limited access dressing and suction-assisted dressing. *Indian J Plast Surg* 2012;45:302-15.
4. Kumar P. Advanced wound care using limited access dressing (LAD). *JSWCR* 2015;8:1-3.
5. Kumar P. Diabetic foot salvage by limited access dressing (LAD). *J Diabetes Metab* 2014;5:365.
6. Kumar P. Limited access dressing and wound infection. *Plast Aesthet Res* 2015;2:237-8.
7. Kumar P. Limited Access Dressing. *Wounds* 2008;20:49-59.
8. Management of axillary defect of hidradenitis suppurativa using keystone design islanded perforator flap--a simple and durable option. The Free Library. 2018 Akshantala Enterprises Private Limited 10 Jan. 2020. Available from: <https://www.thefreelibrary.com/Journal+of+Evolution+of+Medical+and+Dental+Sciences/2018/January/8-p54797> [Last accessed on 15 May 2020]
9. Gravvanis A, Kyriakopoulos A, Kateros K, Tsoutsos D. Flap reconstruction of the knee: a review of current concepts and a proposed algorithm. *World J Orthop* 2014;5:603-13.

Review

Open Access



# Stem cells and tissue engineering in plastic surgery: an update

Gregory R. D. Evans, Alan D. Widgerow

Department of Plastic Surgery, University of California, Irvine, Orange, CA 92868, USA.

**Correspondence to:** Prof. Gregory R. D. Evans, Department of Plastic Surgery, University of California, Irvine, 200 S Manchester Suite 650, Orange, CA 92868, USA. E-mail: [gevans@hs.uci.edu](mailto:gevans@hs.uci.edu)

**How to cite this article:** Evans GRD, Widgerow AD. Stem cells and tissue engineering in plastic surgery: an update. *Plast Aesthet Res* 2020;7:28. <http://dx.doi.org/10.20517/2347-9264.2019.53>

**Received:** 7 Nov 2019 **First Decision:** 18 Feb 2020 **Revised:** 19 Feb 2020 **Accepted:** 6 Mar 2020 **Published:** 30 May 2020

**Science Editors:** Yi-Lin Cao, Raúl González-García **Copy Editor:** Jing-Wen Zhang **Production Editor:** Tian Zhang

## Abstract

Stem cells and tissue engineering have made great strides in plastic surgery. This review of the literature evaluates some current background information and recent advances in our laboratory to bring these areas more into the clinical setting.

**Keywords:** Stem cells, plastic surgery, fat

## INTRODUCTION

Plastic surgeons are innovative. The specialty has been founded on cutting technology, new ways to look at procedures and adaptive behavior. Plastic surgeons have embraced the field of regenerative medicine and have attempted to understand stem cell technology and fat grafting and how this might relate and apply to the clinical setting<sup>[1-5]</sup>. Unfortunately despite much progress, there remains significant unanswered questions on clinical applicability and our ability to regulate and adjust these cells for clinical use.

Embryonic stem cells were first isolated and described by Dr. James Thomson at the University of Wisconsin in 1998<sup>[5-13]</sup>. The pluripotent nature of these cells generated a great deal of excitement but with this excitement came concerns on potential embryonic sacrifice along with the potential experimental concern with the use of these cells. Consequently, the use of embryonic stems cells has been isolated to a few discrete clinical trials<sup>[14-20]</sup>.

Induced pluripotent cells (iPS cells) are genetically modified cells that take on the characteristic of embryonic stem cells. The initial excitement over the use of these cells revolved around the opportunity



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





for researchers to utilize a cell with similar pluripotency but with the lack of embryonic moral concerns relating to embryonic stem cells<sup>[21-30]</sup>. However, concerns on genetic modification along with tumorigenic potential has led to caution for clinical indications<sup>[31-56]</sup>.

Adult stem cells, although not as robust as embryonic stem cells, carry significant potential in regenerative medicine and, with their abundance in fat, carry great significance to plastic surgeons<sup>[18,19,37,39,54,56]</sup>. They are capable of transforming into a limited number of cellular phenotypes within a given family and there is some thought that they may induce further local transformation of cells in a microenvironment through paracrine influences. In 2001, Zuk *et al.*<sup>[13]</sup> reported a source of mesenchymal stem cells in abundance in one particular tissue - that being fat. During processing with collagenase, they found an abundance of cells known as the stromal vascular fraction (SVF) that includes red and white blood cells, immune cells, endothelial cells, and stem cell precursors<sup>[56-80]</sup>. Their process of isolation and determination of unique physical identifiers known as clusters of differentiation allowed the identification of a variety of cells that with multipotent potential. Various processing techniques have been utilized to isolate these cells. Collagen isolation is perhaps the most efficient but concerns with the FDA on the use of collagen in the clinical environment and regulations on minimal manipulation have led to the search for alternative options such as other processing techniques, filtering or drying, washing, or centrifugation.

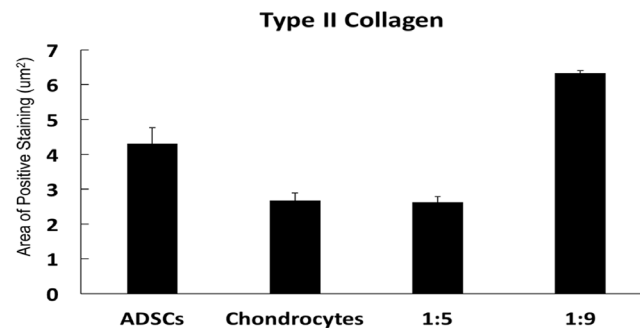
While SVF may contribute to regeneration by its different components, it may also use a paracrine signaling as a means for regeneration based on cross-talk between different cell populations<sup>[11,12]</sup>. Co-culture of SVF with adipocytes has yielded induced progenitor preadipocyte formations. SVF can also promote angiogenesis and neovascularization. We believe that this has great opportunities in the clinical setting for promoting wound healing, particularly in patients with diabetes and other chronic diseases. Co-implantation of SVF with endothelial progenitor cells and adipose derived stem cells (ADSCs) resulted in improved neovascularization potential<sup>[11]</sup>. With lipotransfer there is often significant volume loss but combining with SVF has demonstrated enrichment of fat revascularization, probably through this mechanism of promoting secretion of a diverse array of cytokines. SVF can also be used to create highly vascularized tissue engineered human dermo-epidermal skin substitutes for burn wounds. Rigotti postulated a common sequence of events occurring when SVF is transplanted to radiation-damaged tissue that ultimately results in tissue reperfusion and recovery of some of the damaged skin<sup>[50,81-92]</sup>.

Many of therapeutic studies have also described an initial decrease in inflammation and immune response at the site of SVF injection. When applied to certain disease models, it also tends to decrease inflammatory cytokines and growth factors<sup>[92-117]</sup>.

SVF also has a regenerative capacity, probably through the release of cytokines. SVF increases proliferation of fibroblasts when injected in diabetic foot ulcers. There is also some evidence that it might promote nerve regeneration<sup>[21]</sup>. Diabetic foot disease is a multibillion dollar drain to the health care system, thus utilization of options that could prevent or improve wound healing would be monumental.

Once isolated from adipose tissue, the stromal cell populations represent a diverse collection of cell types. The key concept however is that they are free of lipid and one can mark the cell types with numerous CD (cluster of differentiation) markers. Endothelial precursors are distinctive for bearing the CD 31 antigen and comprise approximately 25%-30% of the mixture of cells. It should be noted however that endothelial precursors bear more markers than just CD 31. Cells for CD 34 markers are considered early multipotent progenitor cells and are considered the true “pre-adipocyte”. Further “pericytes” are thought to give rise to many of the stromal cell populations. Stem cells sense and respond through differentiation to various mechanical cues, growth factors adhesive ligand density, and other factors. It is not only cell differentiation but also cell proliferation, angiogenesis, and cell signaling that are affected by these factors. The rest of this manuscript considers the use of these cells in the clinical setting<sup>[11,12]</sup>.



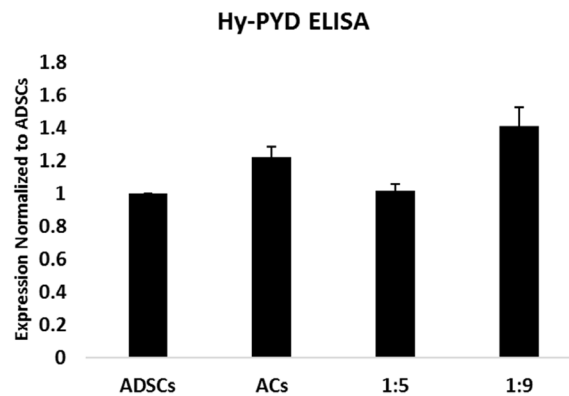


**Figure 1.** The paraffin embedded sections were also stained for type II collagen. The images were quantified, and the 1:9 condition showed the highest level of staining, which was significantly greater than the chondrocytes alone. ADSCs: adipose derived stem cells

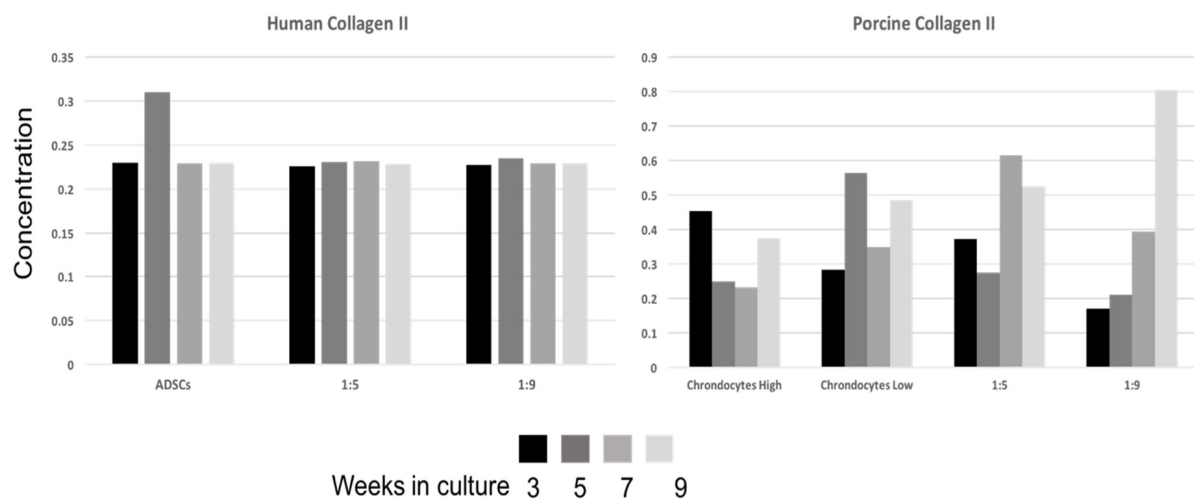
It should be noted that, although clinically there does not appear to be an increased influence on carcinogenesis, several studies indicate that ADSC/ADSC secretomes significantly stimulate proliferation transmigration and 3D invasion of primary normal and tumor epithelial cells<sup>[118-120]</sup>.

Soft Tissue Augmentation and Regeneration is often completed by fat grafting. Variable percentages of absorption occur with fat grafting and volume estimation and preservation may not be optimal<sup>[25]</sup>. The SVF can provide various growth factors such as vascular endothelial growth factor that can promote neo-vascularization as well as other growth factors through a technique coined as cell-assisted lipotransfer<sup>[62,63]</sup>. Utilization of ways to preserve and maintain fat moving forward under the FDA guidelines will help us utilize these options for future clinical studies.

Other studies in our laboratory involve the utilization of ADSCs as a source for chondrocyte progenitors. Porcine chondrocytes can be successfully isolated, expanded, frozen, and thawed, but past a certain point they lose their chondrogenic features. Co-culturing these chondrocytes with ADSCs on AAM (allograft adipose matrix) was hypothesized to enhance their chondrogenic features. Chondrocytes were co-cultured on disc with varying concentrations. After 10 weeks in culture, the discs were paraffin imbedded and stained with H&E. The 1:5 and 1:9 conditions (chondrocytes:ADSCs) demonstrated evidence of extracellular matrix deposition with the 1:9 condition showing a more compact tissue structure, indicating potential chondrogenesis. The paraffin imbedded sections were also stained for type II collagen [Figure 1] with the 1:9 condition showing the highest level of staining, which was significantly greater than the chondrocytes alone. Because the chondrocytes were from porcine and the ADSCs were from human, the contribution of each cell type to chondrogenesis was able to be determined. Hy-PYD (Hydroxylysyl Pyridinoline) assesses collagen crosslinking [Figure 2]. Here, the expression of Hy-PYD was assessed from the discs by enzyme-linked immunosorbent assay (ELISA) and the 1:9 condition increased the expression over the chondrocytes alone. The 1:9 condition was significantly greater than the 1:5 condition. This indicated that ADSCs were stimulating the chondrocytes to be more chondrogenic. The supernatants were collected from the co-culture wells for each condition. ELISA tests were conducted to assess the secretion of type II collagen using species specific antibodies. The human collagen II results were normalized to the chondrocytes because they were presumed to show no expression, and the porcine collagen II results were normalized to the ADSCs, which were presumed to show no expression. The ELISAs were conducted using the supernatants from the collections at Weeks 3, 5, 7, and 9 [Figure 3]. For the human collagen II expression, there was essentially no expression throughout the experiment. However, for the porcine expression, there was a time-dependent increase for the 1:9 co-culture condition, with the nine-week reading showing the highest expression of any of the supernatants tested. The other conditions did not reveal any distinct pattern, indicating that the 1:5 co-culture condition was not different from culturing the chondrocytes alone. In summary, porcine ADSCs can be successfully isolated, expanded, frozen, and



**Figure 2.** Because the chondrocytes were from porcine and the ADSCs were from human, we determined the contribution of each cell type to chondrogenesis. Hy-PYD assesses collagen crosslinking. Here, the expression of Hy-PYD was assessed from the discs by ELISA, and the 1:9 condition increased the expression over the chondrocytes alone. The 1:9 condition was significantly greater than the 1:5 condition. This indicated that ADSCs were stimulating the chondrocytes to be more chondrogenic. ADSCs: adipose derived stem cells; ELISA: enzyme-linked immunosorbent assay



**Figure 3.** The supernatants were collected from the co-culture wells for each condition. Then, ELISA tests were conducted to assess the secretion of type II collagen using species specific antibodies. The human collagen II results were normalized to the chondrocytes because they were presumed to show no expression, and the porcine collagen II results were normalized to the ADSCs, which were presumed to show no expression. The ELISAs were conducted using the supernatants from the collections at Weeks 3, 5, 7, and 9. For the human collagen II expression, there was essentially no expression throughout the experiment. However, for the porcine expression, there was a time-dependent increase for the 1:9 co-culture condition, with the nine-week reading showing the highest expression of any of the supernatants tested. The other conditions did not reveal any distinct pattern, indicating that the 1:5 co-culture was not different from culturing the chondrocytes alone. All quantitative data are expressed as the mean  $\pm$  SD. A Student's *t*-test was performed to assess the differences between the groups. A *P*-value  $< 0.05$  was considered statistically significant. ADSCs: adipose derived stem cells; ELISA: enzyme-linked immunosorbent assay

thawed, but past a certain point they lose their chondrogenic features. Co-culturing these chondrocytes with ADSCs on AAM enhanced their chondrogenic features, revealing more structural changes, an increase in Hy-PYD and type II collagen staining, and secretion in the 1:9 (Chondrocytes:ADSCs) co-culture condition. Chondrocytes contribute to the production of collagen, and the presence of the ADSCs increases this production when these cells were cultured on AAM<sup>[118-125]</sup>.

Recent studies have utilized newer techniques for soft tissue augmentation. Rigotti evaluated a noninvasive technique, which he calls biological morphogenetic surgery, that can enlarge or reduce the shape and

volume of soft tissues by utilizing cannulas and augmentation with fat cells. He also noted an increase in adipose tissue under tissue expanders placed in rats below the latissimus muscle. The thought is that the tensile pressure associated with the tissue expander leads to adipose deposits<sup>[121,122]</sup>.

Wound healing issues are a multibillion dollars business and the use of ADSCs offer potential therapeutic options. ADSCs have been promoted as favorable candidates for wound therapies and they secrete numerous growth factors and cytokines critical in repair. Recent studies have indicated that ADSCs may reverse or improve radiation-induced lesions as well as atrophy and scarring<sup>[94]</sup>. Animal studies suggest that the release of keratinocyte growth factor and the differentiation of ADSCs toward endothelial and epithelial cell line phenotypes may be the mechanisms of action. Further, the angiogenic properties of ADSCs may also benefit complications secondary to ischemia. Autogenous transplantation has demonstrated some promise in improving ankle-brachial index and transcutaneous oxygen pressure. ADSCs may also be useful for the treatment of pathological wound healing in the context of hypertrophic scar formation<sup>[20]</sup>.

In addition, SVF and ADSCs have been used to promote extracellular matrix (ECM) synthesis, the groundwork for wound healing. The extracellular matrix acts as a potent scaffold in many tissue types, accelerating the regenerative functions of nearby cells. It is comprised of structural proteins such as collagen, laminin, fibronectin, and elastin, which are commonly secreted by the fibroblast<sup>[12]</sup>. Furthermore, the ECM contributes to the growth of vascular networks by mediating morphogenesis and migration speeds during angiogenesis. Since the SVF contains matrix-secreting fibroblasts and other stromal cells, the application of SVF is potentially advantageous for laying down the foundations for wound healing<sup>[11]</sup>.

Finally, skin rejuvenation may have promise with ADSC use. It may be possible that these cells could reverse atrophic and photo-damaged cells. Animals studies have demonstrated that subcutaneous ADSC injections increase dermal thickness and collagen density in aged mice and perhaps reduced wrinkles induced by UVB-irradiation<sup>[11,12]</sup>.

Our laboratory has also studied the mechanism of fat formation using acellularized adipose matrix by deconstructing adipo-induction in this acellularized adipose matrix (AAM). It has been observed that AAM injected subcutaneously in an area relatively devoid of fat may initiate lipogenesis. Because of this observation, our global aim was to delineate the sequence of events occurring following implantation of AAM to the final process of adipogenesis. We wanted to compare adipoinduction of adipose/fascia complex to that of adipose fraction alone - analyzing proteomics, bioinformatics, early and late cellular infiltrates, cytokines, adipokines, and enzymes (related to macrophage phenotype and lipoproteins). In addition, we examined the genesis of the adipocytes required to achieve a lipofilling effect, and detailed the involvement of stromal and stem cells in recruiting host tissue to fill the void via adipogenesis, neovascularization and fibrosis<sup>[120]</sup>.

Recently, several groups have started to examine the use of human AAM as a scaffold for tissue engineering, which shows great promise as a vehicle for adipose stem cell delivery as well as a construct that promotes soft tissue regeneration through acellular mechanisms<sup>[119]</sup>. AAM secretes growth factors with adipo- and angio-inductive characteristics [vascular endothelial growth factor, bFGF (fibroblast growth factors), platelet-derived growth factor, and TGF- $\beta$  (transforming growth factor)] and recruits preadipocytes, pericytes, and other cells responsible for local tissue regeneration. Degradation products of the ECM trigger a change in macrophage phenotype that can bring about progenitor cell recruitment<sup>[120]</sup>.

Micronanobubbles (MNBs) technology is another area of exploration in fat graft survival. The bubbles afford the opportunity oxygenate tissues without the expense of utilizing hyperbaric chambers or other nonmobile options. What are the properties that make MNBs unique? MNBs are very small bubbles

in solution that slowly release their oxygen gas into the solution in which they are formed, providing a revolutionary new modality for tissue oxygenation. Strong data have demonstrated improved wound oxygenation, transplant survival and preservation times. This may have major implications for plastic surgery: oxygenation of lipoaspirate and improved fat graft survival<sup>[117]</sup>.

## CONCLUSION

Fat and stem cell technology offer tremendous clinical applications in plastic surgery. We still need to continue to work on the science of this technology. Only by understanding the molecular and tissue interactions will we be able to modify and utilize this technology to its full advantage.

## DECLARATIONS

### Authors' contributions

Dr Evans and Widgerow contribution was equal

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

Both authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:633-76.
2. Lowry WE, Richter L, Yachechko R, Pyle AD, Tchieu J, et al. Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc Natl Acad Sci U S A* 2008;105:2883-8.
3. Hanna J, Markoulaki S, Schorderet P, Carey BW, Beard C, et al. Direct reprogramming of terminally differentiated mature B lymphocytes to pluripotency. *Cell* 2008;133:250-64.
4. Aasen T, Raya A, Barrero MJ, Garreta E, Consiglio A, et al. Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. *Nat Biotechnol* 2008;26:1276-84.
5. Takenaka C, Nishishita N, Takada N, Jakt LM, Kawamata S. Effective generation of iPS cells from CD 34+ cord blood cells by inhibition of p53. *Exp Hematol* 2010; 38:154-62.
6. Phanthong P, Raveh-Amit H, Li T, Kitiyanant Y, Dinnyes A. Is aging a barrier to reprogramming? Lessons from induced pluripotent stem cells. *Biogerontology* 2013;14:591-602.
7. Gonzalez F, Barragan Monasterio M, Tiscornia G, Montserrat Pulido N, et al. Generation of mouse-induced pluripotent stem cells by transient expression of a single nonviral polycistronic vector. *Proc Natl Acad Sci USA* 2009;106:8918-22.
8. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science* 2008;322:949-53.
9. Steinemann D, Gohring G, Schlegelberger B. Genetic instability of modified stem cells - a first step towards malignant transformation. *Am J Stem Cells* 2013;2:39-51.
10. Mayshar Y, Ben-David U, Lavon N, Biancotti JC, Yakir B, et al. Identification and classification of chromosomal aberrations in human

- induced pluripotent stem cells. *Cell Stem Cell* 2010;7:521-31.
11. Guo J, Nguyen A, Banyard DA, Fadavi D, Toronto JD, et al. Stromal vascular fraction: a regenerative reality? Part 2: mechanisms of regenerative action. *J Plast Reconstr Aesthet Surg* 2016;69:180-8.
  12. Nguyen A, Guo J, Fadavi D, Banyard D, Toronto J, et al. Stromal vascular fraction - a regenerative reality? Part 1 - current concepts and review of the literature. *J Plast Reconstr Aesthet Surg* 2016;69:170-9.
  13. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng* 2001;7:211-28.
  14. Riordan NH, Ichim TE, Min WP, Wang H, Solano F, et al. Non-expanded adipose stromal vascular fraction cell therapy for multiple sclerosis. *J Transl Med* 2009;7:29.
  15. Yoshimura K, Sato K, Aoi N, Kurita M, Hirohi T, et al. Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. *Aesthetic Plast Surg* 2008;32:48-55.
  16. van Dijk A, Naaijken BA, Jurgens WJ, Nalliah K, Sairras S, et al. Reduction of infarct size by intravenous injection of uncultured adipose derived stromal cells in a rat model is dependent on the time point of application. *Stem Cell Res* 2011;7:219-29.
  17. Atalay S, Coruh A, Deniz K. Stromal vascular fraction improves deep partial thickness burn wound healing. *Burns* 2014;40:1375-83.
  18. Rajashekhar G, Ramadan A, Abburi C, Callaghan B, Traktuev DO, et al. Regenerative therapeutic potential of adipose stromal cells in early stage diabetic retinopathy. *PLoS One* 2014;9:e84671.
  19. Chung MT, Zimmermann AS, Paik KJ, Morrison SD, Hyun JS, et al. Isolation of human adipose-derived stromal cells using laser-assisted liposuction and their therapeutic potential in regenerative medicine. *Stem Cells Transl Med* 2013;2:808-17.
  20. You HJ, Han SK. Cell therapy for wound healing. *J Korean Med Sci* 2014;29:311-9.
  21. Jarajapu YP, Grant MB. The promise of cell-based therapies for diabetic complications: challenges and solutions. *Circ Res* 2010;106:854-69.
  22. Tocco I, Widgerow AD, Lalezari S, Banyard D, Shaterian A, et al. Lipotransfer: the potential from bench to bedside. *Ann Plast Surg* 2014;72:599-609.
  23. Kakagia D, Pallua N. Autologous fat grafting: in search of the optimal technique. *Surg Innov* 2014;21:327-36.
  24. Eto H, Ishimine H, Kinoshita K, Watanabe-Susaki K, Kato H, et al. Characterization of human adipose tissue-resident hematopoietic cell populations reveals a novel macrophage subpopulation with CD34 expression and mesenchymal multipotency. *Stem Cells Dev* 2013;22:985-97.
  25. Karacaoglu E, Kizilkaya E, Cermik H, Zienowicz R. The role of recipient sites in fat-graft survival: experimental study. *Ann Plast Surg* 2005;55:63-8.
  26. Matsumoto D, Sato K, Gonda K, Takaki Y, Shigeura T, et al. Cell-assisted lipotransfer: supportive use of human adipose-derived cells for soft tissue augmentation with lipoinjection. *Tissue Eng* 2006;12:3375-82.
  27. Tanikawa DY, Agüena M, Bueno DF, Passos-Bueno MR, Alonso N. Fat grafts supplemented with adipose-derived stromal cells in the rehabilitation of patients with craniofacial microsomia. *Plast Reconstr Surg* 2013;132:141-52.
  28. Peltoniemi HH, Salmi A, Miettinen S, Mannerström B, Saariniemi K, et al. Stem cell enrichment does not warrant a higher graft survival in lipofilling of the breast: a prospective comparative study. *J Plast Reconstr Aesthet Surg* 2013;66:1494-503.
  29. Kakudo N, Tanaka Y, Morimoto N, Ogawa T, Kushida S, et al. Adipose-derived regenerative cell (ADRC)-enriched fat grafting: optimal cell concentration and effects on grafted fat characteristics. *J Transl Med* 2013;11:254.
  30. Kamakura T, Ito K. Autologous cell-enriched fat grafting for breast augmentation. *Aesthetic Plast Surg* 2011;35:1022-30.
  31. Chatterjee S, Laliberte M, Blelloch S, Ratanshi I, Safneck J, et al. Adipose-derived stromal vascular fraction differentially expands breast progenitors in tissue adjacent to tumors compared to healthy breast tissue. *Plast Reconstr Surg* 2015;136:414e-25.
  32. Mandel K, Yang Y, Schambach A, Glage S, Otte A, et al. Mesenchymal stem cells directly interact with breast cancer cells and promote tumor cell growth in vitro and in vivo. *Stem Cells Dev* 2013;22:3114-27.
  33. Rowan BG, Gimble JM, Sheng M, Anbalagan M, Jones RK, et al. Human adipose tissue-derived stromal/stem cells promote migration and early metastasis of triple negative breast cancer xenografts. *PLoS One* 2014;9:e89595.
  34. Zimmerlin L, Donnenberg AD, Rubin JP, Basse P, Landreneau RJ, et al. Regenerative therapy and cancer: in vitro and in vivo studies of the interaction between adipose-derived stem cells and breast cancer cells from clinical isolates. *Tissue Eng Part A* 2011;17:93-106.
  35. Charles-de-Sá L, Gontijo-de-Amorim NF, Maeda Takiya C, Borojevic R, Benati D, et al. Antiaging treatment of the facial skin by fat graft and adipose-derived stem cells. *Plast Reconstr Surg* 2015;135:999-1009.
  36. Bourin P, Bunnell BA, Casteilla L, Dominici M, Katz AJ, et al. Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). *Cytotherapy* 2013;15:641-8.
  37. Banyard DA, Salibian AA, Widgerow AD, Evans GR. Implications for human adipose-derived stem cells in plastic surgery. *J Cell Mol Med* 2015;19:21-30.
  38. Gir P, Oni G, Brown SA, Mojallal A, Rohrich RJ. Human adipose stem cells: current clinical applications. *Plast Reconstr Surg* 2012;129:1277-90.
  39. Liras A. Future research and therapeutic applications of human stem cells: general, regulatory, and bioethical aspects. *J Transl Med* 2010;8:131.
  40. Tonnard P, Verpaele A, Peeters G, Hamdi M, Cornelissen M, et al. Nanofat grafting: basic research and clinical applications. *Plast Reconstr Surg* 2013;132:1017-26.
  41. Stuzin JM. Discussion: nanofat grafting: basic research and clinical applications. *Plast Reconstr Surg* 2013;132:1027-8.



42. Pereira Lopes FR, Camargo de Moura Campos L, Dias Corrêa J Jr, Balduino A, Lora S, et al. Bone marrow stromal cells and resorbable collagen guidance tubes enhance sciatic nerve regeneration in mice. *Exp Neurol* 2006;198:457-68.
43. Mohammadi R, Sanaei N, Ahsan S, Rostami H, Abbasipour-Dalivand S, et al. Repair of nerve defect with chitosan graft supplemented by uncultured characterized stromal vascular fraction in streptozotocin induced diabetic rats. *Int J Surg* 2014;12:33-40.
44. Papalia I, Raimondo S, Ronchi G, Magaouda L, Giacobini-Robecchi MG, et al. Repairing nerve gaps by vein conduits filled with lipoaspirate-derived entire adipose tissue hinders nerve regeneration. *Ann Anat* 2013;195:225-30.
45. Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). *Br J Pharmacol* 2011;164:1079-106.
46. Ricco JB, Thanh Phong L, Schneider F, Illuminati G, Belmonte R, et al. The diabetic foot: a review. *J Cardiovasc Surg (Torino)* 2013;54:755-62.
47. Han SK, Kim HR, Kim WK. The treatment of diabetic foot ulcers with uncultured, processed lipoaspirate cells: a pilot study. *Wound Repair Regen* 2010;18:342-8.
48. Lv SS, Liu G, Wang JP, Wang WW, Cheng J, et al. Mesenchymal stem cells transplantation ameliorates glomerular injury in streptozotocin-induced diabetic nephropathy in rats via inhibiting macrophage infiltration. *Int Immunopharmacol* 2013;17:275-82.
49. Perbeck L, Celebioglu F, Svensson L, Danielsson R. Lymph circulation in the breast after radiotherapy and breast conservation. *Lymphology* 2006;39:33-40.
50. Rigotti G, Marchi A, Galiè M, Baroni G, Benati D, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. *Plast Reconstr Surg* 2007;119:1409-22.
51. Gimble JM, Bunnell BA, Chiu ES, Guilak F. Concise review: Adipose-derived stromal vascular fraction cells and stem cells: let's not get lost in translation. *Stem Cells* 2011;29:749-54.
52. Laass MW, Roggenbuck D, Conrad K. Diagnosis and classification of Crohn's disease. *Autoimmun Rev* 2014;13:467-71.
53. Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol* 2006;3:390-407.
54. Dalal J, Gandy K, Domen J. Role of mesenchymal stem cell therapy in Crohn's disease. *Pediatr Res* 2012;71:445-51.
55. Thesleff T, Lehtimäki K, Niskakangas T, Mannerström B, Miettinen S, et al. Cranioplasty with adipose-derived stem cells and biomaterial: a novel method for cranial reconstruction. *Neurosurgery* 2011;68:1535-40.
56. Sándor GK, Tuovinen VJ, Wolff J, Patrikoski M, Jokinen J, et al. Adipose stem cell tissue-engineered construct used to treat large anterior mandibular defect: a case report and review of the clinical application of good manufacturing practice-level adipose stem cells for bone regeneration. *J Oral Maxillofac Surg* 2013;71:938-50.
57. Wilson SM, Goldwasser MS, Clark SG, Monaco E, Bionaz M, et al. Adipose-derived mesenchymal stem cells enhance healing of mandibular defects in the ramus of swine. *J Oral Maxillofac Surg* 2012;70:e193-203.
58. Mehrkens A, Saxer F, Güven S, Hoffmann W, Müller AM, et al. Intraoperative engineering of osteogenic grafts combining freshly harvested, human adipose-derived cells and physiological doses of bone morphogenetic protein-2. *Eur Cell Mater* 2012;24:308-19.
59. Araña M, Gavira JJ, Peña E, González A, Abizanda G, et al. Epicardial delivery of collagen patches with adipose-derived stem cells in rat and minipig models of chronic myocardial infarction. *Biomaterials* 2014;35:143-51.
60. Khalpey Z, Janardhanan R, Konhilas J, Hemphill C, et al. First in man: adipose-derived stromal vascular fraction cells may promote restorative cardiac function. *Am J Med* 2014;127:e11-2.
61. Gimble JM, Katz AJ, Bunnell BA. Adipose-derived stem cells for regenerative medicine. *Circ Res* 2007;100:1249-60.
62. Yoshimura K, Shigeura T, Matsumoto D, Sato T, Takaki Y, et al. Characterization of freshly isolated and cultured cells derived from the fatty and fluid portions of liposuction aspirates. *J Cell Physiol* 2006;208:64-76.
63. Yoshimura K. Cell-assisted lipotransfer for breast augmentation: grafting of progenitor-enriched fat tissue. In: Shiffman MA, editors. *Autologous fat transfer*. Springer, Berlin, Heidelberg; 2010. pp. 261-71.
64. ClinicalTrials.gov. ClinicalTrials.gov Search of Stromal Vascular Fraction. Available from: <https://clinicaltrials.gov/ct2/results?term=stromal+vascular+fraction&pg=2> [Last accessed on 11 Mar 2020]
65. Gentile P, Orlandi A, Scioli MG, Di Pasquali C, Bocchini I, et al. Concise review: adipose-derived stromal vascular fraction cells and platelet-rich plasma: basic and clinical implications for tissue engineering therapies in regenerative surgery. *Stem Cells Transl Med* 2012;1:230-6.
66. Jurgens WJ, Kroeze RJ, Zandieh-Doulabi B, van Dijk A, Renders GA, et al. One-step surgical procedure for the treatment of osteochondral defects with adipose-derived stem cells in a caprine knee defect: a pilot study. *Biores Open Access* 2013;2:315-25.
67. Semon JA, Zhang X, Pandey AC, Alandete SM, Maness C, et al. Administration of murine stromal vascular fraction ameliorates chronic experimental autoimmune encephalomyelitis. *Stem Cells Transl Med* 2013;2:789-96.
68. Astori G, Vignati F, Bardelli S, Tubio M, Gola M, et al. "In vitro" and multicolor phenotypic characterization of cell subpopulations identified in fresh human adipose tissue stromal vascular fraction and in the derived mesenchymal stem cells. *J Transl Med* 2007;5:55.
69. Watson JE, Patel NA, Carter G, Moor A, Patel R, et al. Comparison of markers and functional attributes of human adipose-derived stem cells and dedifferentiated adipocyte cells from subcutaneous fat of an obese diabetic donor. *Adv Wound Care (New Rochelle)* 2014;3:219-28.
70. Sumi M, Sata M, Toya N, Yanaga K, Ohki T, et al. Transplantation of adipose stromal cells, but not mature adipocytes, augments ischemia-induced angiogenesis. *Life Sci* 2007;80:559-65.
71. Zeyda M, Farmer D, Todoric J, Aszmann O, Speiser M, et al. Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. *Int J Obes (Lond)* 2007;31:1420-8.

72. Tiemessen MM, Jagger AL, Evans HG, van Herwijnen MJ, John S, et al. CD4+CD25+Foxp3+ regulatory T cells induce alternative activation of human monocytes/macrophages. *Proc Natl Acad Sci U S A* 2007;104:19446-51.
73. Choi JS, Kim BS, Kim JY, Kim JD, Choi YC, et al. Decellularized extracellular matrix derived from human adipose tissue as a potential scaffold for allograft tissue engineering. *J Biomed Mater Res A* 2011;9:292-9.
74. Eckes B, Nischt R, Krieg T. Cell-matrix interactions in dermal repair and scarring. *Fibrogenesis Tissue Repair* 2010;3:4.
75. Traktuev DO, Prater DN, Merfeld-Clauss S, Sanjeevaiah AR, Saadatzaheh MR, et al. Robust functional vascular network formation in vivo by cooperation of adipose progenitor and endothelial cells. *Circ Res* 2009;104:1410-20.
76. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8:315-7.
77. Sidney LE, Branch MJ, Dunphy SE, Dua HS, Hopkinson A. Concise review: evidence for cd34 as a common marker for diverse progenitors. *Stem Cells* 2014;32:1380-9.
78. Corselli M, Crisan M, Murray IR, West CC, Scholes J, et al. Identification of perivascular mesenchymal stromal/stem cells by flow cytometry. *Cytometry A* 2013;83:714-20.
79. Hager G, Holthöner W, Wolbank S, Husa AM, Godthardt K, et al. Three specific antigens to isolate endothelial progenitor cells from human liposuction material. *Cytotherapy* 2013;15:1426-35.
80. Navarro A, Marin S, Riol N, Carbonell-Uberos F, Miñana MD. Human adipose tissue-resident monocytes exhibit an endothelial-like phenotype and display angiogenic properties. *Stem Cell Res Ther* 2014;5:50.
81. Blaber SP, Webster RA, Hill CJ, Breen EJ, Kuah D, et al. Analysis of in vitro secretion profiles from adipose-derived cell populations. *J Transl Med* 2012;10:172.
82. Chazenbalk G, Bertolotto C, Heneidi S, Jumabay M, Trivax B, et al. Novel pathway of adipogenesis through cross-talk between adipose tissue macrophages, adipose stem cells and adipocytes: evidence of cell plasticity. *PLoS One* 2011;6:e17834.
83. Cianfarani F, Toietta G, Di Rocco G, Cesareo E, Zambruno G, et al. Diabetes impairs adipose tissue-derived stem cell function and efficiency in promoting wound healing. *Wound Repair Regen* 2013;21:545-53.
84. Li J, Gao J, Cha P, Chang Q, Liao Y, et al. Supplementing fat grafts with adipose stromal cells for cosmetic facial contouring. *Dermatol Surg* 2013;39:449-56.
85. Chung MT, Paik KJ, Atashroo DA, Hyun JS, McArdle A, et al. Studies in fat grafting: Part I. Effects of injection technique on in vitro fat viability and in vivo volume retention. *Plast Reconstr Surg* 2014;134:29-38.
86. Premaratne GU, Ma LP, Fujita M, Lin X, Bollano E, et al. Stromal vascular fraction transplantation as an alternative therapy for ischemic heart failure: anti-inflammatory role. *J Cardiothorac Surg* 2011;6:43.
87. Koh YJ, Koh BI, Kim H, Joo HJ, Jin HK, et al. Stromal vascular fraction from adipose tissue forms profound vascular network through the dynamic reassembly of blood endothelial cells. *Arterioscler Thromb Vasc Biol* 2011;31:1141-50.
88. Rehman J, Traktuev D, Li J, Merfeld-Clauss S, Temm-Grove CJ, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation* 2004;109:1292-8.
89. Kollé SF, Fischer-Nielsen A, Mathiasen AB, Elberg JJ, Oliveri RS, et al. Enrichment of autologous fat grafts with ex-vivo expanded adipose tissue-derived stem cells for graft survival: a randomised placebo-controlled trial. *Lancet* 2013;382:1113-20.
90. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circ Res* 2005;97:512-23.
91. Kwon HM, Hur SM, Park KY, Kim CK, Kim YM, et al. Multiple paracrine factors secreted by mesenchymal stem cells contribute to angiogenesis. *Vascul Pharmacol* 2014;63:19-28.
92. Zhu M, Dong Z, Gao J, Liao Y, Xue J, et al. Adipocyte regeneration after free fat transplantation: promotion by stromal vascular fraction cells. *Cell Transplant* 2015;24:49-62.
93. Fu S, Luan J, Xin M, Wang Q, Xiao R, et al. Fate of adipose-derived stromal vascular fraction cells after co-implantation with fat grafts: evidence of cell survival and differentiation in ischemic adipose tissue. *Plast Reconstr Surg* 2013;132:363-73.
94. Paik KJ, Zielins ER, Atashroo DA, Maan ZN, Duscher D, et al. Studies in fat grafting: part v. cell-assisted lipotransfer to enhance fat graft retention is dose dependent. *Plast Reconstr Surg* 2015;136:67-75.
95. Klar AS, Güven S, Biedermann T, Luginbühl J, Böttcher-Haberzeth S, et al. Tissue-engineered dermo-epidermal skin grafts prevascularized with adipose-derived cells. *Biomaterials* 2014;35:5065-78.
96. Barba M, Cicione C, Bernardini C, Michetti F, Lattanzi W. Adipose-derived mesenchymal cells for bone regeneration: state of the art. *Biomed Res Int* 2013;2013:416391.
97. di Summa PG, Kingham PJ, Raffoul W, Wiberg M, Terenghi G, et al. Adipose-derived stem cells enhance peripheral nerve regeneration. *J Plast Reconstr Aesthet Surg* 2010;63:1544-52.
98. Pereira Lopes FR, Lisboa BC, Frattini F, Almeida FM, Tomaz MA, et al. Enhancement of sciatic nerve regeneration after vascular endothelial growth factor (VEGF) gene therapy. *Neuropathol Appl Neurobiol* 2011;37:600-12.
99. Badylak SF. The extracellular matrix as a scaffold for tissue reconstruction. *Semin Cell Dev Biol* 2002;13:377-83.
100. Bosman FT, Stamenkovic I. Functional structure and composition of the extracellular matrix. *J Pathol* 2003;200:423-8.
101. Alberts B. Molecular biology of the cell. 4th ed. New York: Garland Science; 2002. pp. 1548.
102. Sheetz MP, Felsenfeld DP, Galbraith CG. Cell migration: regulation of force on extracellular-matrix-integrin complexes. *Trends Cell Biol* 1998;8:51-4.
103. Friedl P, Zanker KS, Bocker EB. Cell migration strategies in 3-D extracellular matrix: differences in morphology, cell matrix interactions, and integrin function. *Microsc Res Tech* 1998;43:369-78.
104. Bauer AL, Jackson TL, Jiang Y. Topography of extracellular matrix mediates vascular morphogenesis and migration speeds in

- angiogenesis. *PLoS Comput Biol* 2009;5:e1000445.
105. Choi JS, Yang HJ, Kim BS, Kim JD, Kim JY, et al. Human extracellular matrix (ECM) powders for injectable cell delivery and adipose tissue engineering. *J Control Release* 2009;139:2-7.
  106. Debels H, Gerrand YW, Poon CJ, Abberton KM, Morrison WA, et al. An adipogenic gel for surgical reconstruction of the subcutaneous fat layer in a rat model. *J Tissue Eng Regen Med* 2017;11:1230-41.
  107. Choi JH, Bellas E, Vunjak-Novakovic G, Kaplan DL. Adipogenic differentiation of human adipose-derived stem cells on 3D silk scaffolds. *Methods Mol Biol* 2011;702:319-30.
  108. Chernousov MA, Yu WM, Chen ZL, Carey DJ, Strickland S. Regulation of Schwann cell function by the extracellular matrix. *Glia* 2008;56:1498-507.
  109. Brown BN, Londono R, Tottey S, Zhang L, Kukla KA, et al. Macrophage phenotype as a predictor of constructive remodeling following the implantation of biologically derived surgical mesh materials. *Acta Biomater* 2012;8:978-87.
  110. Mills SJ, Cowin AJ, Kaur P. Pericytes, mesenchymal stem cells and the wound healing process. *Cells* 2013;2:621-34.
  111. Caplan AI, Correa D. The MSC: an injury drugstore. *Cell Stem Cell* 2011;9:11-5.
  112. Narayanan AS, Page RC, Swanson J. Collagen synthesis by human fibroblasts. Regulation by transforming growth factor-beta in the presence of other inflammatory mediators. *Biochem J* 1989 ;260:463-9.
  113. Newman AC, Nakatsu MN, Chou W, Gershon PD, Hughes CC. The requirement for fibroblasts in angiogenesis: fibroblast-derived matrix proteins are essential for endothelial cell lumen formation. *Mol Biol Cell* 2011;22:3791-800.
  114. Newman AC, Chou W, Welch-Reardon KM, Fong AH, Popson SA, et al. Analysis of stromal cell secretomes reveals a critical role for stromal cell-derived hepatocyte growth factor and fibronectin in angiogenesis. *Arterioscler Thromb Vasc Biol* 2013;33:513-22.
  115. Bianchi F, Maioli M, Leonardi E, Olivi E, Pasquinelli G, et al. A new nonenzymatic method and device to obtain a fat tissue derivative highly enriched in pericyte-like elements by mild mechanical forces from human lipoaspirates. *Cell Transplant* 2013;22:2063-77.
  116. Traktuev DO, Merfeld-Clauss S, Li J, Kolonin M, Arap W, et al. A population of multipotent CD34-positive adipose stromal cells share pericyte and mesenchymal surface markers, reside in a periendothelial location, and stabilize endothelial networks. *Circ Res* 2008;102:77-85.
  117. Sayadi LR, Banyard DA, Ziegler ME, Obagi Z, Prussak J, et al. Topical oxygen therapy & micro/nanobubbles: a new modality for tissue oxygen delivery. *Int Wound J* 2018;15:363-74.
  118. Kengelback-Weigand A, Tasbihi K, Strissel PL, Schmid R, Marques JM, et al. Plasticity of patient-match normal mammary epithelial cell is dependent on autologous adipose-derived stem cells. *Sci Rep* 2019;9:10722.
  119. Schmid R, Wolf K, Robering JW, Strauss S, Strissel PL, et al. ADSCs and adipocytes are the main producers in the autotaxin-lysophosphatidic acid axis of breast cancer and healthy mammary tissue in vitro. *BMC Cancer* 2018;18:1273.
  120. Tong J, Mou S, Xiong L, Wang Z, Wang R, et al. Adipose-derived mesenchymal stem cells formed acinar-like structure when stimulated with breast epithelial cells in three-dimensional culture. *PLoS One* 2018;13:e0204077.
  121. Rigotti G, Chirumbolo S. Biological morphogenetic surgery: a minimally invasive procedure to address different biological mechanisms. *Aesthetic Surg J* 2019;39:745-55.
  122. Rigotti G, Chirumbolo S, Cicala F, Parnigotto PP, Nicolato E, et al. Negative pressure from an internal spiral tissue expander generates new subcutaneous adipose tissue in an in vivo animal model. *Aesthet Surg J* 2020;40:448-459.
  123. Ziegler ME, Sorensen AM, Banyard DA, Evans GRD, Widgerow AD. Improving in vivo cartilage generation by co-culturing adipose-derived stem cells and chondrocytes on an allograft adipose matrix framework. *Plast Reconstr Surg* 2019;83:583-8.
  124. Ziegler ME, Sorensen AM, Banyard DA, Tylutki T, Chnari E, et al. Deconstructing Allograft Adipose and Fascia Matrix: implications for improving angiogenesis related to procedures for tissue supplementation and volumization stem cells. USA: Lohrasb Ross Sayadi, MD; 2017.
  125. Banyard DA, Borad V, Amezcua E, Wirth GA, Evans GR, et al. Preparation, characterization, and clinical implications of human decellularized adipose tissue extracellular matrix (hDAM): a comprehensive review. *Aesthet Surg J* 2016;36:349-57.

Review

Open Access



# Secondary damage in trauma and limited access dressing: a review

Pramod Kumar<sup>1</sup>, Akriti Gupta<sup>2</sup>, Apoorva Gupta<sup>3</sup>

<sup>1</sup>Consultant Plastic Surgeon, King Fahd Central Hospital, Jazan 82666, Saudi Arabia.

<sup>2</sup>Resident Physician (Pathology), University of Virginia, Charlottesville, VA 22903, USA.

<sup>3</sup>Physician (Internal Medicine), Asante Rogue Regional Medical Center, Medford, OR 97504, USA.

**Correspondence to:** Dr. Pramod Kumar, Department of Plastic Surgery, King Fahad Central Hospital, Jazan 82666, Saudi Arabia.  
E-mail: pkumar 86@hotmail.com

**How to cite this article:** Kumar P, Gupta A, Gupta A. Secondary damage in trauma and limited access dressing: a review. *Plast Aesthet Res* 2020;7:29. <http://dx.doi.org/10.20517/2347-9264.2019.71>

**Received:** 7 Dec 2019 **First Decision:** 21 Apr 2020 **Revised:** 25 Apr 2020 **Accepted:** 6 May 2020 **Published:** 18 Jun 2020

**Science Editor:** Raúl González-García **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

## Abstract

Secondary damage in trauma may increase morbidity, mortality and the cost of treatment considerably. This article reviews the literature of 46 relevant articles on this topic. We hope to provide a better understanding of the various mechanisms that can lead to secondary damage following major trauma and aim to improve the management of such in trauma patients. We also explore the utility of limited access dressing and its ability to minimize and treat secondary musculoskeletal trauma. Four interdependent cellular mechanisms have been described that contribute and perpetuate secondary tissue damage - lysosomal, protein/enzyme denaturation, membrane permeability and mitochondrial. Systemic changes are mainly due to systemic hypoxia and the systemic inflammatory response syndrome. Limited access dressing appears to be an efficient and cost-effective method for the management of secondary damage, as evidenced by the reduced number of debridements, shorter wound coverage time, and reduction in total length of hospital stay while lowering treatment costs and improving quality of care.

**Keywords:** Trauma, secondary damage, limited access dressing

## INTRODUCTION

Cells are complex interconnected systems that work together to maintain a well-regulated micro-environment that is indispensable for their survival. Trauma to a single cell can affect overall homeostasis



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



of the immediate environment. The extent of injury and tissue characteristics determine the extent of biochemical damage to adjacent cells, leading to cell death and tissue dysfunction. Secondary damage in trauma can increase patient morbidity, mortality and the cost of treatment considerably. A better understanding and control of secondary damage is thus essential for improved outcomes in trauma patients, which will, in turn, reduce morbidity and render management cost-efficient. In this article, we review the existing literature and analyze possible areas where standard medical and surgical intervention along with wound management using Limited Access Dressing (LAD) could lead to better outcomes.

## METHOD

Secondary damage in trauma patients was defined. We looked up the relevant literature by using “Secondary Damage in Trauma” and “Limited Access Dressing” on PubMed and Google search engines. Relevant articles from molecular biology, physiology and pathophysiology were also reviewed to explain the mechanism of secondary damage in trauma. A total of 46 relevant articles were reviewed analyzed to find areas where standard medical and surgical intervention, along with wound management using LAD, could lead to better outcomes.

### Definition

secondary injury, in simple words “by-stander” damage, of cells result from a secondary insult with destructive and biochemical mechanisms triggered by the mechanical destruction of tissue following direct trauma (the primary insult). Secondary damage may develop immediately (within hours) following the primary insult, and last from a few days up to weeks after. For instance, necrosis of peripheral tissues surrounding the damaged tissue can develop late after crush injuries<sup>[1]</sup>. Cells or tissue with ultra-structural damage at the time of trauma may die within hours to a few days following trauma. It is still controversial however, whether such cell death should be included under primary or secondary damage<sup>[2]</sup>. Secondary damage may also involve local or distant cells/tissues. From a biomedical point of view, it can occur due to hemorrhagic shock, compartment syndrome and/or ischemic necrosis<sup>[1,2]</sup>.

### Mechanism of secondary damage

Secondary damage to local tissue: This was explained by Knight’s Sport Injury Model (1970)<sup>[2]</sup> that was later updated based on improved biochemical understanding of tissue damage.

Secondary damage to distant tissue: This mainly occurs due to systemic hypoxia following hemorrhage or due to pro-inflammatory cytokines following systemic inflammatory response syndrome (SIRS)<sup>[3]</sup>.

### *Mechanism of local secondary tissue injury*

#### *Knight’s sport injury model (1970)*

Knight’s Sport Injury Model (Secondary Injury Model) was first described in the mid 70’s to account for the series of events that occur following injury in athletes<sup>[2,4]</sup>. This model effectively explains the possible mechanism that is triggered due to the loss of cellular hemostasis occurring in adjacent, uninjured tissues following primary injury and cell death.

It could be speculated that the outcomes of a primary insult/trauma is immediate cell death or permanent ultra-structural changes that lead to death of injured cells over time. The physiological response to cell death, in theory, could affect the functionality of uninjured cells. The physiologic stress leading to tissue damage is called a secondary injury.

Knight hypothesized that secondary injuries are primarily caused by two mechanisms: (1) hypoxic injury causing oncosis (cell swelling) and acidosis; and (2) enzymatic (lysosomal enzymes) injury.



### *Hypoxic injury causing oncosis and acidosis*

Secondary hypoxic injury occurs due to: hemorrhage, intravascular thrombosis, reduced blood flow from inflammation following increased viscosity, increased intravascular pressure following hematoma and muscle spasm.

Causes of secondary hypoxic injury are (1) anaerobic respiration - depending upon the susceptibility of the involved tissues to hypoxia, glycolic pathways of ATP production lasts from few minutes to 6 h. During aerobic respiration, ATP production is hampered, and acidosis occurs; (2) ATP dependent  $\text{Na}^+ \text{K}^+$  ATPase pump failure<sup>[5]</sup> -  $\text{Na}^+$  and  $\text{Ca}^{2+}$  influx due to ATP dependent  $\text{Na}^+$  pump failure leads to cellular homeostatic mechanism failure, leading to oncosis, cell death and necrosis.

Hypoxia leads to reduced ATP production. Hence, initial cryotherapy in musculoskeletal trauma is beneficial, as cold helps reduce ATP demand.

### *Enzymatic (lysosomal) injury*

Enzymes released from lysosomes of the injured and dead tissues: (1) acid hydrolases and phospholipases: these enzymes lyse the cell membrane by cleaving the hydrocarbon chain of membrane phospholipids; and (2) proteases: proteases inactivate protein by cleaving peptide bonds.

Effect of enzyme on tissues: acid hydrolases, phospholipase and proteases can cause loss of cell membrane integrity and lead to increased hydropic swelling (oncosis) followed by cell death.

Also, trauma leads to inflammation and neutrophils are acute inflammatory cells and the first line of cellular defense. Human neutrophil proteins (defensins; HNP-1 to 3) are bactericidal agents and interfere with the function of smooth muscle cells in vessels, are prothrombotic, and may inhibit angiogenesis<sup>[6]</sup>.

### *Updated secondary local tissue injury model*

#### *Mechanism of secondary ischemic injury to local tissue*

The updated secondary injury model is based on improved biochemical understanding of tissue damage. It has now been proven that ischemia resulting from vascular injury, rather than hypoxia, plays a more significant role in driving secondary damage. Hypoxia poses only one problem of inadequate oxygen supply, whereas ischemia presents with three possible consequences<sup>[2]</sup>: (1) hypoxia - leads to anaerobic respiration and thus reduced ATP (adenosine triphosphate); (2) low glucose supply to tissues, leading to reduced ATP; and (3) acidosis that causes reduced cellular ability to produce ATP.

Hypoxia also affects mitochondrial function and leads to neural tissue damage as early as within 4 min<sup>[5]</sup>, and musculoskeletal injury in approximately three hours<sup>[7]</sup>.

#### *Mechanism for secondary direct cell injury to local tissue*

There are four possible mechanisms for secondary direct cell injury to local uninjured tissues: (1) lysosomal mechanisms (due to the release of destructive enzymes that require low pH for activation); (2) protein/enzyme denaturation mechanisms (due to low pH); (3) membrane permeability mechanisms (due to failure of sodium-potassium-ATPase pump) - this leads to cellular swelling; and (4) mitochondrial mechanisms (due to power house failure).

All these mechanisms are basically due to two types of changes - hypoxic and enzymatic.

### *Lysosomal mechanisms*

Lysosomes, the main digestive organelle in a eukaryotic cell, is packed with catabolic enzymes maintained at an acidic intraluminal pH. Under physiological conditions, these highly destructive enzymes, such as

lipases, proteases and cathepsins, are contained within the lysosomal complex by the lysosomal membrane. In theory, externalization of lysosomal enzymes to the neutral pH of the cytoplasm would render these enzymes inactive. However, it is now known that a few forms of cathepsins remain active even at neutral pH and can trigger a cascade of events that lead to cell death from the lysosomal pathway<sup>[8]</sup>. The acidity of the local microenvironment can further activate the lysosomal enzymes released due to lysosomal damage.

There are various stimuli that can lead to increased permeability of the lysosomal membrane and externalization of catalytic enzymes. Lysosomal membrane permeabilization may be caused by various stimuli but the most common mechanism is by destruction of the lipid organization and oxidative damage to membrane bound proteins. Ischemic-reperfusion injury following acute trauma may lead to oxidative stress and membrane damage and destabilization from reactive oxygen species. The hydroxyl radical can cause lipid peroxidation and damage to membrane bound proteins. In addition, reactive oxygen species may also contribute by altering the lysosomal mechanism and activating calcium channels<sup>[9]</sup>.

By releasing highly destructive enzymes (e.g., phospholipase A, cathepsin B) following trauma, cell membranes and cellular proteins are damaged. Lysosomal enzymes cause intracellular cell death when released but when the enzymes become extracellular, secondary damage to adjacent tissue occurs. The normal extracellular pH (approximately 7.2) hampers the action of the majority of lysosomal enzymes but acidic environments ( $\text{pH} \leq 5$ ) provide optimal conditions for their functioning. Experimentally, it has also been shown that inhibitors of lysosomal proteolytic pathways produce beneficial effects on injured musculoskeletal tissue<sup>[2]</sup>.

#### *Protein/enzyme denaturation mechanisms*

Metabolic failure leads to low pH that can cause denaturation of cellular proteins (enzymes)<sup>[5]</sup>. Denaturation of enzymes leads to loss of cellular functionality with the end-result of cell death. Although denaturation seems like an ultimate step in cell death, multiple factors can cause denaturation. Also, a low pH activates many lysosomal enzymes, leading to secondary damage by enzymatic mechanisms. Hence, there is an overlap of enzymatic and denaturation mechanisms that act together to produce secondary damage.

#### *Membrane permeability mechanisms*

Changes in membrane permeability cause: (1) oncosis (cellular swelling) leading to cells bursting; and (2) failure of ion pumps (sodium-potassium-ATPase pump)/changes in voltage gradient and the resulting uncontrolled influx of ions ( $\text{Na}^+$  and  $\text{Ca}^{2+}$ ) leads to cellular death. Increased intracellular  $\text{Ca}^{2+}$  following  $\text{Ca}^{2+}$  influx causes activation of enzyme phospholipase, leading to disruption of the phospholipid membrane and cell death<sup>[10]</sup>.

#### *Mitochondrial mechanisms*

Metabolic failure through mitochondrial (power plant of the cell) damage<sup>[11]</sup> is one of the leading causes of cell death. Mitochondrial failure resulting in insufficient ATP may trigger other lethal pathophysiological processes leading to cell death. This indicates overlap of various theories in this regard.

Causes of mitochondrial injury are: (1) hypoxic or ischemic injury, the oxidative production of ATP is reduced in hypoxia and becomes inadequate for mitochondrial or cellular homeostasis<sup>[2,5,7,11]</sup>. Hypoxia activates a number of phospholipases and proteases that cause progressive failure of mitochondrial ion pumps and damage to the mitochondrial membrane. Also, a number of (intracellular) proteins (stress proteins-heat shock protein 70 family; ubiquitin) that are expressed<sup>[12]</sup> induce protein catabolism within the injured cells; (2) oxidative or reperfusion injury, post-hypoxia reperfusion<sup>[5,11,13]</sup> produces enormous amounts of oxygen-derived free radicals that exceed normal antioxidant defenses and tissue damage results. Re-perfusion free radical damage of the mitochondrial membrane leads to cell powerhouse (mitochondria)

failure. Vasodilatation and hyper perfusion caused by nitric oxide in the post-injury period may accentuate the reperfusion injury; and (3) calcium influx injury, intra-cellular calcium levels increase due to energy dependent  $\text{Na}^+ \text{K}^+$  pump failure and thus, mitochondria act as calcium sinks<sup>[11,14,15]</sup>. Increased mitochondrial  $\text{Ca}^{2+}$  leads to activation of calcium-dependent proteases and phospholipases. Increased mitochondrial  $\text{Ca}^{2+}$  leads to opening of the permeability transition pore, and inner mitochondrial membrane channel. This results in free passage of small molecules, osmotic swelling leading to outer membrane rupture, ATP depletion and apoptotic mitochondrial cell death.

### *Mechanism of systemic secondary tissue injury*

Systemic hypoxia: systemic hypoxia can cause tissue hypoxia of various organs/tissues. Cellular and tissue hypoxia may lead to shock and various types of complications<sup>[16]</sup>.

Delayed neurological changes in high voltage electric burns may be due to changes in the endothelium of small vessels supplying nervous tissues<sup>[17]</sup>. Kidney and heart muscle are damaged due to the release of various intracellular chemicals, hemoglobin and myoglobin<sup>[18]</sup>.

SIRS: follows major trauma, leading to a phase of inflammatory response which involves the synthesis of acute phase proteins by the liver, complement activation and the release of pro- (IL-1, TNF alpha, IL-6, IL-8) and anti-inflammatory cytokines (IL-10, IL-13). If the balance between the pro- and anti-inflammatory cytokines is disrupted, it leads to the development of SIRS and increases morbidity and mortality<sup>[19-22]</sup> [Figure 1].

The auto amplification of cytokine production following major trauma/surgery can occur due to overwhelming multiple organ dysfunction syndrome (MODS) and/or severe infection, leading to a cytokine storm<sup>[23]</sup> [Figure 2]. Infection with exaggerated inflammatory responses induce more cells that produce cytokines and act as catalysts to the cytokine storm<sup>[24,25]</sup>.

Very high level of circulatory pro-inflammatory cytokines, as in a cytokine storm, may cause uncontrolled, auto destruction that leads to life threatening MODS.

Immune depression: cytokines are known to cause immune depression in musculoskeletal trauma<sup>[26]</sup>.

Microvascular dysfunction and increased tissue pressure can occur following musculoskeletal trauma and lead to secondary soft tissue damage and compromised skeletal muscle function<sup>[27]</sup>.

### **Clinical implications of secondary injury**

Repeated debridement and consequent exposure of vital structures - due to secondary injury following initial and surgical trauma, new necrosis occurs, requiring repeated debridement.

Complex reconstructive procedures are required on several occasions to cover vital exposed structures following repeated debridement.

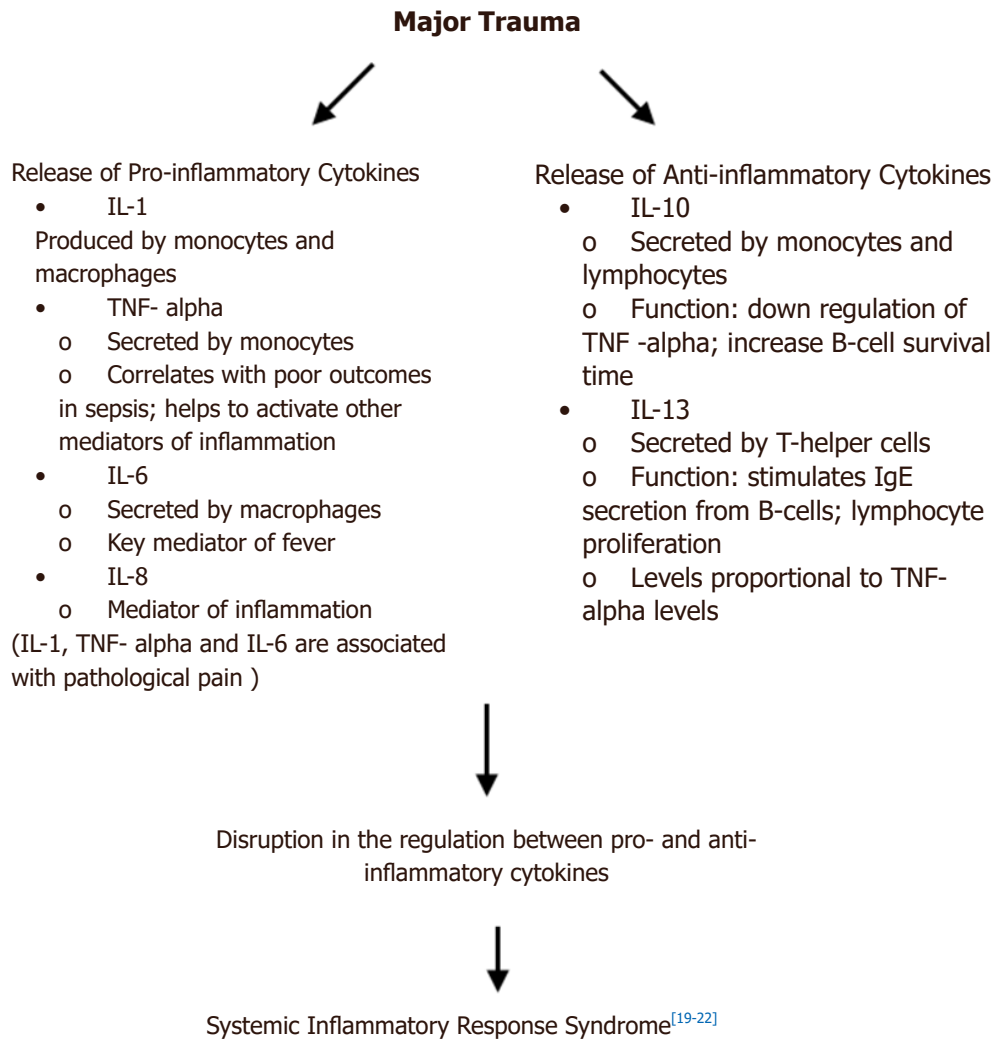
SIRS - Pro-inflammatory cytokines are produced due to tissue damage that increase several times following infection, and if absorbed in large quantities, may produce SIRS, MODS and multiple organ failure.

Immobilization, edema and inflammation lead to functional loss and stiff joints<sup>[28]</sup>.

Missed injuries like spinal injuries may lead to serious crippling injuries<sup>[29]</sup>.

### **Clinical strategy against secondary damage in trauma**

In the management of complex surgical problems, a surgeon has three options<sup>[30]</sup>: (1) avoidance (laissez-faire); (2) aggressive approach; or (3) temporizing maneuvers.



**Figure 1.** Pathogenesis of systemic inflammatory response syndrome

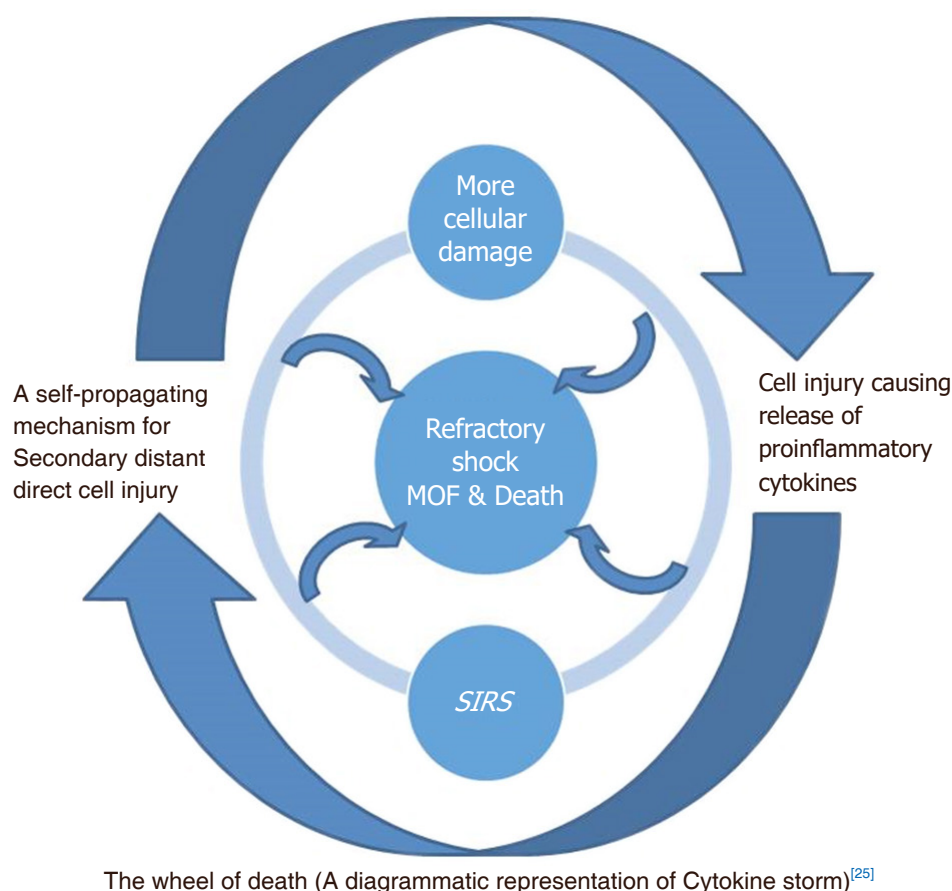
Avoidance leaves natural biochemical mechanism to overcome the damage but in contrast, the insult from an aggressive approach to counter secondary damage may aggravate the problem.

Hence, to counter secondary damage in trauma cases, the best clinical strategy is to employ temporizing maneuvers.

In temporizing maneuvers, damage control methodologies include: (1) quick action to stabilize a patient's local and systemic conditions; (2) prevention of further secondary damage; and (3) after the acute recovery phase is over, secondary definitive management is started.

Rapid and temporary stabilization is achieved by using LAD. Delaying the onset and progress of secondary damage has been attempted by using cryotherapy, but there is a lack of data related to the temperature and duration of cryotherapy required. Secondary, definitive reconstruction may be performed under LAD.

Cryotherapy (exposure to extreme cold) has been used to delay or prevent secondary injury in trauma or sports injury, but optimal tissue temperatures, the duration of cryotherapy, and the need for application pressure to minimize secondary injury following musculoskeletal trauma have yet to be established<sup>[31]</sup>.



**Figure 2.** Diagrammatic representation of cytokine storm - the wheel of death. MOF: multiple organ failure; SIRS: systemic inflammatory response syndrome

Hyperbaric oxygen (HBO): in an experimental study on rats with contused calf muscle injury, following 7 days of treatment with 100% oxygen in a HBO chamber at 2.5 atmospheres pressure for 2 hours/day, HBO was found to reduce edema and inflammation, and accelerated myogenesis. This indicated beneficial effects of HBO against secondary damage. The mechanisms of healing following HBO therapy for muscle injuries have yet to be explained however<sup>[32]</sup>.

Anticytokine and antimediator therapy: has been tried in SIRS and MODS with limited success.

Forced diuresis and dialysis is recommended to prevent and treat renal dysfunction following myoglobinuria/hemoglobinuria<sup>[33]</sup>.

Neurological/neurosurgical management and rehabilitation of neurological secondary injury has been advised<sup>[34,35]</sup>.

Advanced wound management using special modern dressing significantly reduces infection rates and the need for corrective surgeries<sup>[36]</sup>.

### LAD protocol in trauma

LAD is applied over the affected part after saline wash. After surgical removal of obviously dead tissue, application of antimicrobial agents, and complete hemostasis, LAD is applied. Pressure bandaging and appropriate splints are applied as required. Continuous or intermittent negative pressure is applied as per



protocol or as required. The minimum required effective negative pressure produces folds of plastic of LAD that lie snugly over the wound or skin under LAD; higher pressures are guided by pain complaints by the patient and bleeding from the wound. Physiotherapy and occupational therapy may be started at the earliest. Obviously dead tissues are removed surgically and a new LAD is reapplied every 5-7 days. The LAD may be changed if significant leakage occurs. Daily LAD wash with saline and antimicrobial (Betadine) solution is performed. A secondary, elective procedure is performed (e.g., split skin graft/flap *etc.*) as and when required. Physiotherapy may be required after satisfactory healing<sup>[25,30,37]</sup>.

### **Role of LAD**

LAD provides limited access of wound pathogens to the hospital environment and vice versa. LAD is helpful in preventing and treating secondary damage in trauma by reducing edema and removing harmful enzymes, toxins and other chemicals from the open wound<sup>[37]</sup>.

#### ***Effects of LAD on oncosis (cellular swelling)***

Cellular swelling due to sodium and potassium pump failure obstructs the microvasculature, leading to cell death and necrosis of the affected tissue. Intermittent negative pressure of LAD produces intermittent compression of the part under LAD leading to reduction in edema. This intra LAD compression, if combined with early intra LAD physiotherapy, effectively controls edema. Edema reduction thus improves the circulation of adjacent tissues<sup>[37]</sup>.

#### ***Effects of LAD on pH***

In a RCT (randomized control trial)<sup>[38]</sup>, 42 patients with chronic wounds in each group (LAD, conventional dressing) were studied for wound surface pH. On the 10th post-operative day, the LAD group showed a significant ( $P = 0.048$ ) reduction in pH as compared to the conventional dressing group (LAD group  $0.41 \pm 0.26$  vs. conventional group  $0.83 \pm 0.52$ ) with the mean wound surface pH ( $\pm$  SD) in the LAD group  $7.5 \pm 0.43$ .

Following trauma, the release of lysosomal enzymes is responsible for secondary damage. Lysosomal enzyme activity (lysosomal digestion) is optimal in acidic pH ( $< 5$ ), reduced at near neutral pH, and is nearly de-activated at a pH of 7.2<sup>[39]</sup>. Lysosomal enzymes are not only removed by LAD but also deactivated, or its activity reduced by a change in pH under LAD. Hence, LAD may prove to be an effective tool to control secondary damage following trauma.

#### ***LAD provides a safe environment during waiting or temporization***

during the waiting period, infection and SIRS may pose a difficult problem but is controlled effectively with LAD.

1. Control of infection. LAD controls infection in the following ways<sup>[37,40]</sup>: wound isolation and safe disposal of drainage. The important feature of LAD design helps to control infection.

Prevention of wound invasion: negative pressure of LAD provides an alternate channel for the movement of microorganisms. Intermittent or continuous negative pressure reduces bacterial concentrations ( $< 10^5$ /g tissue) to a level that prevents invasion.

Mechanical disruption of quorum sensing by negative pressure occurs as negative pressure prevents the desired concentration of bacterial chemicals through intermittent/continuous removal.

Mechanical disruption of biofilm: Higher intra-LAD negative pressure can cause disruption of the biofilm and expose bacteria in the niche environment to negative pressure.

MDR (multi drug resistant) organisms can be effectively treated: Multi-drug resistant organisms are not resistant to the negative pressure of LAD.

## 2. Control of SIRS

LAD reduces systemic symptoms and signs of toxicity related to traumatized tissues, burns and gangrene. In a study of two groups comprising 54 burn patients (27 in each of LAD and control groups; at the time of induction, both groups showed no significant difference), there was no statistical difference in SIRS on day 1, but SIRS and organ dysfunction on day 5 was significantly lower (*P*-values of 0.029 and 0.017 respectively) in the LAD treated group<sup>[30,37]</sup>.

### *Protection of ischemia induced oxidative damage in LAD treated wounds*

Ischemia induced anaerobic respiration leads to reduced ATP production, and reduced antioxidant protection<sup>[41]</sup>. Studies on LAD treated burn wounds<sup>[42,43]</sup>, diabetic wounds<sup>[44]</sup> and chronic wounds<sup>[45,46]</sup> have shown significant reduction in oxidative stress (malondialdehyde level), and significant increase in antioxidants and nitric oxide levels.

### *Clinical study to find role of LAD in trauma*

In a case series<sup>[30]</sup> of 20 consecutive cases of musculoskeletal extremity trauma treated with LAD without specific controls, 14 cases had exposed, problematic structures with exposed bone in 8 cases, exposed tendons in 3 cases, exposure of both bone and tendon in 2 cases, and an exposed injured brachial artery in 1. Results were quite encouraging. Edema under LAD in these cases was minimal. There was a reduction in the number of debridements: total number of debridement procedures was 23 in 20 patients (average 1.15/patient; range 0-3). Wound bed preparation time was excellent in 5/18 cases, fair in 11/18 cases and poor in 2/18 cases. Excellent (> 99% of grafted area) graft take was seen in 18/20 cases. Conversion rates from cases that required complex reconstructive procedures (e.g., flap) for exposed vital structures to simple reconstructive procedures (SSG) was  $11/13 \times 100 = 84.6\%$ . Functional recovery of the hand was excellent in 4/10 cases, fair in 2/10 cases, and poor in 4/10 cases.

The average cost of treatment was less than one-third of the treatment cost for similar procedures using wet-to-dry dressing (cost calculation was done based on reduced number of debridements, reduced anesthetic requirement, excellent graft take, reduced post-treatment physiotherapy, and rehabilitation costs).

From an administrative point of view, the quality of care was improved due to the reduction in required resources in emergency.

## CONCLUSION

It was concluded that in addition to available medical and surgical interventions, substituting conventional closed dressings with LAD in cases of musculoskeletal trauma helps in reducing secondary damage as evidenced by the reduced number of debridements, reduced wound coverage time, and reduction in total length of hospital stay while lowering treatment costs and improving quality of care.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to the conception and design of the study and performed data analysis and interpretation: Kumar P

Made substantial contributions to data acquisition and performed data analysis and interpretation: Gupta A, Gupta A

**Availability of data and materials**

Not applicable.

**Financial support and sponsorship**

None.

**Conflicts of interest**

All authors declared that there are no conflicts of interest.

**Ethical approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Copyright**

© The Author(s) 2020.

**REFERENCES**

1. Bogens RB, Liu-Snyder P. Understanding Secondary Injury. *Q Rev Biol* 2012;87:89-127.
2. Merrick MA. Secondary injury after musculoskeletal trauma: a review and update. *J Athl Train* 2002;37:209-17.
3. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992;101:1644-55.
4. Knight KL. Cryotherapy in Sports Injury Management. Human Kinetics Champaign, IL: 1995. pp. 3-98
5. Banasik JL. Cell injury, aging and death. In: Costead LC, Banasik JL, editors. *Pathophysiology: Biological and Behavioral Perspectives*. 2nd ed. WB Saunders Philadelphia, PA: 2000. pp. 76-91.
6. Kougias P, Chai H, Lin PH, Yao Q, Lumsden AB, et al. Defensins and cathelicidins: neutrophil peptides with roles in inflammation, hyperlipidemia and atherosclerosis. *J Cell Mol Med* 2005;9:3-10.
7. Belkin M, Brown RD, Wright JG, LaMorte WW, Hobson RW. A new quantitative spectrophotometric assay of ischemia-reperfusion injury in skeletal muscle. *Am J Surg* 1988;156:83-6.
8. Serrano-Puebla A, Boya P. Lysosomal membrane permeabilization in cell death: new evidence and implications for health and disease. *Ann N Y Acad Sci* 2016;1371:30-44.
9. Aits S, Jäättelä M. Lysosomal cell death at a glance. *J Cell Sci* 2013;126:1905-12.
10. Starke PE, Hoek JB, Farber JL. Calcium-dependent and calcium-independent mechanisms of irreversible cell injury in cultured hepatocytes. *J Biol Chem* 1986;261:3006-12.
11. Manjo G, Joris I. Cells, tissues, and disease: principles of general pathology. Blackwell Scientific; Cambridge, MA: 1996.
12. Kim SO, Baines CP, Critz SD, Pelech SL, Katz S, et al. Ischemia induced activation of heat shock protein 27 kinases and casein kinase 2 in the preconditioned rabbit heart. *Biochem Cell Biol* 1999;77:559-67.
13. Sabido F, Milazzo VJ, Hobson RW, Duran WN. Skeletal muscle ischemia-reperfusion injury: a review of endothelial cell-leukocyte interactions. *J Invest Surg* 1994;7:39-47.
14. Bernardi P. Mitochondria in muscle cell death. *Ital J Neurol Sci* 1999;20:395-400.
15. Rizzuto R, Bernardi, Pozzan T. Mitochondria as all round players of the calcium game. *J Physiol* 2000;529:37-47.
16. Kirkman E, Watts S. Haemodynamic changes in trauma. *Br J Anaesth* 2014;113:266-75.
17. Fish R. Electric shock, Part II: nature and mechanisms of injury. *J Emerg Med* 1993;11:457-62.
18. Lee RC. Injury by electrical forces: pathophysiology, manifestations, and therapy. *Curr Probl Surg* 1997;34:677-764.
19. Jaffer U, Wade RG, Gourlay T. Cytokines in the systemic inflammatory response syndrome: a review. *HSR Proc Intensive Care Cardiovasc Anesth* 2010;2:161-75.
20. Socha LA, Gowardman J, Silva D, Correcha M, Petrosky N. Elevation in interleukin 13 levels in patients diagnosed with systemic inflammatory response syndrome. *Intensive Care Med* 2006;32:244-50.
21. Zhang JM, An J. Cytokines, inflammation, and pain. *Int Anesthesiol Clin* 2007;45:27-37.
22. Bates P, Parker P, McFadyen I, Pallister I. Demystifying damage control in musculoskeletal trauma. *Ann R Coll Surg Engl* 2016;98:291-4.
23. Aikawa N. Cytokine storm in the pathogenesis of multiple organ dysfunction syndrome associated with surgical insults. *Nihon Geka Gakkai Zasshi* 1996;97:771-7. (in Japanese)
24. Chousterman BG, Swirski FK, Weber GF. Cytokine storm and sepsis disease pathogenesis. *Semin Immunopathol* 2017;39:517-28.
25. Kumar P. Wheel of death: cytokine cascade (cytokine storm) in cases with extensive wound. *JSWCR* 2013;6:1-2.

26. Reikerås O. Immune depression in musculoskeletal trauma. *Inflamm Res* 2010;59:409-14.
27. Müller M, Disch AC, Zabel N, Haas NP, Schaser KD. Initial intramuscular perfusion pressure predicts early skeletal muscle function following isolated tibial fractures. *J Orthop Surg Res* 2008;3:14.
28. Abghari M, Monroy A, Schubl S, Davidovitch R, Egol K. Outcomes following low-energy civilian gunshot wound trauma to the lower extremities: results of a standard protocol at an urban trauma center. *Iowa Orthop J* 2015;35:65-9.
29. Metak G, Scherer MA, Dannöhl C. Missed injuries of the musculoskeletal system in multiple trauma--a retrospective study. *Zentralbl Chir* 1994;119:88-94. (in German)
30. Kumar PP, Sharma A. The limited access dressing for damage control in trauma patients. *Wounds* 2010;22:188-92.
31. Schaser KD, Stover JF, Melcher I, Lauffer A, Haas NP, et al. Local cooling restores microcirculatory hemodynamics after closed soft-tissue trauma in rats. *J Trauma* 2006;61:642-9.
32. Oyaizu T, Enomoto M, Yamamoto N, Tsuji K, Horie M, et al. Hyperbaric oxygen reduces inflammation, oxygenates injured muscle, and regenerates skeletal muscle via macrophage and satellite cell activation. *Sci Rep* 2018;8:1288.
33. Pezzi M, Giglio AM, Scozzafava A, Serafino G, Maglio P, et al. Early intensive treatment to prevent kidney failure in post-traumatic rhabdomyolysis: case report. *SAGE Open Med Case Rep* 2019;7:2050313X19839529.
34. Walter T, Schwabe P, Schaser KD, Maurer M. Positive outcome after a small-caliber gunshot fracture of the upper cervical spine without neurovascular damage. *Pol J Radiol* 2016;81:134-7.
35. Lammertse DP. Neurorehabilitation of spinal cord injuries following lightning and electrical trauma. *NeuroRehabilitation* 2005;20:9-14.
36. Abdel-Sayed P, Hirt-Burri N, de Buys Roessingh A, Raffoul W, Applegate LA. Evolution of biological bandages as first cover for burn patients. *Adv Wound Care (New Rochelle)* 2019;8:555-64.
37. Kumar P. Exploiting potency of negative pressure in wound dressing using limited access dressing and suction-assisted dressing. *Indian J Plast Surg* 2012;45:302-15.
38. Kumar P, Honnegowda T. Effect of limited access dressing on surface pH of chronic wounds. *Plast Aesthet Res* 2015;2:257.
39. Attaix D, Taillnadier D. The critical role of the ubiquitin-proteasome pathways in muscle wasting in comparison to lysosomal and  $\text{Ca}^{2+}$  dependent systems. In: Bitter EE, Rivert AJ, editors. *Intracellular protein degradation*. JAI Press; Greenwich CT: 1998. pp. 235-66.
40. Kumar P. Limited access dressing and wound infection. *Plast Aesthet Res* 2015;2:237-8.
41. Wu MY, Yiang GT, Liao WT, Tsai AP, Cheng YL, et al. Current Mechanistic Concepts in Ischemia and Reperfusion Injury. *Cell Physiol Biochem* 2018;46:1650-67.
42. Honnegowda TM, Kumar P, Padmanabha Udupa EG, Sharan A, Singh R, et al. A comparative study to evaluate the effect of limited access dressing (LAD) on burn wound healing. *Int Wound J* 2016;13:791-8.
43. Honnegowda TM, Padmanabha Udupa EG, Rao P, Kumar P, Singh R. Superficial burn wound healing with intermittent negative pressure wound therapy under limited access and conventional dressings. *World J Plast Surg* 2016;5:265-73.
44. Honnegowda T, Kumar P, Prabhu K, Kumar A, Rao P, et al. A comparative study to evaluate the effect of limited access dressing on diabetic ulcers. *Plast Aesthet Res* 2015;2:266.
45. Honnegowda TM, Kumar P, Udupa P, Rao P, Bhandary S, et al. Effect of limited access dressing on hydroxyproline and enzymatic antioxidant status in nonhealing chronic ulcers. *Indian J Plast Surg* 2014;47:216-20.
46. Honnegowda TM, Kumar P, Padmanabha Udupa EG, Sharan A, Singh R, et al. Effects of limited access dressing in chronic wounds: A biochemical and histological study. *Indian J Plast Surg* 2015;48:22-8.

Original Article

Open Access



# Lympho-SPECT/CT as a tool to evaluate postoperative outcomes after LVA for lymphedema repair

Jose M. Lasso

Plastic and Reconstructive Surgery, Hospital General Universitario Gregorio Marañón, Madrid 28007, Spain.

**Correspondence to:** Dr. Jose M. Lasso, Plastic and Reconstructive Surgery, Hospital General Universitario Gregorio Marañón, C/ Dr Esquerdo 46, Madrid 28007, Spain. E-mail: josemaria.lasso@salud.madrid.org

**How to cite this article:** Lasso JM. Lympho-SPECT/CT as a tool to evaluate postoperative outcomes after LVA for lymphedema repair. *Plast Aesthet Res* 2020;7:30. <http://dx.doi.org/10.20517/2347-9264.2019.75>

**Received:** 23 Dec 2020 **First Decision:** 19 Mar 2020 **Revised:** 29 Apr 2020 **Accepted:** 4 Jun 2020 **Published:** 18 Jun 2020

**Science Editor:** Xiao Long **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

**Aim:** To describe findings when comparing lympho-SPECT-CT images before and after lymphovenous anastomosis (LVA) surgeries and to correlate these results with pre- and post-operative volume changes in the limbs of patients.

**Methods:** An observational, prospective, longitudinal study was designed. 20 consecutive patients were treated for lymphedema by means of LVA between 2015 and 2018. All were affected by secondary lymphedema (ISG II-III) following lymphadenectomy, radiation or both. All patients received preoperative rehabilitation as well as radiotherapy after oncological surgery. Limb volume was measured before surgery and at one year later. LVA was performed under general anesthesia with ICG guidance. ICG was also used to evaluate postoperative outcomes. Lympho-SPECT-CT was performed in all subjects at their first consultation and at one year after every surgical intervention. Description of findings included an absence of lymph nodes, new lymph node activity in anatomical areas and new lymphatic activity in extra-anatomical areas.

**Results:** Limb volume decreased in 19 patients after LVA. Six patients showed preoperative linear ICG patterns, combined with areas presenting with another type of pattern. After LVA, the linear pattern was observed in 11 patients. SPECT-CT/lymphoscintigraphy before surgery showed a total absence of lymph nodes, except in two cases, in whom small nodes in anatomical locations were described. After LVA, we observed new landmarks in 16 patients corresponding to lymphatic circulation that was not present in preoperative studies. In six cases, new



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





lymphatic activity compatible with lymph nodes was detectable after LVA. The Spearman correlation coefficient was negative when circumferences and lympho-SPECT-CT were tested ( $P = 0.02$ ).

**Conclusion:** Results showed a postoperative decrease in volume that correlated inversely with lympho-SPECT/CT findings. Lympho-SPECT/CT provided additional information related to accurate identification and the anatomical location of lymphatic structures that were not observed before reconstructive surgery. It can be a complementary test to conventional lymphoscintigraphy.

**Keywords:** Lymphedema, supermicrosurgery, lymphovenous anastomosis, LVA, lympho-SPECT/CT, ICG

## INTRODUCTION

Lymphedema is a debilitating disease in which drainage of the lymphatic system is impaired and affects a considerable number in the population. In developed countries, this is prevalent after pelvic or axillary radiotherapy, and lymph node dissection following oncologic surgery of the breast or pelvis. Unfortunately, secondary lymphedema and especially, primary lymphedema are still pathological conditions that cannot be treated definitively. Treatment prospects however, have improved for patients in the last decade due to new surgical techniques.

Current surgical treatments are based on microsurgical techniques<sup>[1-4]</sup>. The two main procedures are: lymph node transfer and lymphovenous anastomosis (LVA). Preoperative clinical evaluation of the patients and imaging studies of the lymphatic system are essential when surgery is planned for. On the other hand, postoperative imaging methods are crucial for understanding the efficacy of surgery.

Among these methods, lympho-SPECT/CT seems to be effective in evaluating lymphatic flow and to document lymph node regeneration. Previous publications have found that it should be a principal tool to complete functional evaluation of lymphedema<sup>[5]</sup>.

We report a prospective study in 20 patients with secondary lymphedema that was treated with LVA. The degree of lymphedema ranged from II to III (ISL classification). Our main goal was to describe new findings using lympho-SPECT-CT images before and after surgery and to correlate these results with the respective volume changes.

## METHODS

An observational, prospective, longitudinal study was designed. 20 consecutive patients treated with LVA for lymphedema at the Hospital Gregorio Marañón in Madrid between 2015 and 2018 were included. All were affected by secondary lymphedema of the lower or upper limbs following surgical lymphadenectomy, radiation or both. Patients were randomly selected in out-patient hospital. The inclusion criteria were: secondary lymphedema of a limb; no previous reconstructive surgery of the lymphatic system; patients with previous rehabilitation therapy were considered for inclusion in the study; those with an ISL classification of lymphedema of degree II and II; and lymphedema had to be established for at least one year before reconstructive microsurgery.

### Clinical evaluation

Pre- and postoperative evaluation was based on imaging and clinical examination. Limb circumference was measured at every 5 cm: point 1 - mid-palm or mid-foot, point 2 - bony landmarks (ulnar styloid process/lateral malleolus), C3-C8 - every 5 cm above point 2. The variable used for evaluation and follow-up was the *circumference difference*, which is equal to the sum of the differences between the involved

and uninvolved limb at all points. The circumference difference was registered at first visit and at each postoperative visit after LVA. Results were considered stable and registered for analysis at the one-year-postoperative visit after surgery.

The clinical examination also included a record of complications, episodes of cellulitis and an enquiry about subjective symptoms. Subjective perception of the disease was evaluated with a patient survey that consisted of four questions utilizing a five-point Likert-type scale<sup>[6]</sup> and a fifth enquiry about overall treatment satisfaction (Satisfaction with limb weight, appearance, firmness, ability to perform daily activities: scores ranging from 1-5, being 5 the highest score; and would you undergo surgery again? yes/no). The questionnaire was developed at our center and has been standardized for use in all lymphedema patients since 2008.

### **Surgical protocol (LVA-ICG)**

In the operating room, ICG (VERDYE™; Diagnostic Green GmbH, Aschheim-Dornach, Germany) was injected intradermally into the first and fourth web spaces (0.1 mL per injection of a 25 mg/mL solution mixed with 5 mL of water for injection), 15 minutes later, circumferential fluorescent images of the lymphatic drainage channels were obtained using an infrared camera system (Photodynamic Eye, PDE™; Hamamatsu Photonics K.K., Hamamatsu, Japan). Lymphography patterns were described at this moment. Linear patterns or patterns that showed dynamic drainage of ICG were marked on the skin. ICG fluorescent images were obtained both before and during ICG-SL surgery and at every year postoperatively. Findings were labeled according to Yamamoto's dermal backflow classification and contrasted with postoperative SPECT-CT/lymphoscintigraphy results. It was found that this ICG lymphangiography staging system had a significant correlation with the clinical stage of lymphedema<sup>[7]</sup>. In the proximal part of the affected limb, dermal backflow patterns were more frequently found than linear patterns. The dermal backflow pattern is further divided into splash, stardust and diffuse patterns.

LVAs were performed under general anesthesia in all cases, with specific material and 12/0 monofilament sutures, under microscope magnification. Anastomoses were performed end-to-end and end-to-side, depending on the availability of recipient veins. Skin closure was achieved with superficial non-absorbable sutures (4/0) without subcutaneous sutures.

### **Postoperative care**

Oral antibiotics were prescribed for five days after surgery. Patients wore a soft non-elastic bandage that was replaced every 3 days for 3 weeks, under surgeons' supervision. Subsequently, from the fourth week, customized compression sleeves were placed for three months. Lymphatic drainage was performed twice a week during this period. After drainage, patients wore elastic stockings as instructed. On the other hand, conservative therapy prior to LVA consisted of lymphatic drainage that started three weeks before (twice per week).

### **Nuclear medicine**

Lympho-SPECT/CT was performed in all subjects at the first consultation and at every year after surgical intervention. Lymphography was done with 46 MBq Tc-99m of nanocolloidal human albumin (Nanocoll, GE Healthcare<sup>®</sup>) injected into the subcutaneous plane at the second web space in each limb (both healthy and affected) after dilution in 0.15 mL of 0.9% sodium chloride. As 85% of colloids are smaller than 80 nm, a 30-gauge needle was required. The interval between injections was less than 30 s.

As patients may have severe alteration of lymphatic flow due to fibrosis or ectasia, they were asked to walk around or elevate the arms for 30 min before obtaining planar images. Conventional lymphoscintigraphy was then performed: anterior and posterior planar images from shoulder to hand (hip to foot) were

acquired to obtain early (30 min) and late (2 h) images with a gamma camera (GAMMG<sup>c</sup>) and low energy-high resolution collimator (LEHR). The acquisition from each time point proceeded over 10 min.

SPECT-CT images were acquired over 30 min, starting at 3 h after injection of the nucleotide using a dual-head combined SPECT/CT camera (Optima<sup>TM</sup> NM/CT640, General Electric) with the following parameters: 128 × 128 matrix, rotation of 360°, 4° view angle, 25 s per projection; slice thickness was 2.5 mm. Two nuclear medicine specialists reviewed all images regarding the detectability and number of lymph nodes.

Description of imaging findings included the absence of nodes, improved drainage of the lymph node basin, new lymphatic drainage in extra-anatomical position, and traces of the nucleotide constituting spots along the lymphatic system of the limb. Data acquired were compared with postoperative SPECT-CT/lymphoscintigraphy results.

### Statistical analysis

Categorical variables were described as numbers and percentages. Continuous variables were described as median (interquartile range). Lympho-SPECT-CT findings were described as: absence of nodes (0); traces of the nucleotide constituting spots along the lymphatic system of the limb (1); improved drainage of the lymph node basin (2); and new lymphatic drainage in extra-anatomical position - elbow, supraclavicular, parascapular (3). Correlation between continuous variables was evaluated using the Spearman coefficient. Statistical significance was defined as  $P < 0.05$ . IBM SPSS software was used for calculation of the above.

## RESULTS

The patients' age ranged from 25 to 76 years old, with a mean of 48.3 years. Sixteen and four patients had upper and lower limb lymphedema respectively. Eighteen patients were female.

The duration of disease from diagnosis to LVA surgery ranged from 15 months to 11 years. Fifteen patients had lymphadenectomy and postoperative radiotherapy (14 in the axilla and 1 in the groin); four had sentinel node biopsy and postoperative radiotherapy (2 axilla, 2 groin) and a single patient had radiotherapy (in the groin after pelvic surgery). A mean of 4.35 (range, 2-10) anastomoses were performed in each affected limb. In all cases, follow-up was more than 12 months after surgery (range, 12-24 months). All patients underwent preoperative rehabilitation, with different criteria. Six of them did not improve with non-surgical therapy while the rest worsened when therapy was discontinued [Table 1].

After LVA, all patients reported a subjective decrease in weight and/or firmness of the limb, and an improvement in their quality of life, with a mean increase in overall satisfaction of 4.3 points in a 20-point survey. The improvement in "satisfaction with limb appearance" was the most significant. All patients responded "yes" to the question "Would you undergo surgery again?" after completing sequential therapy (LVA and rehabilitation).

No severe postoperative complications have been reported to date. Four patients had frequent episodes of cellulitis preoperatively that required hospital admission (three per year or more). None of them have had repeated limb infection except an obese woman with lower limb lymphedema who smoked and did not follow postoperative recommendations.

With respect to volume decrease, the circumference measurements reached near-normal values (less than 6 cm difference) in 15 patients [Table 2].

**Table 1. Progress is measured in months. When expressed in years, it is indicated along the table**

Patient	Age	Sex	Involved limb	ISL classification	Progress of lymphedema	Number of LVA	Preoperative rehabilitation
1	32	F	Upper right	II	25 months	3	Temporarily effective
2	45	F	Upper right	II	24 months	3	Temporarily effective
3	35	M	Lower left	II	3 years	4	Temporarily effective
4	41	F	Upper right	II	18 months	5	Non effective
5	38	F	Upper left	II	4 years	4	Temporarily effective
6	25	F	Lower right	III	15 months	4	Non effective
7	56	F	Upper right	II	25 months	2	Temporarily effective
8	39	F	Upper right	II	11 years	5	Temporarily effective
9	45	F	Upper left	II	3 years	4	Temporarily effective
10	67	F	Upper left	III	40 months	5	Non effective
11	45	F	Lower right	II	38 months	6	Temporarily effective
12	29	M	Upper left	II	3 years	3	Temporarily effective
13	76	F	Lower right	II	28 months	10	Temporarily effective
14	50	F	Upper left	II	24 months	4	Temporarily effective
15	62	F	Upper right	II	4 years	4	Non effective
16	65	F	Upper left	III	5 years	5	Non effective
17	60	F	Upper left	II	6 years	4	Temporarily effective
18	49	F	Upper left	II	25 months	5	Temporarily effective
19	53	F	Upper right	II	23 months	3	Non effective
20	55	F	Upper right	II	30 months	4	Temporarily effective

Temporarily effective: Patients worsened when non-surgical therapy was discontinued; Non effective: Patients non responders to non-surgical therapy. LVA: lymphovenous anastomosis

**Table 2. Results for each patient 1 year after LVA procedure (cm)**

Patient	Circumference difference preLVA (cm)	Circumference difference postLVA (cm)	Satisfaction before surgeries	Satisfaction after surgeries
1	70	58	9	11
2	7.3	6	10	12
3	17	13.5	13	17
4	11.2	11	6	12
5	17	15	7	10
6	30	22	9	15
7	10	8.5	8	14
8	41	39	6	10
9	37	30	8	12
10	23	22.5	7	16
11	10	8	14	19
12	15	12.5	11	17
13	18	12	9	15
14	50	41	11	17
15	20	15	8	14
16	42	43	14	16
17	15	12.3	17	20
18	25	21.2	16	19
19	22	19	8	12
20	32.5	30	12	18

Satisfaction: sum of responses in survey before and after global treatment with lymphovenous anastomosis (LVA)

Figures 1 and 2 show volume reduction in the upper limb of a patient with lymphedema after breast cancer (patient 2).

Table 3 shows pre- and post-operative ICG and SPECT-CT/lymphoscintigraphy results for all patients in our study. Findings of SPECT-CT/lymphoscintigraphy before surgery showed a total absence of lymph



**Figure 1.** Pre-operative view of a ISL second degree lymphedema of the right upper limb showing a more affected cubital area. Red arrow



**Figure 2.** Post-operative view of a ISL second degree lymphedema of the right upper limb. The cubital area improved after lymphovenous anastomosis (red arrow).

nodes, except in two cases, in which several nodes in anatomical (axilla or groin) locations were described. These presented with a smaller size than the healthy, contralateral ones (Figure 3, patient 3).

In 16 patients, there were new landmarks corresponding to lymphatic circulation that were not present in pre-operative studies. In two patients, there were new spots that presented in unexpected anatomical areas, which may be considered as new lymphatic pathways. In six cases, images compatible with new functional lymph nodes were detectable after LVA (Figure 4, patient 14).

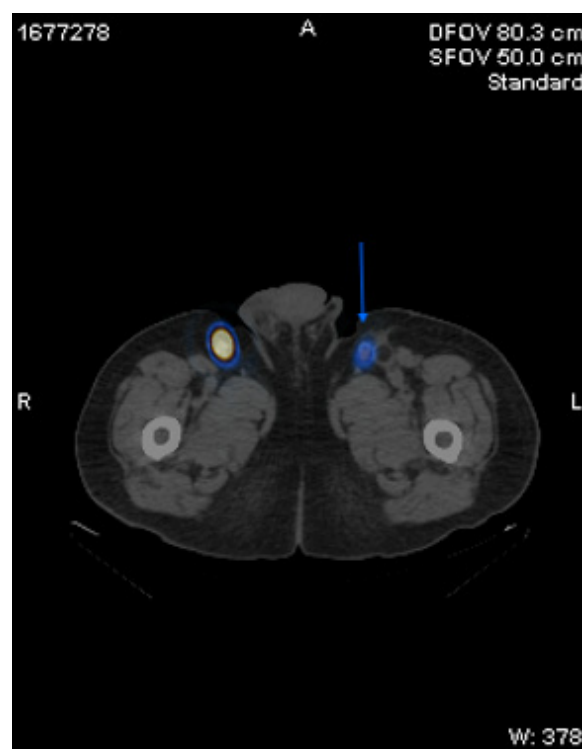
When ICG evaluation was performed before surgery, most patients presented with a diffuse or splash pattern. In six cases, there were segments of a linear pattern in combination with another pattern. One year later, several changes were observed in these patients. Eleven presented with a linear pattern, with four in combination with another pattern [Table 3]. Lymph nodes were not detected with this technique however.

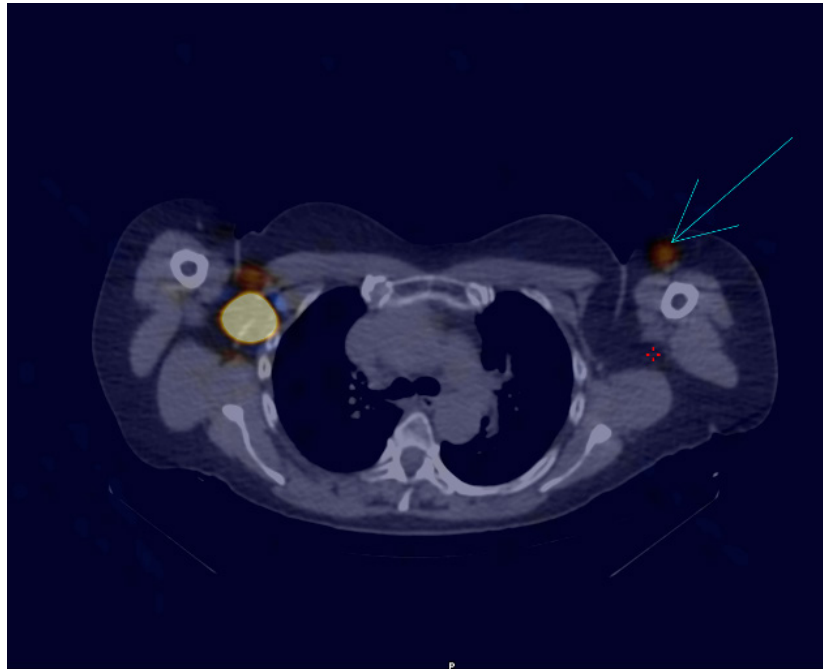


**Table 3. Fluorescence patterns are registered according to the images precepted 15 min after ICG injection**

	PRE-LVA fluorescence pattern	POST-LVA fluorescence pattern	Baseline SPECT-CT/lymphoscintigraphy	Post LVA SPECT-CT/lymphoscintigraphy
1	Diffuse/stardust	Stardust	-	-
2	Diffuse/stardust	Diffuse/linear in some areas	-	++
3	Diffuse/stardust	Stardust	-	+
4	Stardust	Linear	-	+*
5	Stardust	Linear	-	+
6	Linear, stardust	Linear - slow progression	-	+
7	Linear/stardust	Linear	-	++
8	Diffuse	Diffuse	-	+
9	Stardust/splash	Splash	-	+
10	Linear/splash	Linear	+	+
11	Linear/stardust	Linear	-	++
12	Diffuse	Diffuse	-	+
13	Splash	Splash	-	-
14	Stardust	Linear/splash	-	++*
15	Diffuse/splash	Splash	-	+
16	Diffuse	Linear/diffuse	-	-
17	Linear/stardust	Linear	-	++
18	Stardust	Stardust	-	+
19	Linear/splash	Linear	-	+
20	Splash	Splash	-	+

SPECT-CT/lymphoscintigraphy results are described at baseline and 1 year after lymphovenous anastomosis (LVA). Results are marked as follows: -: No detectable migration of the tracer from the site of injection to the groin or axilla, lymphatic leakage or dermal backflow; +: The report of new images of lymphatic flow along the limb; ++: The presence of lymph nodes at any point in the limb; \*The presence of extraanatomical lymphatic flow; Note that fluorescence patterns are detected 15 min after injection and SPECT-CT/lymphoscintigraphy images are obtained 3 h after injection. This evaluation was performed the day before LVA and 1 year after it. Patterns are labeled according to Yamamoto's dermal backflow classification<sup>[11]</sup>

**Figure 3.** Pre-operative lympho-SPECT showing a lymph node at the root of the affected limb (left limb) that is smaller than the contralateral one (blue arrow).



**Figure 4.** New lymph node at the arm of the patient with lymphedema of the left upper limb, at one year after lymphovenous anastomosis (blue arrow). The contralateral side (right axilla) is marking a functional nodal group

Figure 5 shows that in patient number 8, the stardust pattern had changed and new reticular and linear superficial patterns were observed after LVA. When lympho-SPECT/CT was correlated with these images, new images that were not visible before surgery could be observed.

The Spearman correlation coefficient was significantly negative when circumferences and lympho-SPECT-CT were tested ( $P = 0.02$ ).

## DISCUSSION

Lymphoscintigraphy is a specific, simple and reliable technique that offers useful information of lymphatic function, allowing the examiner to detect lymphatic flow obstruction, dilated vessels, collateral lymphatic flow and the presence, malfunction or absence of lymph nodes<sup>[8-10]</sup>. Dermal uptake is a pathognomonic sign of lymphedema in limbs, and this finding is seen in most cases<sup>[11,12]</sup>. However, lymphoscintigraphy has limitations such as a two-dimensional view that does not allow projection onto anatomical landmarks. On the other hand, some artefacts can be observed when fibrosis, dermal backflow or accumulation in deeper vessels is seen<sup>[13]</sup>.

SPECT-CT is currently combined with lymphoscintigraphy for the detection of sentinel lymph nodes in the diagnosis of several tumours<sup>[14]</sup>. In lymphedema, SPECT-CT/lymphography systems provide integrated functional and morphological information, which allows better localization of the depth of vessels and lymph nodes. It is useful for accurate evaluation of anatomical differences between lymphatic vessels and veins and as well, it can provide better understanding of dermal backflow<sup>[15]</sup>.

SPECT-CT might be useful for predicting treatment efficacy when staging lymphedema<sup>[16-18]</sup>. In fact, combined CT-imaging has allowed better comprehension of the pathophysiology of lymphedema. Many studies have also proposed using SPECT-CT imaging beyond diagnosis by reporting its value in microsurgical treatment, which is essential for understanding the behavior of the lymphatic system after LVA or lymph node transfer.



**Figure 5.** Lympho-SPECT, lymphography and ICG evaluation in patient 8. A: Software reconstruction showing a positive node in the right infraclavicular area and elbow after lymphovenous anastomosis (LVA). Orange and yellow arrows are marking the new activity. We can also observe the right breast implant for reconstruction, as well as lymphography under three-dimensional reconstruction (blue arrow); B: development of linear and reticular patterns one year after LVA in the forearm of this patient

In this study, we evaluated the changes in lymphatic drainage one year after LVA and corroborated the effectiveness of surgical treatment. Our study population included a small group of randomly selected patients that were included in a prospective study. After analysis of lympho-SPECT-CT findings, we reported that in a majority of patients, regardless of the degree of lymphedema, there was evidence corresponding to new lymphatic circulation in images of the limbs (upper and lower limbs). We believe that LVA was not only useful in the reestablishment of lymphatic drainage through the venous by-passes, but also in developing collateral circulation (especially in two cases with extraanatomical landmarks). This could be explained in relation to the activation of new routes of drainage<sup>[19]</sup>. Cases with new lymph nodes may indicate that drainage is greater, as has been demonstrated in experimental models<sup>[20]</sup>. Interestingly, ICG findings are in concordance with these positive landmarks but we could not compare qualitative variables with quantitative ones to perform a better statistical analysis. In seven cases, we clearly observed that the linear pattern<sup>[21,22]</sup> obtained after LVA was accompanied by more remarkable findings on lympho-SPECT-CT.

On the other hand, we were able to evaluate the correlation between clinical parameters such as the volume of the limbs and findings on lympho-SPECT. In this case, we observed an inverse correlation coefficient with statistical significance, indicating that the reduction of limb volume that is observed after LVA is associated with a significant increase in the number of landmarks that support improvement and reestablishment of lymphatic drainage. There are many factors influencing the final result. This is a heterogeneous group of patients, with different ages and time of evolution of lymphedema. Our work includes both upper and lower limb lymphedema of different ISL stages, which make comparisons difficult. It has also been demonstrated that LVA studies have a poor quality of evidence and high risk of bias, and therefore, it is difficult to draw reliable conclusions on the clinical effectiveness of LVA<sup>[23]</sup>.

Nevertheless, LVA seems to be safe for the treatment of primary and secondary lymphedema. If a reduction of volume can be demonstrated after LVA, we may think that lymphatic drainage has improved. In order to prove this, nuclear medicine testing would be an objective method to do so. If we are able to demonstrate such improvement by means of functional and anatomical landmarks (the development of new lymph nodes or the establishment of collateral circulation) that were not visible before surgery, we would then be in a position to confirm that surgery has been effective as it has been demonstrated that <sup>99m</sup>Tc-phytate lymphoscintigraphy with SPECT-CT can provide both functional and morphological information simultaneously in patients with upper-limb lymphedema<sup>[24]</sup>. Using this modality, SPECT-CT may accurately reflect lymphodynamic conditions of the limbs after LVA. Furthermore, the surgical therapeutic efficacy could be estimated quantitatively by comparing pre- and postoperative findings.

In conclusion, our results have showed a postoperative decrease in volume of limbs with lymphedema that correlated inversely with lympho-SPECT/CT findings. Lympho-SPECT/CT provided additional information related to the accurate identification and anatomical location of lymphatic structures that were not observed before reconstructive surgery. It should be a complementary test to conventional lymphoscintigraphy.

## DECLARATIONS

### Authors' contributions

The author contributed solely to the article.

### Availability of data and materials

Data supporting these findings can be found. Data can be deposited into the data repositories of the hospital, which can be accessed but not sent externally except for legal reasons.

## Financial support and sponsorship

None.

## Conflicts of interest

The author declared that there are no conflicts of interest.

## Ethical approval and consent to participate

Patients signed and gave informed consent for every operation and participation.

## Consent for publication

The authors and patients gave consent for publication.

## Copyright

© The Author(s) 2020.

## REFERENCES

1. Koshima I, Kawada S, Moriguchi T, Kajiura Y. Ultrastructural observation of lymphatic vessels in lymphedema in human extremities. *Plast Reconstr Surg* 1996;97:397-405.
2. Koshima I, Inagawa K, Urushibara K, Moriguchi T. Supermicrosurgical lymphaticovenular anastomosis for the treatment of lymphedema in the upper extremities. *J Reconstr Microsurg* 2000;16:437-42.
3. Koshima I, Nanba Y, Tsutsui T, Takahashi Y, Itoh S. Long-term follow-up after lymphaticovenular anastomosis for lymphedema in the legs. *J Reconstr Microsurg* 2003;19:209-15.
4. Koshima I, Nanba Y, Tsutsui T, Takahashi Y, Itoh S, et al. Minimal invasive lymphaticovenular anastomosis under local anesthesia for leg lymphedema: is it effective for stage III and IV? *Ann Plast Surg* 2004;53:261-6.
5. Blum KS, Radtke C, Knapp WH, Pabst R, Gratz KF. SPECT-CT: a valuable method to document the regeneration of lymphatics and autotransplanted lymph node fragments. *Eur J Nucl Med Mol Imaging* 2007;34:1861-7.
6. Likert R. A technique for the measurement of attitudes. *Arch Psychol* 1932;22:55.
7. Yamamoto T, Narushima M, Doi K, Oshima A, Ogata F, et al. Characteristic indocyanine green lymphography findings in lower extremity lymphedema: the generation of a novel lymphedema severity staging system using dermal backflow patterns. *Plast Reconstr Surg* 2011;127:1979-86.
8. Szuba A, Shin WS, Strauss HW, Rockson S. The third circulation: radionuclide lymphoscintigraphy in the evaluation of lymphedema. *J Nucl Med* 2003;44:43-57.
9. Williams WH, Witte CI, Witte MH, McNeill GC. Radionuclide lymphangioscintigraphy in the evaluation of peripheral lymphedema. *Clin Nucl Med* 2000;25:451-64.
10. Maegawa K, Miami T, Yamamoto Y, Satake T, Kobayashi S. Types of lymphoscintigraphy and indications for lymphaticovenous anastomosis. *Microsurgery* 2010;30:437-42.
11. Witte CL, Witte MH, Unger EC, Williams WH, Bernas MJ, et al. Advances in imaging of lymph flow disorders. *Radiographics* 2000;20:1697-19.
12. International Society of Lymphology. The diagnosis and treatment of peripheral lymphedema: 2013 consensus document of the international society of lymphology. *Lymphology* 2013;46:106-19.
13. Baulieu F, Bourgeois P, Maruani A, Belgrado JP, Tauveron V, et al. Contributions of SPECT/CT imaging to the lymphoscintigraphic investigations of the lower limb lymphedema. *Lymphology* 2013;46:106-19.
14. Cheville AL, Brinkmann DH, Ward SB, Durski J, Laack NN, et al. The addition of SPECT/CT lymphoscintigraphy to breast cancer radiation planning spares lymph nodes critical for arm drainage. *Int J Rad Onc Bio Phys* 2013;85:971-7.
15. Iimura T, Fukushima Y, Kumita S, Ogawa R, Hyakusoku H. Estimating lymphodynamic conditions and lymphovenous anastomosis efficacy using 99mTc-phytate lymphoscintigraphy with SPECT-CT in patients with lower-limb lymphedema. *Plast Reconstr Surg Glob Open* 2015;3:e404.
16. Weiss M, Baumeister RG, Frick A, Wallmichrath J, Bartenstein P, et al. Primary lymphedema of the lower limb: the clinical utility of single photon emission computed tomography/CT. *Korean J Radiol* 2015;16:188-95.
17. Pecking AP, Albérini JL, Wartski M, Edeline V, Cluzan RV. Relationship between lymphoscintigraphy and clinical findings in lower limb lymphedema (LO): toward a comprehensive staging. *Lymphology* 2008;41:1-10.
18. Campisi C, Boccardo F. Microsurgical technique for lymphedema treatment: Derivative lymphatic-venous microsurgery. *World J Surg* 2004;28:609-13.
19. Brouwer OR, Vermeeren L, van der Ploeg IM, Valdés Olmos RA, Loo CE, et al. Lymphoscintigraphy and SPECT/CT in multicentric and multifocal breast cancer: does each tumour have a separate drainage pattern? Results of a Dutch multicentre study (MULTISENT). *Eur J Nucl Med Mol Imaging* 2012; 39:1137-43.



20. Blum KS, Hadamitzky C, Gratz KF, Pabst R. Effects of autotransplanted lymph node fragments on the lymphatic system in the pig model. *Breast Cancer Res Treat* 2010;120:59-66.
21. Narushima M, Yamamoto T, Ogata F, Yoshimatsu H, Mihara M, et al. Indocyanine green lymphography findings in limb lymphedema. *J Reconstr Microsurg* 2016;32:72-9.
22. Chen WF, Zhao H, Yamamoto T, Hara H, Ding J. Indocyanine green lymphographic evidence of surgical efficacy following microsurgical and supermicrosurgical lymphedema reconstructions. *J Reconstr Microsurg* 2016;32:688-98.
23. Rosian K, Michal Stanak M. Efficacy and safety assessment of lymphovenous anastomosis in patients with primary and secondary lymphoedema: a systematic review of prospective evidence. *Microsurgery* 2019;39:763-72.
24. Visconti G, Hayashi A, Tartaglione G, Salgarello M, Yamamoto T. Innovative surgical treatment of peripheral lymphedema after breast cancer surgery. *Transl Cancer Res* 2018;7:S365-78.

Review

Open Access



# Stem cells and tissue engineering: an alternative treatment for craniofacial congenital malformations and articular degenerative diseases

Cristina Velasquillo<sup>1,\*</sup>, Antonio Madrazo-Ibarra<sup>1,\*</sup>, Claudia Gutiérrez-Gómez<sup>1,2</sup>, Marcia Rosario Pérez-Dosal<sup>3</sup>, Yaaziel Melgarejo-Ramírez<sup>1</sup>, Clemente Ibarra<sup>4</sup>

<sup>1</sup>Biología, Instituto Nacional de Rehabilitación LGII, Ciudad de México 14389, México.

<sup>2</sup>Hospital General Dr. Manuel Gea González and Instituto Nacional de Rehabilitación LGII, Ciudad de México 14080, México.

<sup>3</sup>Médico Adscrito al Servicio de Cirugía Plástica, Instituto Nacional de Pediatría, Ciudad de México 04530, México.

<sup>4</sup>Dirección General, Instituto Nacional de Rehabilitación LGII, Ciudad de México 14389, México.

\*These authors contributed equally to this work as first.

**Correspondence to:** Dr. Cristina Velasquillo, Biología, Instituto Nacional de Rehabilitación LGII, Ciudad de México 14389, México. E-mail: mvelasquillo@ciencias.unam.mx; and Dr. Clemente Ibarra, Dirección General, Instituto Nacional de Rehabilitación LGII, Ciudad de México 14389, México. E-mail: clementeibarra@yahoo.com

**How to cite this article:** Velasquillo C, Madrazo-Ibarra A, Gutiérrez-Gómez C, Pérez-Dosal MR, Melgarejo-Ramírez Y, Ibarra C. Stem cells and tissue engineering: an alternative treatment for craniofacial congenital malformations and articular degenerative diseases. *Plast Aesthet Res* 2020;7:31. <http://dx.doi.org/10.20517/2347-9264.2020.30>

**Received:** 4 Mar 2020 **First Decision:** 15 May 2020 **Revised:** 2 Jun 2020 **Accepted:** 9 Jun 2020 **Published:** 24 Jun 2020

**Science Editor:** Yi-Lin Cao **Copy Editor:** Cai-Hong Wang **Production Editor:** Tian Zhang

## Abstract

The life quality of patients with craniofacial malformations is severely affected by the physical disabilities caused by the malformation itself, but also by being subjected to bullying, which leads to a series of relevant psychological and societal effects that have an economic impact on the health sector. Orofacial clefts, notably cleft lip (CL), cleft palate, and microtia, are the most common craniofacial birth defects in humans and represent a substantial burden, both personal and societal. On the other hand, osteoarthritis is a widespread degenerative disease that is becoming more common due to the extension of the human lifespan and to an increase in injuries in young people as a result of their lifestyle. Advances in tissue engineering as a part of regenerative medicine offer new hope to patients that can benefit from new tissue engineering therapies based on the supportive action of tailored 3D biomaterials and the synergic action of stem cells that can be driven to the process of bone and cartilage regeneration. This review provides an update on recent considerations for stem cells and studies on the use of advanced biomaterials and cell therapies for the regeneration of craniofacial congenital malformations and articular degenerative diseases.

**Keywords:** Mesenchymal stromal cells, microtia, cartilage, cleft lip, cleft palate



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



## INTRODUCTION

According to the National Cancer Institute, degenerative disease is a pathology in which the function or structure of the affected tissues or organs worsens over time<sup>[1]</sup>. Unfortunately, neither most degenerative diseases nor craniofacial congenital malformation diseases have a cure, so they evolve until patients become severely disabled. Since stem cells became an alternative treatment, they have changed the course of these diseases. Their applications are currently being tested and have shown positive results in several of these diseases.

Stem cells are cells with self-renewal and differentiation abilities. Mesenchymal stem cells (MSC) are adult stem cells that are not hematopoietic and can be found in several tissues, such as adipose tissue, bone marrow, and umbilical cord, to mention some examples. According to the International Society for Cell Therapy (ISCT), MSC must (1) be plastic-adherent; (2) express CD105, CD73, and CD90; (3) lack CD45, CD34; and (4) differentiate into osteoblasts, adipocytes, and chondroblasts<sup>[2]</sup>; however, these criteria do not suffice to justify their therapeutic potential<sup>[3]</sup>. Besides their differentiation ability, MSC have paracrine activity in angiogenesis, cellular activation/proliferation, and immunomodulation<sup>[4,5]</sup>. Since they were first introduced in 1970 by Friedenstein, MSC have changed the treatment of individuals with orthopedic, hematologic, oncologic, ophthalmologic and dermatologic conditions. They have been used mainly to replace cell lines that have been lost or destroyed or to modify the behavior of other cells.

In this paper, we will briefly describe the applications of MSC in common degenerative and congenital diseases in Mexico.

## DEFINING THE REGENERATIVE POTENTIAL OF MSC BEYOND BIOLOGY

For many years, the use of autologous cells isolated directly from biopsies was the only alternative for tissue engineering applications. Fully differentiated cells tend to lose cellular features if they are exposed to a constant cellular division. These cellular features include changes in the extracellular matrix (ECM), protein synthesis, altered metabolism, and dedifferentiation. Regenerative therapies commonly need a high number of cells, leading to the search of cells with high regenerative potential and no risk of morphological features loss. Mesenchymal stromal cells have become a promising alternative since they are one of the first cells in cellular lineage with unlimited fashion propagation and an extensive differentiation ability<sup>[3]</sup>.

The analysis of the potential of MSC for therapeutic purposes can be conducted at different stages. Typically, the mesenchymal phenotype according to the ISCT criteria should be verified; however, additional surface markers have been described, which include being positive for CD29, and negative for CD14, CD11b CD19, CD79 alpha, and HLA-DR surface markers. Differentiation protocols can also be analyzed based on the expression of these markers in chondrogenic, adipogenic, or osteogenic lineages. For example, osteogenic differentiation can be confirmed with alkaline phosphatase activity, calcium release after osteogenic stimulation, catalase (osteoclast inhibitor), and glutathione peroxidase 3 (osteogenic biomarker) expression<sup>[6]</sup>. Transcriptional analysis at mRNA levels is another alternative to track the therapeutic potential of MSC. It is possible to estimate cellular growth and colony-forming potential quantifying the MSC marker STRO-1 and the platelet-derived growth factor receptor A (PDGFR-alpha). A transcriptional increase of Twist-related protein-1 (TWIST-1) and Twist-related protein-2 (DERMO-1) has also been described as crucial for MSC growth and development<sup>[7]</sup>.

There has been a continuous debate about whether autologous or heterologous cells are the most adequate source of MSC in regenerative therapies for congenital and craniofacial diseases. Their immunomodulatory ability is a relevant aspect exerted through the inhibition of T-cell proliferation, which regulates the immune response, and is also involved in the alloimmune response. Autologous MSC have been shown to decrease *in vitro* alloimmune response in host autologous cells in transplanted murine models. It has

been proposed that the homing activity of MSC creates immune-privileged sites that limit the infiltration of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in tissues, thus limiting damage and promoting regeneration<sup>[8]</sup>. Meanwhile, heterologous MSC from bone marrow (BM-MSC) have been used for the treatment of pseudoarthrosis and have been proved to promote healing of femoral fractures in a claudication animal model. Heterologous BM-MSC reached the lesion 24 h after being infused, and later promoted a periosteal reaction that lead to fracture consolidation and cartilage formation 120 days after the infusion. In comparison, BM-MSC alone formed a fibro-osteoid tissue<sup>[9]</sup>. These effects lead us to elucidate that the use of autologous versus allogeneic MSC will depend on the required clinical outcome.

In recent years, clinical implications and advantages in the use of stromal vascular fraction (SVF) have opened new alternatives for tissue engineering in craniofacial or degenerative diseases. The differences between SVF and adipose-derived MSC (AD-MSC) are that an SVF is a freshly harvested, heterogeneous population of cells directly isolated from lipoaspirates by mechanical or enzymatic disaggregation that contains stromal cells (15%-30%), erythrocytes, granulocytes, monocytes, pericytes, and endothelial cells<sup>[10]</sup>. AD-MSC are a cultured, more homogeneous subpopulation of cells resulting from a culture selection and *in vitro* expansion. On the other hand, compared to BM-MSC, adipose tissue contains 100-500 fold more MSC, and SVF contains 4-6 fold more MSC, whose therapeutic impact, angiogenic stimulation, T-cell regulation and reduction of IL-10 production represent a feasible source for tissue engineering<sup>[11]</sup>.

Although there are still difficulties to establish the proper dose and clinical safety protocols, there is no doubt of the potential of AD-MSC to accelerate healing processes. Therapeutic efforts for the treatment of degenerative diseases have moved research groups to develop semi-automated, surgically-closed systems to obtain SVF during surgeries with minimal laboratory equipment requirements that will enable the application and implantation of autologous or heterologous MSC for tissue engineering<sup>[12]</sup>.

### Degenerative diseases

A degenerative disease is a pathology in which the function or structure of the affected tissues or organs worsens over time<sup>[1]</sup>. As mentioned earlier, stem cells have changed the course of these diseases and have become an alternative treatment for degenerative disorders.

### Osteoarthritis

Osteoarthritis (OA) is a condition that causes joints to hurt and become stiff. It is the most common cause of arthritis worldwide, and it mainly affects knees (85%), hips, hands, and feet. Approximately 240 million people in the world have OA<sup>[13]</sup>. 5% of adults worldwide have either hip or knee OA. These numbers will increase as the population ages and the obesity rates increases<sup>[14]</sup>. Pain is the main symptom that typically leads patients to seek medical care and guides clinicians into treatment decision-making as well. Pain can be so intense, patients become unable to work, making OA the fourth leading cause of years lived with disability worldwide<sup>[15]</sup>.

OA has been part of the changes of articular cartilage, but that concept has evolved, now considering the whole joint<sup>[16,17]</sup>. Some of the structural damages of joints are (1) loss of cartilage; (2) osteophyte formation; (3) subchondral bone changes; and (4) meniscal alterations<sup>[17]</sup>. Chondral erosions caused by overload or abnormal joint kinematics turn into fissures. In an attempt to repair these lesions, hypertrophic chondrocytes increase their synthetic activity, but, by doing that, they increase the production of proinflammatory mediators and degradation products. These molecules stimulate surrounding synovium, increasing its proliferation, and proinflammatory response as well. All inflammation mediators favor endochondral ossification, causing bone overgrowth and osteophyte formation. Pain comes from the peripheral nociceptors sensing ongoing tissue injury, as well as inflammation in the joint<sup>[16]</sup>.

Nowadays, treatment towards OA is oriented towards minimizing pain, optimizing function, and modifying the process of joint damage. Pain control, as mentioned earlier, is what guides the physician's decision into which treatment to use. Analgesics and anti-inflammatory medications are the mainstay treatment, accompanied by lifestyle modifications such as weight loss and physical therapy/activity<sup>[18]</sup>. Since no medication has been shown to stop the process of OA, measures have been taken to prevent it. Focal cartilage lesions, if left untreated, tend to quickly progress into osteoarthritis.

A retrospective study performed in the National Institute of Rehabilitation in Mexico reported that 61% of the patients undergoing arthroscopic surgery had focal chondral lesions in the knee, with 74% of these being grade III-IV ICRS/Outerbridge<sup>[19]</sup>. Cartilage repair techniques, such as microfractures, autologous chondrocyte implantation, and mosaicplasty have shown to delay the appearance of OA, as well as the need for total joint replacement after chondral injuries in young adults<sup>[20-23]</sup>. Some biological therapies have been researched, including drugs that promote chondrogenesis and osteogenesis<sup>[24]</sup>, matrix degradation inhibitors, apoptosis inhibitors, and anti-inflammatory cytokines<sup>[25]</sup>; however, none of them have demonstrated sufficient symptom improvement to be included in the standard of care<sup>[26]</sup>.

Mesenchymal stem cells have turned into the most explored therapeutic drug in cell-based OA treatment due to their ability to differentiate to chondrocytes and their immunomodulatory properties<sup>[27]</sup>. Furthermore, they have been used in different ways to try and modify the course of the disease.

#### *MSC seeded on scaffolds*

Cartilage implants: by taking advantage of the differentiation capacity of MSC to chondrocytes, MSC have been similarly used for cartilage lesion repair as matrix-assisted autologous chondrocyte implants. Previous studies using chondrocytes seeded on collagen or polyglycolic-acid matrixes have shown good mid- to long-term clinical and magnetic resonance imaging (MRI) outcomes, as well as the ability to delay degenerative changes in the knee<sup>[28-31]</sup>.

A few years ago, the United States Food and Drug Administration approved MACI, a porcine collagen membrane seeded with autologous chondrocytes, for the treatment of focal chondral lesions in the knee<sup>[32]</sup>. Okano *et al.*<sup>[33]</sup> came up with the "cell sheet technology" consisting of multiple cell layers placed on top of another (instead of using a matrix), taking advantage of the intact ECM produced by the cultured chondrocytes and their adhesion factors. This innovative technique has been shown to form hyaline cartilage in preclinical studies and is currently undergoing clinical studies in Japan<sup>[34-36]</sup>. Even though these techniques have had great outcomes, they involve two surgical procedures: one to obtain the cartilage biopsy and the second one for the implantation. This makes the intervention expensive and may increase the risk of surgical complications. MSC seeded on a 3-dimensional scaffold or using the cell sheet technology can help solve this problem. Due to endogenous cell stimulation, MSC differentiate into cartilage, forming a cartilage-like tissue repair<sup>[37]</sup>. Several clinical and preclinical studies using MSC seeded on matrixes have shown positive results in forming cartilage-like tissue and alleviating symptoms<sup>[38,39]</sup>.

In 2015, Kim *et al.*<sup>[40]</sup> conducted a comparative matched paired analysis comparing injected vs surgically implanted MSC in patients with knee osteoarthritis. Patients were evaluated with Patient-Reported Outcome Measures (PROMs), as well as a second-look arthroscopy. After a minimum follow-up of 24 months, patients who underwent MSC implantation showed better clinical and second-look arthroscopic outcomes. Despite the positive findings with this technique, it is usually employed to repair small defects and does not address larger areas related to OA. Problems related to the acquisition of autologous MSC and the risk of graft-versus-host reactions with allogeneic MSC have limited their use in clinical studies.

Meniscus repair: menisci play an important role in load-bearing and load transmission to the cartilage and subchondral bone. Approximately 15% of knee lesions are associated with damage to the meniscus<sup>[41]</sup>.



Meniscal lesions generate knee instability and further cartilage damage favoring the development of OA. Treatment for meniscal lesions is decided depending on the complexity and the location of the damage. Repair strategies are used when the rupture is small, located in the vascular areas, and the meniscus can be stabilized intra-articularly. However, partial meniscectomy or complete meniscectomy is required in complex lesions. Meniscectomies cause an increase of 235% contact pressure<sup>[42]</sup>, as well as an increase in OA incidence<sup>[43-45]</sup>. The use of meniscal substitutes after partial meniscectomy has shown symptom relief, as well as a slow decrease of articular degeneration; however, they do not prevent it<sup>[46,47]</sup>.

Leroy *et al.*<sup>[46]</sup> reported a decrease in scaffold dimensions leading to a concern about the scaffold's capacity in the long term. The use of MSC in combination with meniscal substitutes have become of great interest due to the evidence of meniscal-like tissue formation after implantation in rats, pigs, and rabbits<sup>[48,49]</sup>. Olivos-Meza *et al.*<sup>[50]</sup> conducted a comparative study between patients who received meniscal substitution with acellular polyurethane meniscal scaffolds (APS) vs. polyurethane scaffold enriched with peripheral blood MSC (MPS). They evaluated femoral and tibial articular cartilage status using MRI T2-mapping 3, 6, 9, and 12 months after surgery, as well as clinical evaluation using PROMs. No differences were observed between APS and MPS during the 12-month follow-up; however, a longer follow-up is needed to see the scaffold degeneration and tissue formation.

MSC exosomes: exosomes are extracellular vesicles that function as intercellular communication vehicles transferring lipids, nucleic acids (mRNA and microRNAs) and proteins to generate a response in recipient cells<sup>[51]</sup>. Exosomes are rich in microRNA, which can bind specific sites in transcribed mRNA, modifying their expression and transduction<sup>[51,52]</sup>. These properties have been studied to promote cartilage regeneration and decrease pro-inflammatory molecules in OA<sup>[53-57]</sup>. Tao *et al.*<sup>[56]</sup> and Toh *et al.*<sup>[58]</sup> reported several microRNAs (140-5p, 23b, 92a, 125b, 320, 145, 22 and 221) derived from human synovial MSC, which promote cartilage regeneration, OA suppression, and cartilage/extracellular matrix homeostasis in preclinical studies. The exosomes' potential for OA treatment, good tolerance, and minimal risk of immunogenicity and toxicity has made them one of the most important hotspots for future research. However, further studies describing how to obtain large-scale purified exosomes as well as their clinical efficacy and biosecurity are still needed.

Intra-articular injections: intra-articular injections of MSC have become the main modality of cell therapy research for OA treatment due to their simple application thanks to their anti-inflammatory, immune-regulatory, and regenerative abilities. MSC can be either injected with no other components or mixed with hyaluronic acid (HA), platelet-rich plasma (PRP), or saline solution, to mention some examples. Preclinical studies have shown cartilage repair, reduction in proinflammatory cytokines, and improved imaging, morphology, and histology<sup>[59,60]</sup>. Mixed injections with PRP/MSC or HA/MSC have shown significantly better results on the repaired cartilage than individual uses of any of them.

Several clinical trials have been developed worldwide using MSC derived from the stromal vascular fraction (SVF), umbilical cord (UC-MSC), adipose tissue (AD-MSC) or bone-marrow (BM-MSC), the latter being the most common site. BM-MSC have shown a better chondrogenic ability compared to AD-MSC<sup>[61]</sup> and have shown an improvement in cartilage quality and knee function, as well as a decrease in pain and other symptomatology<sup>[27]</sup>. Most clinical trials that use AD-MSC and SVF have been conducted using mixed injections combined with PRP. Results have been positive, showing an increase in cartilage thickness, significant positive changes in MRI, and symptomatology improvement<sup>[62]</sup>. Few trials have been done using UC-MSC. Cartistem<sup>®</sup> is the first approved allogeneic cell treatment for OA in the world. It was approved by the Ministry of Food and Drug Safety in Korea and is now commercially available<sup>[63]</sup>. It uses UC-MSC combined with sodium hyaluronate. Up to 5000 patients have been treated with Cartistem<sup>®</sup> and around 97.67% of them have shown improved quality of life<sup>[63,64]</sup>.

### **Congenital anomalies**

Congenital anomalies, also known as birth defects, are structural or functional anomalies that occur during intrauterine life<sup>[65]</sup>. These defects can be identified prenatally, at birth, or even during later infancy. They occur in 2%-4% of live births<sup>[66]</sup> and are more common in stillborn spontaneous miscarriages. Approximately 50% of all congenital anomalies are not linked to a specific cause<sup>[65]</sup>; however, they are commonly caused by genetic abnormalities and/or environmental exposures. Genetic abnormalities include chromosomal alterations (e.g., Down syndrome) or single-gene/monogenic disorders. The latter have different modes of inheritance such as autosomal dominant, autosomal recessive, or X-linked<sup>[67]</sup>. On the other hand, environmental exposure to a teratogen, any agent that causes abnormalities in the form or function of the fetus, can produce cell death, alter normal growth of tissues, or interfere with normal cellular differentiation, resulting in a congenital anomaly<sup>[68]</sup>.

Birth defects are divided depending on the pathophysiology of the defect: (1) malformation when the intrinsic development is abnormal; (2) deformation when extrinsic mechanical forces modify a normally formed structure; (3) disruption when a vascular defect causes a malformation; or (4) dysplasia when there is an abnormal organization of cells into tissues<sup>[68]</sup>. These defects can be isolated or present in syndromes or associated patterns that may affect one or more organ systems. A lot of preventive measures, as well as treatment measures, have been focused on these anomalies due to their medical, surgical, psychological, and cosmetic significance.

### **Congenital microtia**

Congenital microtia is the incomplete formation or growth of the auricle, leading to the small or deformed auricle. It may occur as an isolated condition or as part of a syndrome or spectrum of anomalies. Microtia severity ranges from a complete absence of the auricle (anotia) to a mild size discrepancy. Most of the time, microtia occurs unilaterally (79%-93%), the right side being the most affected side<sup>[69]</sup>. It is associated with hearing loss of the ipsilateral ear, but normal hearing in the unaffected ear. Speech and language development are usually normal. Individuals with microtia, however, are at a higher risk of communication delay and attention deficit disorders<sup>[70,71]</sup>.

The etiology of microtia is poorly understood, though there is strong evidence supporting the importance of environmental causes such as altitude, and gestational exposure to certain drugs<sup>[72-75]</sup>. Ethnicity has been reported to be an important consideration due to the high incidence and prevalence of microtia among Asians, Hispanics, and Native Americans. In Mexico, the World Health Organization and the Mexican Registry and Epidemiological Surveillance of External Congenital Malformations (RYVEMCE) reported a prevalence of 6.15-7.37 cases per 10,000 childbirths, being one of the countries with the highest prevalence of microtia worldwide<sup>[72,75]</sup>. Due to the psychological and functional implications related to microtia, there have been several studies focusing on the surgical treatment and biotechnology measures needed to recreate an auricle as similar as possible to the native one.

Auricle reconstruction with autologous rib cartilage remains the gold standard for patients with microtia/anotia. Tanzer *et al.*<sup>[76]</sup> and Brent *et al.*<sup>[77]</sup> described this technique as an alternative to allogeneic implants in the late 1950s, overcoming several problems associated with these implants. Sculpted autologous costal cartilage graft is one of the most challenging procedures in plastic and reconstructive surgery since the surgeon has to handcraft the cartilage trying to create an ear similar in appearance to the contralateral one. Grafts have good long-term durability and grow concomitantly as the patient ages<sup>[77]</sup>. However, costal cartilage grafts are not as consistent as synthetic implants: they require long operative time, harvesting results in donor-site morbidity, and, occasionally, there is an insufficient source of cartilage.

Tissue engineering techniques emerged as an alternative treatment. The idea of preformed ear structures seeded with cells goes back to the 1940s when Peer *et al.*<sup>[78]</sup> started using diced cartilage placed inside an

auricle shaped mold. Research started focusing on scaffolds that could promote cell proliferation, as well as matrix production. Decades later, research focused on finding the ideal scaffold that would induce cellular proliferation and cartilage tissue formation. This was proved by Vacanti *et al.*<sup>[79]</sup> and Rodriguez *et al.*<sup>[80]</sup>, who conducted several preclinical studies showing that polyglycolic acid (PGA) + polylactic acid (PLA) would promote *in vitro* cell proliferation and matrix production, and *in vivo* cartilage formation after implantation. Mice were implanted with 3D ear-shaped scaffolds seeded with chondrocytes. After 12 weeks, scaffolds were almost entirely degraded; however, the neo-tissue maintained the original 3D structure and demonstrated histological cartilage appearance. These studies were the introduction of biotechnology to regenerative medicine<sup>[81]</sup>.

The combination of seeded auricular chondrocytes (AuCs) to scaffolds and the computer-assisted design/computer-aided manufacturing (CAD/CAM) technology<sup>[82-84]</sup> led to the start of clinical studies. The first clinical application was done in Shanghai in 2018 by Guangdong Zhou *et al.*<sup>[84]</sup>, where 5 patients with unilateral microtia were implanted with 3D printed PCL + PGA scaffolds seeded with autologous chondrocytes from the cartilage remnants of the microtia. 2.5 years later, they reported the follow-up of one patient showing the formation of cartilaginous tissue after histologic evaluation, the transition from a stiff graft to a more flexible one over the time, and the degradation of the scaffold without losing the original ear shape.

Currently, autologous chondrocytes from the microtia auricle are being isolated, expanded, and seeded onto the constructs, showing normal elastic cartilage on histology<sup>[85]</sup>. However, monolayer expansion of chondrocytes results in dedifferentiation<sup>[80,86]</sup>, limiting the capacity to generate robust cartilage, and needs extensive 3D construct culture before implantation<sup>[84,87]</sup>. Mesenchymal stem cells have the potential of massive expansion and the ability to differentiate into chondrocytes through co-culture or co-implantation<sup>[88]</sup>.

Studies have been done using articular cartilage co-cultures with MSC, though little is known about AuCs and MSC. Pre-clinical *in vivo* studies have shown the formation of cartilage, but the impact of these studies is limited due to the use of non-human cells, the lack of specific markers for elastic cartilage, and the absence of mechanical evaluation<sup>[89-94]</sup>. Cohen *et al.*<sup>[95]</sup> conducted a comparative preclinical study evaluating cartilage formation in constructs using human AuCs vs human AuCs and MSC in a 1:1 ratio. The study showed that the auricular cartilage generated in the 1:1 constructs was similar in structure, histology, biochemical development, and mechanical properties to discs containing only AuCs and native human auricular cartilage after 3 months *in vivo*. To date, no clinical study using AuCs in combination with MSC has been conducted. However, these findings suggest MSCs could solve several problems related to cartilage culture and could bring other benefits related to their immunomodulatory/anti-inflammatory potential.

### Cleft lip and palate

Cleft lip (CL) and palate (CLP) are common congenital malformations in Mexico, with an incidence of 1 in 800 births<sup>[96]</sup>. Up to 2003, CLP had a prevalence of 139,000 affected children throughout the country, with approximately 10 new cases identified daily<sup>[97]</sup>.

Patients with CLP undergo (on average) 4 surgical procedures during their lifetime: (1) lip closure and primary nasal repair; (2) palate closure; (3) alveolar bone graft; and (4) rhinoseptoplasty<sup>[98]</sup>. The alveolar bone graft is the placing of bone in the primary palate to restore the continuity of the maxillary arch and separate the oral and nasal cavity<sup>[99,100]</sup>. This allows adequate dental hygiene, promotes harmonic facial growth, and provides the necessary bone matrix for the eruption of the lateral and canine incisors<sup>[101,102]</sup>.

The best donor area for the bone graft is the iliac crest, where approximately 3-8 cm<sup>3</sup> of bone are obtained. Several problems are associated with this procedure, such as recipient area alterations (lack of integration,

bone sequestration, infection or bone resorption), donor area complications (hematoma, infection, abnormal scar, pain and temporary inability to walk)<sup>[103]</sup>, and insufficient bone graft<sup>[104,105]</sup>. In these cases, the possibility of synthetic bone substitutes (silicone, polytetrafluoroethylene, polyethylene, polyester, polyamides, acrylic, metals, cyanoacrylate, resins)<sup>[106-111]</sup>, or natural bone substitutes (calcium phosphate, granules) has been suggested<sup>[112-119]</sup>.

The use of cell-based therapy represents one of the most advanced methods to approach craniofacial abnormalities. Several animal models have been used to test alveolar cleft-grafting materials including mice, rabbits, cats, dogs, goats, sheep, and monkeys. Studies have shown heterogeneous results in terms of biocompatibility, bone regeneration capacity, integration, resorption, and mechanical resistance due to the physicochemical characteristics of each material<sup>[120,121]</sup>. Existing systematic reviews support the ability of bone regeneration on these materials for the treatment of small periodontal bone defects, but recommend further studies on major bone defects such as palatal fissures<sup>[122-124]</sup>.

Scaffolds, as in all biotechnology-related applications, have been a major research topic regarding CLP. The ideal scaffold should have macro-geometry, micro-architecture, bioactivity, and appropriate mechanical properties<sup>[125]</sup>. The first two characteristics have been addressed with the introduction of 3D printed scaffolds. A head CT scan is performed in patients with CLP, and a scaffold with the patient's exact macroscopic geometry is created. Bioactivity and mechanical properties are determined by the scaffold material. Several different materials like polycaprolactone (PCL) with hydroxyapatite and platelet-derived growth factor-BB<sup>[125]</sup>, cryogels<sup>[126]</sup>, demineralized bone matrices, PLA, among others, have been tested to evaluate bone regeneration and cellular migration<sup>[127-131]</sup>. Today, the use of bioceramics, such as calcium phosphate, in combination with biomimetic polymer scaffolds, folic acid derivatives, morphogens, and stem cells are currently considered the most promising alternatives for CLP regeneration<sup>[127]</sup>.

The use of mesenchymal stem cells is emerging as an alternative treatment or in combination with previously-described therapies for patients with CLP. As mentioned earlier, MSC can be obtained from different parts of the body such as adipose tissue, bone marrow, and umbilical cord. The generation of an artificial alveolar cleft and the implantation of teeth in the regenerated bone region have been accomplished in dog models using BM-MSC<sup>[132-134]</sup>. Ahn *et al.*<sup>[135]</sup> reported the first case of regeneration of an alveolar cleft defect. Patient-specific 3D-printed bioresorbable polycaprolactone (PCL) scaffolds were seeded with iliac BM-MSC and showed 45% defect regeneration 6 months after transplantation, with a 75% bone mineral density compared to the surrounding bone. AD-MSC, due to their availability and easy handling, are excellent candidates for tissue engineering in CLP patients. Preclinical studies comparing bone regeneration between AD-MSC and autogenous bone graft in canine maxillary alveolar cleft models showed no significant differences, meaning AD-MSC can be an acceptable alternative<sup>[136]</sup>. However, clinical studies are needed to confirm their efficacy and reproducibility in humans.

Unlike other alternatives, MSC derived from dental tissues have been studied for CLP patients due to their higher accessibility and less invasive retrieval. Lee *et al.*<sup>[137]</sup> reported that stem cells from human exfoliated deciduous teeth (SHEDs) have mineralization potential after expressing bone-specific osteogenic markers following insertion into *ex vivo*-cultured embryonic palatal shelves and *in novo* culture. Furthermore, Nakajima *et al.*<sup>[138]</sup> compared the bone regeneration ability of SHEDs, BM-MSC, and dental pulp stem cells in mice. They concluded that after 12 weeks of transplantation, the ratio of new bone formation was not significantly different among these groups. However, SHED produced the largest osteoid and widely distributed collagen fibers. Up until now, no clinical studies have been conducted using SHEDs. Although a huge effort has been devoted to the use of tissue engineering as a solution for treating bone defects, more evidence is still needed.

## CONCLUSION

Mesenchymal stem cells are an emerging alternative for tissue engineering therapies. Besides their differentiation ability, they also express paracrine functions, which have shown to be immunomodulatory and anti-inflammatory. Taking advantage of these functions, MSC have been studied in different fields for the medical treatment of degenerative and congenital diseases. Despite favorable findings in preclinical studies, more clinical studies following all the steps described in translational medicine are needed to address their efficacy, safety, and clinical application. The complexity of these technologies must be considered carefully, and every country must follow a single regulatory pathway.

## DECLARATIONS

### Authors' contributions

The authors would like to thank Mariana Rodríguez for copy-editing this paper.

### Authors' contributions

Written and analyzed all topics, Veasquillo mainly in microtia: Velasquillo C, Madrazo-Ibarra A

Mayor contribution on microtia studies: Gutiérrez-Gómez C

Cleft lip and palate: Pérez-Dosal MR

Mayor contribution on stem cells applications in tissue engineering: Melgarejo-Ramírez Y

Mayor contribution articular degenerative diseases: Madrazo-Ibarra A, Ibarra C

### Availability of data and materials

Reviewed several papers already published.

### Financial support and sponsorship

This research work was supported by SECTEI under the project SECTEI-/183/2019 (10160c19).

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. NCI Dictionary of Cancer Terms [Internet]. National Cancer Institute. 2011. Available from: <https://www.cancer.gov/publications/dictionaries/cancer-terms> [Last accessed on 12 Jun 2020]
2. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006;8:315-7.
3. Blau HM, Daley GQ. Stem cells in the treatment of disease. *N Engl J Med* 2019;380:1748-60.
4. Glenn JD. Mesenchymal stem cells: emerging mechanisms of immunomodulation and therapy. *World J Stem Cells* 2014;6:526.
5. Maumus M, Jorgensen C, Noël D. Mesenchymal stem cells in regenerative medicine applied to rheumatic diseases: role of secretome and exosomes. *Biochimie* 2013;95:2229-34.
6. Niu CC, Lin SS, Yuan LJ, Chen LH, Pan TL, et al. Identification of mesenchymal stem cells and osteogenic factors in bone marrow aspirate and peripheral blood for spinal fusion by flow cytometry and proteomic analysis. *J Orthop Surg* 2014;9:32.
7. Samsonraj RM, Rai B, Sathiyathan P, Puan KJ, Röttschke O, et al. Establishing criteria for human mesenchymal stem cell potency:



- establishing criteria for hMSC potency. *Stem Cells* 2015;33:1878-91.
8. Ben Nasr M, Vergani A, Avruch J, Liu L, Kefaloyianni E, et al. Co-transplantation of autologous MSC delays islet allograft rejection and generates a local immunoprivileged site. *Acta Diabetol* 2015;52:917-27.
9. Ferreira ML, Silva PC, Alvarez Silva LH, Bonfim DC, Conilho Macedo Müller LC, et al. Heterologous mesenchymal stem cells successfully treat femoral pseudarthrosis in rats. *J Transl Med* 2012;10:51.
10. Rodriguez RL, Frazier T, Bunnell BA, Mouton CA, March KL, et al. Arguments for a different regulatory categorization and framework for stromal vascular fraction. *Stem Cells Dev* 2020;29:257-62.
11. Chu DT, Nguyen Thi Phuong T, Tien NLB, Tran DK, Minh LB, et al. Adipose tissue stem cells for therapy: an update on the progress of isolation, culture, storage, and clinical application. *J Clin Med* 2019;8:917.
12. Lander EB, Berman MH. Autologous stromal vascular fraction: a new era of personal cell therapy. *Stem Cells Res Dev Ther* 2018;4:1-6.
13. Osteoarthritis Research Society International (OARSI) [Internet]. Available from: <https://www.oarsi.org/> [Last accessed on 12 Jun 2020]
14. GBD Compare | IHME Viz Hub [Internet]. Available from: <http://vizhub.healthdata.org/gbd-compare> [Last accessed on 12 Jun 2020]
15. Woolf AD, Pfleger B. Burden of major musculoskeletal conditions. *Bull World Health Organ* 2003;81:646-56.
16. Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. *Lancet* 2019;393:1745-59.
17. Martel-Pelletier J, Barr AJ, Cicuttini FM, Conaghan PG, Cooper C, et al. Osteoarthritis. *Nat Rev Dis Primer* 2016;2:16072.
18. McAlindon TE, Bannuru RR, Sullivan MC, Arden NK, Berenbaum F, et al. OARSI guidelines for the non-surgical management of knee osteoarthritis. *Osteoarthritis Cartilage* 2014;22:363-88.
19. Villalobos Córdoba FE, Izaguirre A, Almazan A, Cruz F, Pérez Jiménez FJ, et al. Articular cartilage injuries in 1,309 knee arthroscopies, a public health problem in a developing country? *Osteoarthritis Cartilage* 2007;15:B102.
20. Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, et al. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. *N Engl J Med* 1994;331:889-95.
21. Everhart JS, Campbell AB, Abouljoud MM, Kirven JC, Flanigan DC. Cost-efficacy of knee cartilage defect treatments in the United States. *Am J Sports Med* 2019;363546519834557.
22. Orth P, Gao L, Madry H. Microfracture for cartilage repair in the knee: a systematic review of the contemporary literature. *Knee Surg Sports Traumatol Arthrosc* 2020;28:670-706.
23. Thorlund JB, Juhl CB, Roos EM, Lohmander LS. Arthroscopic surgery for degenerative knee: systematic review and meta-analysis of benefits and harms. *BMJ* 2015;350:h2747.
24. Lohmander LS, Hellot S, Dreher D, Krantz EFW, Kruger DS, et al. Intraarticular sprifermin (recombinant human fibroblast growth factor 18) in knee osteoarthritis: a randomized, double-blind, placebo-controlled trial: sprifermin effects in knee osteoarthritis. *Arthritis Rheumatol* 2014;66:1820-31.
25. Zhang W, Ouyang H, Dass CR, Xu J. Current research on pharmacologic and regenerative therapies for osteoarthritis. *Bone Res* 2016;4:15040.
26. Poulet B, Staines KA. New developments in osteoarthritis and cartilage biology. *Curr Opin Pharmacol* 2016;28:8-13.
27. Wang AT, Feng Y, Jia HH, Zhao M, Yu H. Application of mesenchymal stem cell therapy for the treatment of osteoarthritis of the knee: a concise review. *World J Stem Cells* 2019;11:222-35.
28. Ibarra C, Izaguirre A, Villalobos E, Masri M, Lombardero G, et al. Follow-up of a new arthroscopic technique for implantation of matrix-encapsulated autologous chondrocytes in the knee. *Arthrosc J Arthrosc Relat Surg* 2014;30:715-23.
29. Villalobos E, Madrazo-Ibarra A, Martínez V, Olivos-Meza A, Velasquillo C, et al. Arthroscopic matrix-encapsulated autologous chondrocyte implantation: a pilot multicenter investigation in Latin America. *Cartilage* 2020;11.
30. Brittberg M, Recker D, Ilgenfritz J, Saris DBF, Summit Extension Study Group. Matrix-applied characterized autologous cultured chondrocytes versus microfracture: five-year follow-up of a prospective randomized trial. *Am J Sports Med* 2018;46:1343-51.
31. Schuette HB, Kraeutler MJ, McCarty EC. Matrix-assisted autologous chondrocyte transplantation in the knee: a systematic review of mid- to long-term clinical outcomes. *Orthop J Sports Med* 2017;5:232596711770925.
32. MACI (Autologous Cultured Chondrocytes on a Porcine Collagen Membrane). Available from: <https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/maci-autologous-cultured-chondrocytes-porcine-collagen-membrane> [Last accessed on 12 Jun 2020]
33. Okano T, Yamada N, Sakai H, Sakurai Y. A novel recovery system for cultured cells using plasma-treated polystyrene dishes grafted with poly(N-isopropylacrylamide). *J Biomed Mater Res* 1993;27:1243-51.
34. Mitani G, Sato M, Lee JIK, Kaneshiro N, Ishihara M, et al. The properties of bioengineered chondrocyte sheets for cartilage regeneration. *BMC Biotechnol* 2009;9:17.
35. Kaneshiro N, Sato M, Ishihara M, Mitani G, Sakai H, et al. Bioengineered chondrocyte sheets may be potentially useful for the treatment of partial thickness defects of articular cartilage. *Biochem Biophys Res Commun* 2006;349:723-31.
36. Sato M, Yamato M, Hamahashi K, Okano T, Mochida J. Articular cartilage regeneration using cell sheet technology. *Anat Rec (Hoboken)* 2014;297:36-43.
37. Demoor M, Ollitrault D, Gomez-Leduc T, Bouyoucef M, Hervieu M, et al. Cartilage tissue engineering: Molecular control of chondrocyte differentiation for proper cartilage matrix reconstruction. *Biochim Biophys Acta* 2014;1840:2414-40.
38. Barron V, Merghani K, Shaw G, Coleman CM, Hayes JS, et al. Evaluation of cartilage repair by mesenchymal stem cells seeded on a PEOT/PBT scaffold in an osteochondral defect. *Ann Biomed Eng* 2015;43:2069-82.
39. Qi BW, Yu AX, Zhu SB, Zhou M, Wu G. Chitosan/poly(vinyl alcohol) hydrogel combined with Ad-hTGF-β1 transfected mesenchymal stem cells to repair rabbit articular cartilage defects. *Exp Biol Med* 2013;238:23-30.

40. Kim YS, Kwon OR, Choi YJ, Suh DS, Heo DB, et al. Comparative matched-pair analysis of the injection versus implantation of mesenchymal stem cells for knee osteoarthritis. *Am J Sports Med* 2015;43:2738-46.
41. Majewski M, Susanne H, Klaus S. Epidemiology of athletic knee injuries: a 10-year study. *The Knee* 2006;13:184-8.
42. Dhollander A, Verdonk P, Verdonk R. Treatment of painful, irreparable partial meniscal defects with a polyurethane scaffold: midterm clinical outcomes and survival analysis. *Am J Sports Med* 2016;44:2615-21.
43. Ahmed AM, Burke DL. In-vitro measurement of static pressure distribution in synovial joints--Part I: tibial surface of the knee. *J Biomech Eng* 1983;105:216-25.
44. Baratz ME, Fu FH, Mengato R. Meniscal tears: the effect of meniscectomy and of repair on intraarticular contact areas and stress in the human knee. A preliminary report. *Am J Sports Med* 1986;14:270-5.
45. Roos H, Laurén M, Adalberth T, Roos EM, Jonsson K, et al. Knee osteoarthritis after meniscectomy: prevalence of radiographic changes after twenty-one years, compared with matched controls. *Arthritis Rheum* 1998;41:687-93.
46. Leroy A, Beaufils P, Faivre B, Steltzen C, Boisrenoult P, et al. Actifit® polyurethane meniscal scaffold: MRI and functional outcomes after a minimum follow-up of 5 years. *Orthop Traumatol Surg Res OTSR* 2017;103:609-14.
47. Schüttler KF, Haberhauer F, Gesslein M, Heyse TJ, Figiel J, et al. Midterm follow-up after implantation of a polyurethane meniscal scaffold for segmental medial meniscus loss: maintenance of good clinical and MRI outcome. *Knee Surg Sports Traumatol Arthrosc Off J ESSKA* 2016;24:1478-84.
48. Angele P, Johnstone B, Kujat R, Zellner J, Nerlich M, et al. Stem cell based tissue engineering for meniscus repair. *J Biomed Mater Res A* 2008;85:445-55.
49. Dutton AQ, Choong PF, Goh JCH, Lee EH, Hui JHP. Enhancement of meniscal repair in the avascular zone using mesenchymal stem cells in a porcine model. *J Bone Joint Surg Br* 2010;92:169-75.
50. Olivos-Meza A, Pérez Jiménez FJ, Granados-Montiel J, Landa-Solís C, Cortés González S, et al. First clinical application of polyurethane meniscal scaffolds with mesenchymal stem cells and assessment of cartilage quality with T2 mapping at 12 months. *Cartilage* 2019;194760351985241.
51. Lai RC, Yeo RWY, Lim SK. Mesenchymal stem cell exosomes. *Semin Cell Dev Biol* 2015;40:82-8.
52. Chen TS, Lai RC, Lee MM, Choo ABH, Lee CN, et al. Mesenchymal stem cell secretes microparticles enriched in pre-microRNAs. *Nucleic Acids Res* 2010;38:215-24.
53. Karlsen TA, Jakobsen RB, Mikkelsen TS, Brinchmann JE. microRNA-140 targets RALA and regulates chondrogenic differentiation of human mesenchymal stem cells by translational enhancement of SOX9 and ACAN. *Stem Cells Dev* 2014;23:290-304.
54. Liang Y, Duan L, Xiong J, Zhu W, Liu Q, et al. E2 regulates MMP-13 via targeting miR-140 in IL-1 $\beta$ -induced extracellular matrix degradation in human chondrocytes. *Arthritis Res Ther* 2016;18:105.
55. Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y, et al. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. *Genes Dev* 2010;24:1173-85.
56. Tao SC, Yuan T, Zhang YL, Yin WJ, Guo SC, et al. Exosomes derived from miR-140-5p-overexpressing human synovial mesenchymal stem cells enhance cartilage tissue regeneration and prevent osteoarthritis of the knee in a rat model. *Theranostics* 2017;7:180-95.
57. Yu XM, Meng HY, Yuan XL, Wang Y, Guo QY, et al. MicroRNAs' involvement in osteoarthritis and the prospects for treatments. *Evid Based Complement Alternat Med* 2015;2015:1-13.
58. Toh WS. MSC exosome as a cell-free MSC therapy for cartilage regeneration: Implications for osteoarthritis treatment. *Dev Biol* 2017;9.
59. Toghraie F, Razmkhah M, Gholipour MA, Faghieh Z, Chenari N, et al. Scaffold-free adipose-derived stem cells (ASCs) improve experimentally induced osteoarthritis in rabbits. *Arch Iran Med* 2012;15:495-9.
60. Zhou J, Wang Y, Liu Y, Zeng H, Xu H, et al. Adipose derived mesenchymal stem cells alleviated osteoarthritis and chondrocyte apoptosis through autophagy inducing. *J Cell Biochem* 2019;120:2198-212.
61. Im GI, Shin YW, Lee KB. Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? *Osteoarthritis Cartilage* 2005;13:845-53.
62. Gentile P, Scioli MG, Bielli A, Orlandi A, Cervelli V. Concise review: the use of adipose-derived stromal vascular fraction cells and platelet rich plasma in regenerative plastic surgery. *Stem Cells* 2017;117-34.
63. MEDIPOST - The Future of Biotechnology [Internet]. Available from: <http://www.medi-post.com/front/eng/stemcell/cartistem.do> [Last accessed on 12 Jun 2020]
64. Park Y, Ha C, Lee C, Yoon YC, Park Y. Cartilage regeneration in osteoarthritic patients by a composite of allogeneic umbilical cord blood-derived mesenchymal stem cells and hyaluronate hydrogel: results from a clinical trial for safety and proof-of-concept with 7 years of extended follow-up. *Stem Cells Transl Med* 2017;6:613-21.
65. Congenital anomalies [Internet]. Available from: <https://www.who.int/news-room/fact-sheets/detail/congenital-anomalies> [Last accessed on 12 Jun 2020]
66. Holmes LB. Congenital malformations. *N Engl J Med* 1976;295:204-7.
67. Corsello G, Giuffrè M. Congenital malformations. *J Matern Fetal Neonatal Med* 2012;25:25-9.
68. Kumar V, Abbas A, Aster J. Robbins basic pathology. 10th Edition. Elsevier; 2017. p. 952.
69. Suutarla S, Rautio J, Ritvanen A, Ala-Mello S, Jero J, et al. Microtia in Finland: comparison of characteristics in different populations. *Int J Pediatr Otorhinolaryngol* 2007;71:1211-7.
70. Eavey RD. Microtia and significant auricular malformation. Ninety-two pediatric patients. *Arch Otolaryngol Head Neck Surg* 1995;121:57-62.
71. Kelley PE, Scholes MA. Microtia and congenital aural atresia. *Otolaryngol Clin North Am* 2007;40:61-80, vi.

72. Aguinaga-Ríos M, Frías S, Arenas-Aranda DJ, Morán-Barroso VF. Microtia-atresia: aspectos clínicos, genéticos y genómicos. *Bol Méd Hosp Infant México* 2014;71:387-95.
73. Anderka MT, Lin AE, Abuelo DN, Mitchell AA, Rasmussen SA. Reviewing the evidence for mycophenolate mofetil as a new teratogen: case report and review of the literature. *Am J Med Genet A* 2009;149A:1241-8.
74. González-Andrade F, López-Pulles R, Espín VH, Paz-y-Miño C. High altitude and microtia in Ecuadorian patients. *J Neonatal-Perinat Med* 2010;3:109-16.
75. Luquetti DV, Leoncini E, Mastroiacovo P. Microtia-anotia: a global review of prevalence rates. *Birth Defects Res A Clin Mol Teratol* 2011;91:813-22.
76. Tanzer RC. Total reconstruction of the external ear. *Plast Reconstr Surg Transplant Bull* 1959;23:1-15.
77. Brent B. The correction of mi-rotia with autogenous cartilage grafts: I. The classic deformity? *Plast Reconstr Surg* 1980;66:1-12.
78. Peer LA. Extended use of diced cartilage grafts. *Plast Reconstr Surg* 1946;1954;14:178-85.
79. Vacanti CA, Vacanti JP. Bone and cartilage reconstruction with tissue engineering approaches. *Otolaryngol Clin North Am* 1994;27:263-76.
80. Rodriguez A, Cao YL, Ibarra C, Pap S, Vacanti M, et al. Characteristics of cartilage engineered from human pediatric auricular cartilage. *Plast Reconstr Surg* 1999;103:1111-9.
81. Otto IA, Melchels FPW, Zhao X, Randolph MA, Kon M, et al. Auricular reconstruction using biofabrication-based tissue engineering strategies. *Biofabrication* 2015;7:032001.
82. Cohen BP, Hooper RC, Puetzer JL, Nordberg R, Asanbe O, et al. Long-term morphological and microarchitectural stability of tissue-engineered, patient-specific auricles in vivo. *Tissue Eng Part A* 2016;22:461-8.
83. Liao HT, Zheng R, Liu W, Zhang WJ, Cao Y, et al. Prefabricated, ear-shaped cartilage tissue engineering by scaffold-free porcine chondrocyte membrane. *Plast Reconstr Surg* 2015;135:313e.
84. Zhou G, Jiang H, Yin Z, Liu Y, Zhang Q, et al. In vitro regeneration of patient-specific ear-shaped cartilage and its first clinical application for auricular reconstruction. *EBioMedicine* 2018;28:287-302.
85. Kamil SH, Vacanti MP, Vacanti CA, Eavey RD. Microtia chondrocytes as a donor source for tissue-engineered cartilage. *Laryngoscope* 2004;114:2187-90.
86. Domm C, Schünke M, Christesen K, Kurz B. Redifferentiation of dedifferentiated bovine articular chondrocytes in alginate culture under low oxygen tension. *Osteoarthritis Cartilage* 2002;10:13-22.
87. Gu Y, Kang N, Dong P, Liu X, Wang Q, et al. Chondrocytes from congenital microtia possess an inferior capacity for in vivo cartilage regeneration to healthy ear chondrocytes. *J Tissue Eng Regen Med* 2018;12:e1737-46.
88. Hendriks J, Riesle J, Ca van B. Co-culture in cartilage tissue engineering. *J Tissue Eng Regen Med* 2007;1:170-8.
89. Cai Z, Pan B, Jiang H, Zhang L. Chondrogenesis of human adipose-derived stem cells by in vivo co-graft with auricular chondrocytes from Microtia. *Aesthetic Plast Surg* 2015;39:431-9.
90. Goh BS, Che Omar SN, Ubaidah MA, Saim L, Sulaiman S, et al. Chondrogenesis of human adipose derived stem cells for future microtia repair using co-culture technique. *Acta Otolaryngol (Stockh)* 2017;137:432-41.
91. Kang N, Liu X, Guan Y, Wang J, Gong F, et al. Effects of co-culturing BMSC and auricular chondrocytes on the elastic modulus and hypertrophy of tissue engineered cartilage. *Biomaterials* 2012;33:4535-44.
92. Morrison KA, Cohen BP, Asanbe O, Dong X, Harper A, et al. Optimizing cell sourcing for clinical translation of tissue engineered ears. *Biofabrication* 2016;9:015004.
93. Pleumeekers MM, Nimeskern L, Koevoet WLM, Karperien M, Stok KS, et al. Cartilage regeneration in the head and neck area: combination of ear or nasal chondrocytes and mesenchymal stem cells improves cartilage production. *Plast Reconstr Surg* 2015;136:762e-74.
94. Zhang X, Xue K, Zhou J, Xu P, Huang H, et al. Chondrogenic differentiation of bone marrow-derived stem cells cultured in the supernatant of elastic cartilage cells. *Mol Med Rep* 2015;12:5355-60.
95. Cohen BP, Bernstein JL, Morrison KA, Spector JA, Bonassar LJ. Tissue engineering the human auricle by auricular chondrocyte-mesenchymal stem cell co-implantation. In: Lammi MJ, editor. *PLoS One* 2018;13:e0202356.
96. Arrendares S, Lisker R. Análisis genético del labio y paladar hendido solo. Estudio en población mexicana. *Rev Invest Clin* 1974;26.
97. Trigos-Micoló I, Figueroa MEG y L. Análisis de la incidencia, prevalencia y atención del labio y paladar hendido en México. *Cir Plástica* 2003;13:35-9.
98. Taher A. Cleft lip and palate: lesions, pathophysiology, and primary treatment. *J Craniofac Surg* 2001;12:200.
99. Vig KW. Alveolar bone grafts: the surgical/orthodontic management of the cleft maxilla. *Ann Acad Med Singapore* 1999;28:721-7.
100. Waite PD, Waite DE. Bone grafting for the alveolar cleft defect. *Semin Orthod* 1996;2:192-6.
101. Lilja J, Kalaaji A, Friede H, Elander A. Combined bone grafting and delayed closure of the hard palate in patients with unilateral cleft lip and palate: facilitation of lateral incisor eruption and evaluation of indicators for timing of the procedure. *Cleft Palate Craniofac J* 2000;37:98-105.
102. da Silva Filho OG, Teles SG, Ozawa TO, Filho LC. Secondary bone graft and eruption of the permanent canine in patients with alveolar clefts: literature review and case report. *Angle Orthod* 2000;70:174-8.
103. Eufinger H, Leppänen H. Iliac crest donor site morbidity following open and closed methods of bone harvest for alveolar cleft osteoplasty. *J Craniomaxillofac Surg* 2000;28:31-8.
104. Dawson KH, Egbert MA, Myall RW. Pain following iliac crest bone grafting of alveolar clefts. *J Craniomaxillofac Sur* 1996;24:151-4.
105. Steinberg B, Padwa BL, Boyne P, Kaban L. State of the art in oral and maxillofacial surgery: treatment of maxillary hypoplasia and

- anterior palatal and alveolar clefts. *Cleft Palate Craniofac J* 1999;36:283-91.
106. Schliephake H, Dard M, Planck H, Hierlemann H, Stern U. Alveolar ridge repair using resorbable membranes and autogenous bone particles with simultaneous placement of implants: an experimental pilot study in dogs. *Int J Oral Maxillofac Implants* 2000;15:364-73.
107. Rüdiger SG, Ehmke B, Hommens A, Karch H, Flemmig TF. Guided tissue regeneration using a polylactic acid barrier. Part I: Environmental effects on bacterial colonization. *J Clin Periodontol* 2003;30:19-25.
108. Mellonig JT, Nevins M, Sanchez R. Evaluation of a bioabsorbable physical barrier for guided bone regeneration. Part II. Material and a bone replacement graft. *Int J Periodontics Restorative Dent* 1998;18:129-37.
109. Lekovic V, Camargo PM, Weinlaender M, Kenney EB, Vasilic N. Combination use of bovine porous bone mineral, enamel matrix proteins, and a bioabsorbable membrane in intrabony periodontal defects in humans. *J Periodontol* 2001;72:583-9.
110. Piette E, Alberius P, Samman N, Linde A. Experience with e-PTFE membrane application to bone grafting of cleft maxilla. *Int J Oral Maxillofac Surg* 1995;24:327-32.
111. Puumanen K, Kellomäki M, Ritsilä V, Böhlting T, Törmälä P, et al. A novel bioabsorbable composite membrane of Polyactive 70/30 and bioactive glass number 13--93 in repair of experimental maxillary alveolar cleft defects. *J Biomed Mater Res B Appl Biomater* 2005;75:25-33.
112. Aichelmann-Reidy ME, Heath CD, Reynolds MA. Clinical evaluation of calcium sulfate in combination with demineralized freeze-dried bone allograft for the treatment of human intraosseous defects. *J Periodontol* 2004;75:340-7.
113. Yukna RA, Krauser JT, Callan DP, Evans GH, Cruz R, et al. Multi-center clinical comparison of combination anorganic bovine-derived hydroxyapatite matrix (ABM)/cell binding peptide (P-15) and ABM in human periodontal osseous defects. 6-month results. *J Periodontol* 2000;71:1671-9.
114. Kiliç AR, Efeoglu E, Yilmaz S. Guided tissue regeneration in conjunction with hydroxyapatite-collagen grafts for intrabony defects. A clinical and radiological evaluation. *J Clin Periodontol* 1997;24:372-83.
115. Rabie AB, Chay SH. Clinical applications of composite intramembranous bone grafts. *Am J Orthod Dentofac Orthop* 2000;117:375-83.
116. Méndez R, López-Cedrún JL, Patiño B, Vázquez I, Martín-Sastre R, et al. Platelet-rich plasma (platelet gel) in secondary alveoloplasty in cleft patients. *Cir Pediatr* 2006;19:23-6.
117. Segura-Castillo JL, Aguirre-Camacho H, González-Ojeda A, Michel-Perez J. Reduction of bone resorption by the application of fibrin glue in the reconstruction of the alveolar cleft. *J Craniofac Surg* 2005;16:105-12.
118. Zybutz MD, Laurell L, Rapoport DA, Persson GR. Treatment of intrabony defects with resorbable materials, non-resorbable materials and flap debridement. *J Clin Periodontol* 2000;27:169-78.
119. Boyne PJ. Application of bone morphogenetic proteins in the treatment of clinical oral and maxillofacial osseous defects. *J Bone Joint Surg Am* 2001;83:S146-50.
120. Peled M, Aizenbud D, Horwitz J, Machtei EE. Treatment of osseous cleft palate defects: a preliminary evaluation of novel treatment modalities. *Cleft Palate-Craniofacial J* 2005;42:344-8.
121. Trejo PM, Weltman R, Caffesse R. Treatment of intraosseous defects with bioabsorbable barriers alone or in combination with decalcified freeze-dried bone allograft: a randomized clinical trial. *J Periodontol* 2000;71:1852-61.
122. Murphy KG, Gunsolley JC. Guided tissue regeneration for the treatment of periodontal intrabony and furcation defects. A systematic review. *Ann Periodontol* 2003;8:266-302.
123. Carpio L, Loza J, Lynch S, Genco R. Guided bone regeneration around endosseous implants with anorganic bovine bone mineral. A randomized controlled trial comparing bioabsorbable versus non-resorbable barriers. *J Periodontol* 2000;71:1743-9.
124. Reynolds MA, Aichelmann-Reidy ME, Branch-Mays GL, Gunsolley JC. The efficacy of bone replacement grafts in the treatment of periodontal osseous defects. A systematic review. *Ann Periodontol* 2003;8:227-65.
125. Nyberg EL, Farris AL, Hung BP, Dias M, Garcia JR, et al. 3D-printing technologies for craniofacial rehabilitation, reconstruction, and regeneration. *Ann Biomed Eng* 2017;45:45-57.
126. Hixon KR, Melvin AM, Lin AY, Hall AF, Sell SA. Cryogel scaffolds from patient-specific 3D-printed molds for personalized tissue-engineered bone regeneration in pediatric cleft-craniofacial defects. *J Biomater Appl* 2017;32:598-611.
127. Martín-Del-Campo M, Rosales-Ibañez R, Rojo L. Biomaterials for Cleft Lip and Palate Regeneration. *Int J Mol Sci* 2019;20.
128. Gerhardt LC, Boccaccini AR. Bioactive glass and glass-ceramic scaffolds for bone tissue engineering. *Mater Basel Switz* 2010;3:3867-910.
129. Janssen NG, de Ruiter AP, van Hout WMMT, van Miegem V, Gawlitta D, et al. Microstructured  $\beta$ -tricalcium phosphate putty versus autologous bone for repair of alveolar clefts in a goat model. *Cleft Palate-Craniofacial J* 2017;54:699-706.
130. Al-Ahmady HH, Abd Elazeem AF, Bellah Ahmed NEM, Shawkat WM, Elmasry M, et al. Combining autologous bone marrow mononuclear cells seeded on collagen sponge with Nano Hydroxyapatite, and platelet-rich fibrin: reporting a novel strategy for alveolar cleft bone regeneration. *J Craniomaxillofac Surg* 2018;46:1593-600.
131. Batool F, Strub M, Petit C, Bugueno IM, Bornert F, et al. Periodontal tissues, maxillary jaw bone, and tooth regeneration approaches: from animal models analyses to clinical applications. *Nanomater Basel Switz* 2018;8.
132. Yoshioka M, Tanimoto K, Tanne Y, Sumi K, Awada T, et al. Bone regeneration in artificial jaw cleft by use of carbonated hydroxyapatite particles and mesenchymal stem cells derived from iliac bone. *Int J Dent* 2012;2012:352510.
133. Tanimoto K, Sumi K, Yoshioka M, Oki N, Tanne Y, et al. Experimental tooth movement into new bone area regenerated by use of bone marrow-derived mesenchymal stem cells. *Cleft Palate Craniofac J* 2015;52:386-94.
134. Sumi K, Abe T, Kunimatsu R, Oki N, Tsuka Y, et al. The effect of mesenchymal stem cells on chemotaxis of osteoclast precursor cells. *J Oral Sci* 2018;60:221-5.

135. Ahn G, Lee JS, Yun WS, Shim JH, Lee UL. Cleft alveolus reconstruction using a three-dimensional printed bioresorbable scaffold with human bone marrow cells. *J Craniofac Surg* 2018;29:1880-3.
136. Pourebrahim N, Hashemibeni B, Shahnaseri S, Torabinia N, Mousavi B, et al. A comparison of tissue-engineered bone from adipose-derived stem cell with autogenous bone repair in maxillary alveolar cleft model in dogs. *Int J Oral Maxillofac Surg* 2013;42:562-8.
137. Lee JM, Kim HY, Park JS, Lee DJ, Zhang S, et al. Developing palatal bone using human mesenchymal stem cell and stem cells from exfoliated deciduous teeth cell sheets. *J Tissue Eng Regen Med* 2019;13:319-27.
138. Nakajima K, Kunimatsu R, Ando K, Ando T, Hayashi Y, et al. Comparison of the bone regeneration ability between stem cells from human exfoliated deciduous teeth, human dental pulp stem cells and human bone marrow mesenchymal stem cells. *Biochem Biophys Res Commun* 2018;497:876-82.



Review

Open Access



# Nerve transfers in distal forearm and in the hand

Alfio Luca Costa<sup>1</sup>, Paolo Titolo<sup>2</sup>, Bruno Battiston<sup>2</sup>, Michele Rosario Colonna<sup>1</sup>

<sup>1</sup>Department of Human Pathology of the Adult, the Child and the Adolescent, University of Messina, Messina 98125, Italy.

<sup>2</sup>Department of Traumatology, Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, Turin 10126, Italy.

**Correspondence to:** Dr. Costa Alfio Luca, Department of Human Pathology of the Adult, the Child and the Adolescent, University of Messina, Via Consolare Valeria 1, Messina 98125, Italy. E-mail: [alfiocosta@hotmail.it](mailto:alfiocosta@hotmail.it)

**How to cite this article:** Costa AL, Titolo P, Battiston B, Colonna MR. Nerve transfers in distal forearm and in the hand. *Plast Aesthet Res* 2020;7:32. <http://dx.doi.org/10.20517/2347-9264.2020.43>

**Received:** 20 Mar 2020 **First Decision:** 21 May 2020 **Revised:** 7 Jun 2020 **Accepted:** 7 Jun 2020 **Published:** 24 Jun 2020

**Science Editor:** Alessandro Thione **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Nerve transfers were used, originally, to restore shoulder and elbow function in brachial plexus lesions. This concept has been developed over the years and applied to distal nerve injuries in which lower functionality was expected because of the gap between the injury site and the target muscle. The aim of this review is to describe nerve transfers in the distal forearm and hand for isolated lesions of the median, ulnar and radial nerves. The different advantages achieved by transposition of a functional nerve stump near the effector muscle have opened up new options for the management of nerve lesions. Some of these alternatives have only been recently reported and a few are exclusively case reports.

**Keywords:** Nerve transfers, nerve injury, hand surgery, babysitting, coaptation, microsurgery

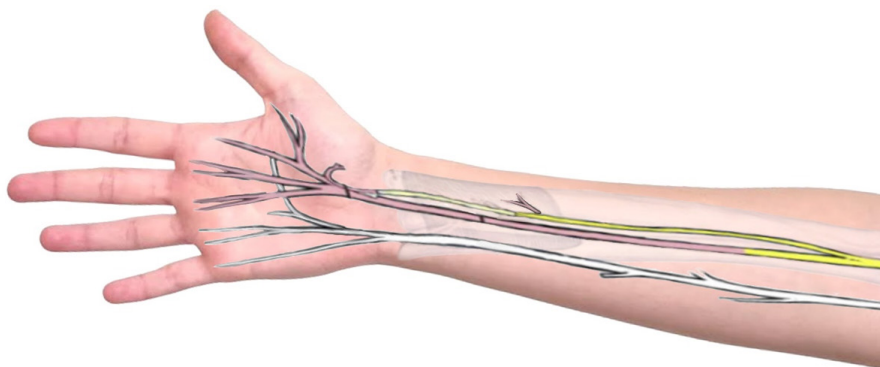
## INTRODUCTION

Nerve transfers were originally adopted for brachial plexus lesions to restore shoulder and elbow function<sup>[1-3]</sup>. This concept has been developed over the years and now, applied to distal nerve injuries<sup>[4]</sup> in which poor functional outcomes were anticipated because of the gap between the injury site and the innervated muscle. The aim of this review is to describe nerve transfers in the distal forearm and hand for isolated lesions of the median, ulnar and radial nerves.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** Anterior interosseous nerve (AIN) for thumb opposition. The axons of AIN at the level of pronator quadratus are coapted to the thenar branch of median nerve, through a nerve graft. Yellow: functional nerve; pink: nonfunctional nerves

## MOTORY NERVE TRANSFERS

### Median nerve

The median nerve provides a large part of sensitivity to the palmar side of the hand, which is critical for fine manipulation. It innervates and enables forearm pronation, has the most important role in wrist and finger flexion, especially the thumb and index fingers, and plays a significant part in thumb opposition.

Depending on the location and severity of nerve injury, different interventions are possible<sup>[5-7]</sup>.

In the distal forearm and in the hand, thumb opposition and restoration of sensibility of the thumb and index finger are the main objectives of reconstruction.

#### *Motor nerve transfers in the distal forearm and hand*

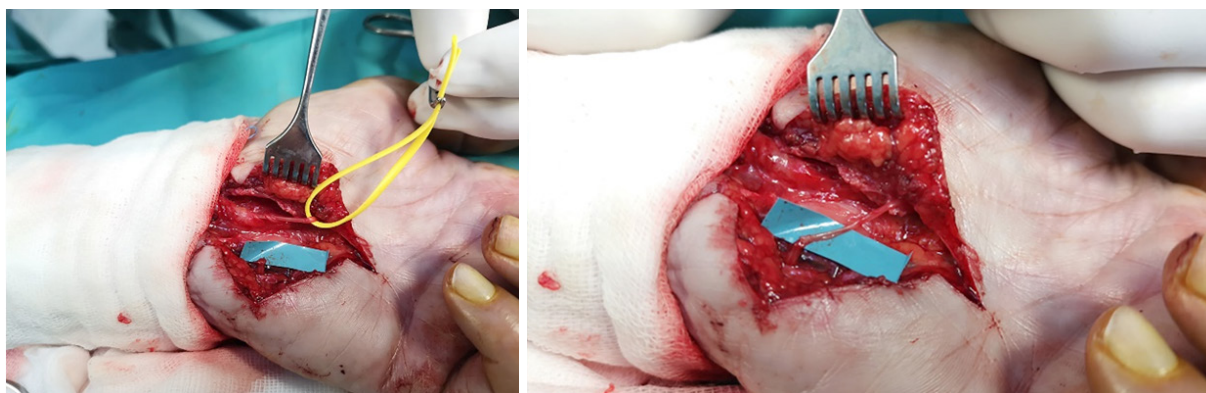
Median nerve injuries, at all levels, are associated with atrophy of the thenar eminence and loss of thumb opposition. Infrequently, atypical innervation patterns from the ulnar nerve can preserve opposition, and these have to be evaluated closely<sup>[6]</sup>.

In lesions of the motor fascicles of the median nerve, direct repair or interposition of a graft remains the treatment of choice.

When the anatomy is significantly altered however, direct repair can lead to suboptimal results as the nerve at this level is mainly sensory and, proximally, the scheme of the motor neurons is undefined<sup>[8-10]</sup>. An interpositional nerve graft could result in abnormal regeneration in which sensory fibers branch out into the motor fibers, and consequently without thenar function.

To obtain opposition of the thumb, tendon transfers are very effective, but it should be emphasized that these procedures require long periods of reeducation and lead to abnormal ergonomics.

If available, the anterior interosseous nerve (AIN) is dissected at the level of the branch to the pronator quadratus and coapted [Figure 1] to the thenar branch of the median nerve (TBMN). The AIN, at this level, is composed mainly of motor fibers with only sensory branches for proprioception of the wrist<sup>[11]</sup>, with a congruous number of axons (distal AIN ~ 900; thenar motor branch ~1,050<sup>[10,12]</sup>). It should also be mentioned that this technique requires the use of a nerve graft, which inevitably leads to a loss of the total number of fibers.



**Figure 2.** Transfer between the motor branch of the abductor digiti quinti (ADQMB) and the thenar branch of the median nerve. This branch is released proximally for 2/3 cm and coapted end to end towards the ADQMB branch

Reinnervation of the muscles of the thenar eminence by direct nerve repair is impossible in high median nerve injuries, due to the long distance that the nerves have to traverse for regeneration. Nerve transfers involving the ulnar (third lumbrical motor branch)<sup>[13]</sup> and radial nerve (motor branch to the extensor digiti minimi and extensor carpi ulnaris) have been proposed, but until a few years ago, results were still ambiguous and consequently, classical tendon transfers were preferred<sup>[4]</sup>. Bertelli *et al.*<sup>[14]</sup> recently described promising results after nerve transfer between the motor branch of the abductor digiti quinti (ADQMB) and the TBMN in which the ADQMB is dissected as distally as achievable, and then coapted to the TBMN without nerve grafting<sup>[14]</sup> [Figure 2].

#### *Anterior interosseous nerve to median recurrent motor branch transfer: technique*

The surgeon opens the carpal tunnel to identify the median nerve and follows it to the origin of the TBMN, close to the thenar eminence. In the distal forearm, the flexor digitorum superficialis and profundus are retracted to expose the pronator quadratus and the median nerve. The AIN and the nerve branch to the pronator quadratus are identified. The pronator quadratus is then dissected superior to the median nerve with intramuscular dissection to obtain the maximum length.

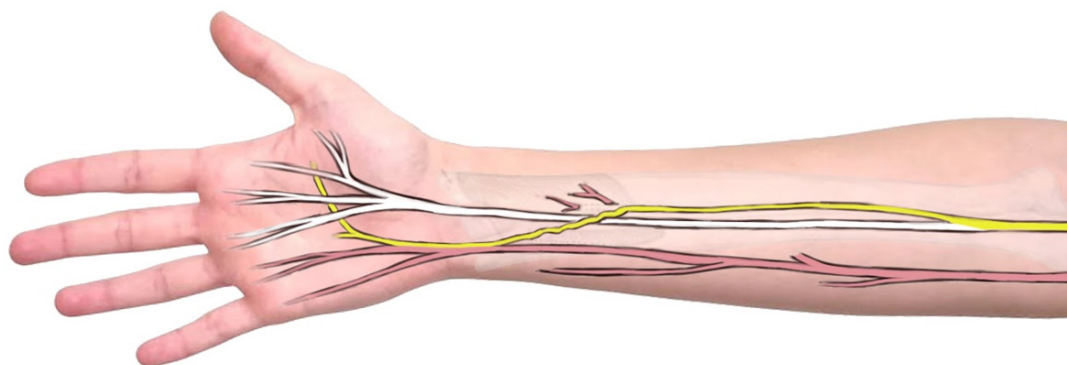
An interpositional nerve graft (frequently the sural nerve or medial antebrachial cutaneous) is usually necessary for tensionless suture. Range of motion of the wrist should be assessed before utilizing the graft to ensure that hand movement will not generate excessive stress on the coaptation.

#### *Abductor digiti quinti motor branch to the recurrent motor branch transfer: technique*

A lazy S incision is made on the lateral margin of the hypothenar region, Guyon's canal is opened, and the motor branch of the ulnar nerve is identified and followed distally. The branch for the abductor digiti minimi is dissected and its function assessed with an electrical stimulator. After that, the surgeon opens the carpal tunnel to visualize the median nerve. In the median nerve, the origin of the TBMN is identified near the thenar eminence. This branch is divided proximally for 2/3 cm and coapted end-to-end towards the ADQMB branch.

### **Ulnar nerve**

As a result of injury to the ulnar nerve, grip and pinch weakness, and sometimes, clawing of the last two ulnar digits occurs<sup>[15,16]</sup>. For proximal injuries, direct coaptation should reestablish sensation to the ulnar digits<sup>[17-20]</sup>. However, after an immediate direct ulnar nerve repair, it is not possible to achieve recovery of the innervation of the intrinsic musculature due to the long distance that the neurons must traverse for nerve regeneration<sup>[18]</sup>. Classical tendon transfers prevent deformities such as clawing, but they are often



**Figure 3.** Anterior interosseous nerve (AIN) to ulnar deep motor transfer. The AIN is followed into the pronator quadratus. Proximal neurolysis of the motor fascicle of the ulnar nerve enables tensionless coaptation. Yellow: functional nerves; pink: nonfunctional nerves

associated with a loss of strength and fluidity of movement. In isolated lesions of the ulnar nerve, various techniques reported in the literature involve the median nerve as a donor of motor and/or sensitive fibers in the distal forearm and in the hand<sup>[21-24]</sup>. The preferred motor donor is the distal AIN to restore functionality of the intrinsic musculature. It is possible to achieve neurolysis of the motor branch of the ulnar nerve for up to 14 centimeters proximally to the radial styloid, allowing adequate length to achieve tensionless coaptation with the AIN donor branch [Figure 3]. In cases of injury to both the ulnar and median nerves, the radial nerve can act as a fiber donor.

#### *Motor nerve transfers in forearm*

When there are no median nerve injuries, the anterior interosseous nerve in its distal portion directed to the pronator quadratus muscle can be used as a fiber donor for the motor component of the ulnar nerve. Brown *et al.*<sup>[25]</sup> performed the first such case in 1991 and several authors have since successfully reported this technique.

This technique is frequently executed end-to-end, and proximal neurolysis of the ulnar nerve avoids the need for a nerve graft. Battiston and Lanzetta showed good results in seven patients who underwent terminal anterior interosseous nerve-to-ulnar motor nerve transfer in distal forearm, proximal to Guyon's canal<sup>[22]</sup>.

Brown and Mackinnon<sup>[4]</sup> have shown that neurolysis up to 14 cm proximal to the radial styloid can be performed for the ulnar nerve.

The reverse end-to-side or “supercharge” nerve transfer<sup>[26,27]</sup> can also improve intrinsic function and allow the ulnar nerve to regenerate spontaneously<sup>[28]</sup>.

Barbour *et al.*<sup>[28]</sup> also suggested through their experience in nerve transfers to the ulnar nerve that supercharged coaptations can keep the motor end plates good, as well as serve as a “babysitter”, until “native parent” axons return.

With validation of the idea that axons can regenerate if a nerve is sutured in end-to-side fashion, supercharged end-to-side (SETS) nerve transfers began to be used<sup>[29]</sup>.

In a retrospective matched-cohort study, Baltzer *et al.*<sup>[30]</sup> compared the outcomes of supercharged end-to-side procedures with the conventional technique in patients with a high ulnar nerve injury. As a result, the group in which the AIN SETS was implemented had better results and recovery of the intrinsic functionality of the hand.

Korciem *et al.*<sup>[31]</sup> directed a prospective study in 21 patients with a high ulnar nerve injury. In 10 patients, the lesion was managed through direct and isolated repair of the ulnar nerve (UR) while in the remaining 11 patients, the repair was associated with a supercharged end to side (SETS) nerve transfer. In the latter group, the patients showed improvement at six months' follow-up, which is a shorter time than necessary to regenerate ulnar nerve fibers from the lesion.

In 2010, Sherif and Amr<sup>[32]</sup> demonstrated that a double bridging nerve graft between the motor components of the ulnar and median nerve in the distal forearm could prevent atrophy of the intrinsic muscle until proximal nerve regeneration can arrive at these effectors.

These authors reported the best results in median nerve effector protection, and a good result regarding the ulnar nerve with the creation of an artificial Martin-Gruber connection through a double end-to-side bridge graft. In the same way, Colonna *et al.*<sup>[33]</sup> reported a double end-to-side coaptation via a nerve graft enabled fibers from the donor median nerve to regenerate the injured ulnar nerve

#### *Anterior interosseous nerve to ulnar motor branch transfer technique*

A lazy S incision at the level of Guyon's canal and dissection of the pronator quadratus muscle allows exposure of the ulnar and median nerves at the level of the distal forearm. At the level of Guyon's canal, it is possible to identify sensory and motor branches of the ulnar nerve; internal neurolysis of the motor fibers of the ulnar nerve proceeds as proximally as possible and finally these are divided. The anterior interosseous nerve is followed as it enters the pronator quadratus muscle and divided as distally as possible. The proximal stump of the anterior interosseous nerve is then coapted end-to-end to the distal stump of the motor branch of the previously dissected ulnar nerve.

#### *Motor nerve transfer in the distal palm*

Barbour *et al.*<sup>[34]</sup> reported transfers from the branch of the posterior interosseous nerve (specifically, branches from the extensor digiti minimi and extensor carpi ulnaris) with sub-optimal results.

This demonstrates the inconsistent pattern of reinnervation seen when reinnervating numerous motor functions with an inadequate number of donor nerves.

The TBMN has been used in recent years as a fiber donor in the palm to restore function of the deep motor branch of the ulnar nerve. Aszmann and Gesselbauer<sup>[35]</sup> built on Riche-Cannieu's ulnar-to-median nerve communication in the palm and proposed a distal babysitting technique via a nerve graft between the thenar branch of the median nerve (donor) and the ulnar nerve "just distal to Guyon's canal". At long-term follow-up at 6 years, they presented very promising results with intrinsic motor function after distal ulnar lesions in three patients.

In 2017, Colonna *et al.*<sup>[36]</sup> suggested using the branch for the first lumbrical as a babysitter for the deep motor branch of the ulnar nerve to avoid intrinsic atrophy. This hypothesis was based on anatomical studies and qualitative and quantitative analysis of nerve fibers.

In 2018, Bertelli *et al.*<sup>[37]</sup> described nerve transfer from the motor branch of the opponens pollicis (OPB) to the deep branch of the ulnar nerve in the terminal division (TDDBUN) to increase pinch strength. With promising results, they suggested combining transfers from the OPB to the TDDBUN and distal AIN to the motor branch of the ulnar nerve for reconstruction.

### **Median nerve**

In sensory nerve transfers for the median nerve, the fundamental aim is to restore sensitivity of the thumb and index finger to ensure pinch and grip functions, which are essential for fine motor tasks<sup>[38]</sup>.





**Figure 4.** The fourth digital nerve is transferred end-to-end to the first digital nerve. The remaining median-dependent distal stumps are coapted end-to-side. Yellow: functional nerves; lighter yellow: sensitive areas; pink: nonfunctional nerves



**Figure 5.** Very distal sensory nerve transfer described by Bertelli: sensory dorsal radial nerve branches coapted to the palmar nerves at the level of the metacarpal-phalangeal joint

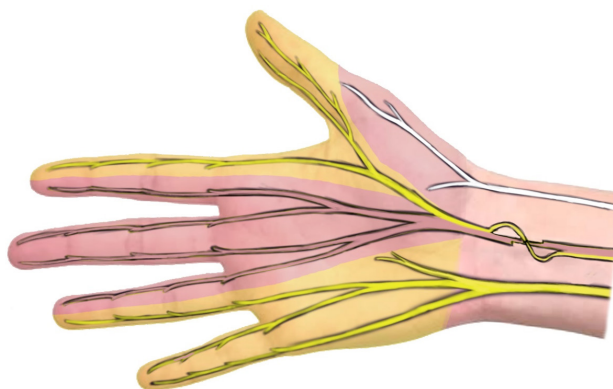
The recovery of sensory function is not influenced by timing as motor function is. However, it must be remembered that a classic nerve graft in a high median lesion translates into recovery times for sensation of more than a year; this also results in long recovery times without protective sensitivity<sup>[39,40]</sup>.

For these reasons, different fiber donors have been considered to restore sensation to the critical median nerve, depending on availability.

In isolated lesions of the median nerve, one possibility is to sacrifice the digital nerve directed to the fourth interdigital space and innervated by the ulnar nerve, to re-innervate the first interdigital space, in particular the ulnar margin of the first finger and the radial margin of the second finger. This nerve transfer is done end-to-end<sup>[4]</sup>. To ensure proprioception in non-critical areas, end-to-side coaptations are performed between the distal stumps of these areas and a functioning sensory branch [Figure 4].

Another option in high median nerve injuries is to use the dorsal sensory branch from the radial nerve<sup>[41,42]</sup>.

Bertelli *et al.*<sup>[43]</sup> described promising results with a “very distal nerve transfer” from dorsal branches of the radial nerve to palmar nerves at the level of the proximal phalanx [Figure 5].



**Figure 6.** Transfer of sensation with transposition of fascicles for the third to the first web space. Yellow: functional nerves; lighter yellow: sensitive areas; pink: nonfunctional nerves; lighter pink: non sensitive areas

In incomplete lesions of the median nerve or high lesions of the brachial plexus (C5-C6)<sup>[44]</sup>, the sensory component for the third interdigital space can be preserved since it originates from a distinct fascicle. This fascicle can be dissected up to the distal forearm and coapted to the distal portion of the fascicle for the first web space in order to restore critical sensitivity between the thumb and index finger [Figure 6]. It is possible to access both the recipient and donor nerves nearby through a single incision. This technique also avoids performing a sensory nerve transfer in the hand, thereby avoiding scarring on the palmar surface of the hand itself. In addition, the repair is quick and easy to achieve<sup>[45]</sup>. The distal stumps of the donor fascicle are also coapted end-to-side to the functional fascicles to maintain protective sensation in the donor site.

#### *Fourth web space digital nerve to first web space digital nerve transfer: technique*

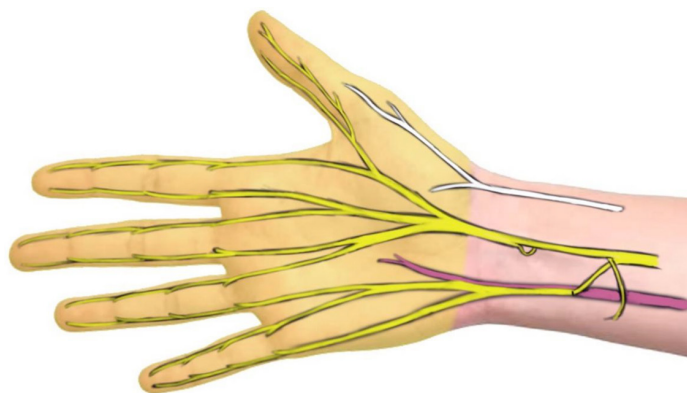
A classic incision is made over the carpal tunnel and extended to the first and fourth web spaces with zigzag Bruner-Type incisions. Under the superficial arterial arch, the branches of the median and ulnar nerves are identified. The branch to the fourth web space is followed and divided as distally as possible, which corresponds to the heads of the metacarpals. The nerve to the first web space is dissected proximally in order to achieve a length that allows tensionless coaptation. When an adequate length is obtained, the median-dependent branch is cut proximally and transferred to the proximal stump of the fourth digital nerve, which is dependent on the ulnar nerve. All other remaining sensory nerves are coapted end-to-side, as in Figure 4, to restore protective sensation.

#### *Very distal sensory nerve transfers in high median nerve lesions: technique*

The surgeon makes a V incision on the radial side of the metacarpophalangeal joint of the second finger. This incision exposes the dorsal sensory branch of the radial nerve and the radial collateral of the digital nerve of the second finger from the median nerve. These are divided in such a way that the proximal stump of the dorsal sensory branch for the second finger can be sutured end-to-end to the distal stump of the radial collateral of the proper digital nerve. Another V-shaped skin incision is performed, centered on the ulnar side of the metacarpophalangeal joint of the first finger, and the dorsal sensory branch and the digital collateral nerve are identified. These are subsequently divided and coapted as previously described for the second finger.

#### **Radial nerve**

The sensibility of the dorsum of the hand can be re-established via the lateral antebrachial cutaneous nerve (LACN) due to its characteristics. The LACN runs near the sensory radial branch of the distal forearm. Its dimensions are suitable for end-to-end coaptation, which can restore a large area of sensation to the back of the hand, by sacrificing a critical distal distribution. The LACN is also expendable and its use does not create significant morbidity along its supplied territory<sup>[4]</sup>.



**Figure 7.** Nerve transfers in an end-to-end strategy to restore ulnar sensation. Protective sensation of the third web space is maintained through end-to-side coaptation of the distal stump on the sensitive portion of the median nerve itself

The back of the hand supplied by the radial nerve is not critical. This has prompted some authors to suggest end-to-side coaptation on the functioning median nerve and indeed, this has been performed in many clinical scenarios<sup>[46-49]</sup>. Experimental data have also shown that the axons transmitted in these end-to-side strategies provide maximum protective sensation<sup>[50,51]</sup>.

Recently, Somsak suggested an end-to-side transfer as a treatment for C5-C6 root avulsions. In addition to the loss of sensation, such patients may experience pain on the dorsoradial aspect of the hand. An end-to-side transfer between the superficial branch of the radial nerve to the ulnovolar portion of the median nerve has shown promise in relieving pain and providing protective sensation<sup>[52]</sup>.

### Ulnar nerve

Sensory nerve transfers for isolated ulnar nerve injuries aim to reestablish protective sensation to the ulnar border of the hand<sup>[53]</sup>. In the literature, several methods have been proposed by different authors to utilize the functioning median nerve in order to provide sensation to the distribution of the ulnar nerve.

In the event of a brachial plexus or high ulnar nerve injury, the nerve directed to the third web space can be used to restore sensation to the ulnar border of the hand, which is more critical<sup>[22]</sup>.

Brown *et al.*<sup>[54]</sup> described end-to-end coaptation between the proximal stump of the nerve of the third web space and the distal stump of the nerve for the fourth web space in the distal forearm.

Furthermore, the dorsal sensory branch of the ulnar nerve is coapted end-to-side to the sensory part of the median nerve after making an epineurial opening [Figure 7].

Flores reported an analogous approach, with the use of an end-to-side technique<sup>[55]</sup>.

The sensitivity of the ulnar side of the hand can, in fact, be restored with end-to-side nerve transfers. This can be done using the median branch to the third web space as a donor. Another possible technique involves coaptation of the sensitive branches of the ulnar nerve with the functional median nerve at the level of the forearm<sup>[16]</sup>. These techniques allow restoration of the sensitivity of the ulnar border of the hand without denervation of the territories supplied by the median nerve.

Oberlin *et al.*<sup>[56]</sup> described coaptation in the distal forearm with an interpositional nerve graft between the LACN and the dorsal branch of the ulnar nerve. Ruchelsman *et al.*<sup>[57]</sup> described a revised technique, which avoids the use of an interpositional graft through dissection for a longer LACN.

**Table 1. Motory nerve transfers in distal forearm and in the hand**

	Recipient	Donor	Modality	Advantage	Disvantage	Ref.
Median N. Lesion	TBMN	AIN (Pronator)	End-to-End	Congruous number of Axon	Not available in high median nerve lesion Interpositional nerve graft needed	[10-12]
	TBMN	EDMNB	End-to-End		Unclear results	[4]
	TBMN	ECUMB	End-to-End		Unclear results	[4]
	TBMN	TLMB	End-to-End		Unclear results	[13]
	TBMN	ADQMB	End-to-End	No need of interpositional nerve graft		[14]
Ulnar N. Lesion	MBUN	AIN (Pronator)	End-to-End	No need of interpositional nerve graft Reliable technique	Dispersion of axons to the hypotenar musculature	[4,22,25]
	MBUN	AIN (Pronator)	SETS	Allow the ulnar nerve to regenerate spontaneously	Unclear results	[30,31]
	MBUN	MBMN	DBNG	Allow the ulnar nerve to regenerate spontaneously	Interpositional nerve graft needed Unclear results	[32]
	MBUN	EDMNB	End-to-End	Low morbidity	Interpositional nerve graft needed Suboptimal results	[34]
	MBUN	ECUMB	End-to-End	Low morbidity	Interpositional nerve graft needed Suboptimal results	[34]
	DMBUN	TBMN	DBNG	Allow the ulnar nerve to regenerate spontaneously	Interpositional nerve graft needed	[35]
	TDDBUN	OPB	End-to-End	Very distal transfer Can restore pinch strength Could be combined with the AIN nerve transfer		[37]

TBMN: Thenar branch of median nerve; AIN: anterior interosseous nerve; EDMNB: extensor digiti minimi motor branch; ECUMB: extensor carpi ulnaris motor branch; TLMB: third lumbrical motor branch; ADQMB: abductor digiti quinti motor branch; MBUN: motor branch of ulnar nerve; SETS: supercharged end to side; MBMN: motor branch of median nerve; DBNG: double bridging nerve graft; DMBUN: deep motor branch of ulnar nerve; TDDBUN: terminal division deep branch of the ulnar nerve; OPB: opponens pollicis branch

Other strategies involve direct end-to-end coaptation including between the palmar cutaneous branch of the median nerve as donors and the ulnar dorsal nerve [22,56,58].

#### *Nerve transfers to restore ulnar sensation: technique*

Nerve transfers to restore sensation of the ulnar nerve are generally performed simultaneously with motor transfers. Sensory fascicles of the ulnar nerve are dissected proximally. Distal to the carpal tunnel, it is possible to recognize the fascicles directed to the third interdigital space. These are dissected proximally to the distal forearm. Here the sensory fascicles of the ulnar nerve and the fascicles directed to the third web space are coapted end-to-end as illustrated in Figure 7. The dorsal cutaneous ulnar branch is divided proximally and transferred, tension free, to the median nerve. Protective sensation of the third web space is maintained through end-to-side coaptation between the distal stump of the fascicle and the sensitive portion of the median nerve itself.

## CONCLUSION

Nerve transfers in the distal forearm and hand appear to be a viable and promising option in patients with peripheral nerve injuries. The numerous advantages offered by transposition of a functional nerve stump near the effector muscle have opened up new alternatives to nerve grafts and tendon transfers, for the treatment of nerve injuries. The surgeon who performs brachial plexus surgery must be able to provide the best treatment for the patient and his needs. The complexity of the anatomical components and the density of the nerve structures in the distal forearm and hand give rise to various reconstructive possibilities. The main nerve transfers of the distal forearm and in the hand have been summarized in Tables 1 and 2. The addition of new concepts such as very distal nerve transfers and end-to-side coaptations have led to new solutions for previous problems in which solutions were more complex and are sometimes associated with

**Table 2. Sensory Nerve Transfers in distal forearm and in the hand**

	Recipient	Donor	Modality	Advantage	Disvantage	Ref.
Median N. Lesion	I Digital Branch +	IV Digital Branch	End-to-End	Restore sensitivity of the thumb and index finger	Loss of sensitivity of the IV web space	[4]
	II and III Digital Branch	Ulnar Sensory Branch	End-to-Side	Proprioceptive sensitivity in non-critical areas		
	Collateral Digital Nerve in the I Web space	Sensory Dorsal Branch of Radial Nerve	End-to-End	Restore sensitivity of the thumb and index finger Maintain proprioceptive sensitivity in donor areas due to an end-to-side coaptation for the distal dorsal stumps	Provide just protective sensitivity	[41-43]
Radial N. Lesion	Component for I digital nerve	Component for III digital nerve	End-to-End	Useful in cases of C5-C6 lesions	Loss of sensitivity of the IV web space	[44]
	Radial Sensory Branch	LACN	End-to-End	Dimensions comparable for the coaptation Donor runs near the recipient		[4]
	Radial Sensory Branch	Median Nerve	End-to-Side	No loss of sensitivity Useful in cases of C5-C6 lesions	Provide just protective sensitivity	[52]
Ulnar N. Lesion	Ulnar Sensory Branch	III Digital Branch	End-to-End	Restore sensitivity of the ulnar border of the hand	Proprioceptive sensitivity in donor areas due to an end-to-side coaptation for the distal stumps	[54]
	Ulnar Sensory Branch	III Digital Branch	End-to-Side	No loss of sensitivity	Provide just protective sensitivity of the ulnar border of the hand	[55]
	Ulnar Sensory Branch	Median nerve branch in forearm	End-to-End	No scar in the hand Restore sensitivity of the ulnar border of the hand	Protective sensitivity for the third web space through an end-to-side strategy	[16]
	Ulnar Sensory Branch	Median nerve branch in forearm	End-to-Side	No scar in the hand lloss of sensitivity	Provide just protective sensitivity of the ulnar border of the hand	[16]
	Dorsal Ulnar Sensory Branch	LACN	End-to-Side	No loss of sensitivity	Provide just protective sensitivity	[56,57]
	Ulnar Sensory Branch	PCBMN	End-to-End	Restore sensitivity of the ulnar border of the hand	Proprioceptive sensitivity in donor areas due to an end-to-side coaptation for the distal stumps	[22,56,58]

LACN: lateral antebrachial cutaneous nerve; PCBMN: palmar cutaneous branch of median nerve

greater morbidity. Such techniques can be found in the recent literature but require further study because some of them remain isolated case reports.

## DECLARATIONS

### Authors' contributions

Concept and design: Colonna MR

Data acquisition, data analysis, manuscript preparation: Costa AL

Critical revision and completion of manuscript: Costa AL, Titolo P, Battiston B, Colonna MR

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declare that there are no conflicts of interest.



## Ethical approval and consent to participate

Not applicable.

## Consent for publication

All patients underwent surgical procedures with informed consent that described in detail the procedure and any alternatives. The patients also signed a separate consent for the processing of sensitive data and the recording of photos and videos for educational, illustrative and research purposes.

## Copyright

© The Author(s) 2020.

## REFERENCES

1. Tung TH, Novak CB, Mackinnon SE. Nerve transfers to the biceps and brachialis branches to improve elbow flexion strength after brachial plexus injuries. *J Neurosurg* 2003;98:313-8.
2. Tung TH, Mackinnon SE. Brachial plexus injuries. *Clin Plast Surg* 2003;30:269-87.
3. Tung TH, Mackinnon SE. Nerve transfers: Indications, techniques, and outcomes. *J Hand Surg Am* 2010;35:332-41.
4. Brown JM, Mackinnon SE. Nerve transfers in the forearm and hand. *Hand Clin* 2008;24:319-40.
5. Imbriglia JE, Hagberg WC, Baratz ME. Median nerve reconstruction. In: Peimer CA, editor. *Surgery of the hand and upper extremity*, vol II. New York: McGraw-Hill; 1996. p. 1381.
6. Davis TRC. Median nerve palsy. In: Green DP, Hotchkiss RN, Pederson WC, editors. *Green's operative hand surgery*. 5th edition. Philadelphia: Elsevier; 2005. p. 1131.
7. Bertelli JA, Soldado F, Lehn VL, Ghizoni MF. Reappraisal of clinical deficits following high median nerve injuries. *J Hand Surg Am* 2016;41:13-9.
8. Humphreys DB, Mackinnon SE. Nerve transfers. *Operat Tech Plast Reconstr Surg* 2003;9:89, 7-11.
9. Mackinnon SE, Dellon AL. Anatomic investigations of nerves at the wrist: I. Orientation of the motor fascicle of the median nerve in the carpal tunnel. *Ann Plast Surg* 1988;21:32-5.
10. Vernadakis AJ, Humphreys DB, Mackinnon SE. Distal anterior interosseous nerve in the recurrent motor branch graft for reconstruction of a median nerve neuroma-in-continuity. *J Reconstr Microsurg* 2004;20:7-11.
11. Haase SC, Chung KC. Anterior interosseous nerve transfer to the motor branch of the ulnar nerve for high ulnar nerve injuries. *Ann Plast Surg* 2002;49:285-90.
12. Ustun ME, Ogun TC, Karabulut AK, Büyükmumcu M. An alternative method for restoring opposition after median nerve injury: an anatomical feasibility study for the use of neurotisation. *J Anat* 2001;198:635-8.
13. Schultz RJ, Aiache A. An operation to restore opposition of the thumb by nerve transfer. *Arch Surg* 1972;105:777-9.
14. Bertelli JA, Soldado F, Rodríguez-Baeza A, Ghizoni MF. Transfer of the motor branch of the abductor digiti quinti for thenar muscle reinnervation in high median nerve injuries. *J Hand Surg Am* 2018;43:8-15.
15. Tse R, Hentz VR, Yao J. Late reconstruction for ulnar nerve palsy. *Hand Clin* 2007;23:373-92.
16. Bertelli JA. Prior to repair functional deficits in above- and below-elbow ulnar nerve injury. *J Hand Surg Am* 2020; doi: 10.1016/j.jhsa.2019.10.033.
17. Weber RV, Mackinnon SE. Upper extremity nerve transfers. In: Slutsky DJ, Hentz VR, editors. *Peripheral nerve surgery: practical applications in the upper extremity*. Philadelphia: Churchill Livingstone Elsevier; 2006. p. 89.
18. Kim DH, Han K, Tiel RL, Murovic JD, Kline DG. Surgical outcomes of 654 ulnar nerve lesions. *J Neurosurg* 2003;98:993-1004.
19. Lester RL, Smith PJ, Mott G, McAllister RM. Intrinsic reinnervation- myth or reality? *J Hand Surg* 1993;18:454-60.
20. Gaul JS Jr. Intrinsic motor recovery: a long-term study of ulnar nerve repair. *J Hand Surg Am* 1982;7:502-8.
21. Wang Y, Zhu S. Transfer of a branch of the anterior interosseous nerve to the motor branch of the median nerve and ulnar nerve. *Chin Med J (Engl)* 1997;110:216-9.
22. Battiston B, Lanzetta M. Reconstruction of high ulnar nerve lesions by distal double median to ulnar nerve transfer. *J Hand Surg Am* 1999;24:1185-91.
23. Haase SC, Chung KC. Anterior interosseous nerve transfer to the motor branch of the ulnar nerve for high ulnar nerve injuries. *Ann Plast Surg* 2002;49:285-90.
24. Novak CB, Mackinnon SE. Distal anterior interosseous nerve transfer to the deep motor branch of the ulnar nerve for reconstruction of high ulnar nerve injuries. *J Reconstr Microsurg* 2002;18:459-64.
25. Brown JM, Yee A, Mackinnon SE. Distal median to ulnar nerve transfers to restore ulnar motor and sensory function within the hand: technical nuances. *Neurosurgery* 2009;65:966-78.
26. Kale SS, Glaus SW, Yee A, Nicoson MC, Hunter DA, et al. Reverse end-to-side nerve transfer: from animal model to clinical use. *J Hand Surg Am* 2011;36:1631-9.e2.
27. Farber SJ, Glaus SW, Moore AM, Hunter DA, Mackinnon SE, et al. Supercharge nerve transfer to enhance motor recovery: a laboratory study. *J Hand Surg Am* 2013;38:466-77.

28. Barbour J, Yee A, Kahn LC, Mackinnon SE. Supercharged end-to-side anterior interosseous to ulnar motor nerve transfer for intrinsic musculature reinnervation. *J Hand Surg Am* 2012;37:2150-9.
29. Boutros S, Nath RK, Yüksel E, Weinfeld AB, Mackinnon SE. Transfer of flexor carpi ulnaris branch of the ulnar nerve to the pronator teres nerve: Histomorphometric analysis. *J Reconstr Microsurg* 1999;15:119-22.
30. Baltzer H, Woo A, Oh C, Moran SL. Comparison of ulnar intrinsic function following supercharge end-to-side anterior interosseous-to-ulnar motor nerve transfer: a matched cohort study of proximal ulnar nerve injury patients. *Plast Reconstr Surg* 2016;138:1264-72.
31. Koriem E, El-Mahy MM, Atiyya AN, Diab RA. Comparison between supercharged ulnar nerve repair by anterior interosseous nerve transfer and isolated ulnar nerve repair in proximal ulnar nerve injuries. *J Hand Surg Am* 2020;45:104-10.
32. Magdi Sherif M, Amr AH. Intrinsic hand muscle reinnervation by median-ulnar end-to-side bridge nerve graft: case report. *J Hand Surg Am* 2010;35:446-50.
33. Colonna MR, Russo A, Galeano M, Delia G, Pajardi GE, et al. "Babysitting" procedures in proximal nerve trunk injuries: two case reports and a review. *Plast Aesthet Res* 2015;2:208-12.
34. Barbour JR, Gontre G, Daliwal G, Mackinnon SE, Tung TH. Transfer of the extensor digit quinti and extensor carpi ulnaris branches of the posterior interosseous nerve to the motor branch of the ulnar nerve to restore intrinsic hand function: case report and anatomic study. *J Hand Surg Am* 2012;38:98-103.
35. Gesslbauer B, Furtmüller GJ, Schuhfried O, Roche AD, Sporer M, et al. Nerve grafts bridging the thenar branch of the median nerve to the ulnar nerve to enhance nerve recovery: a report of three cases. *J Hand Surg Eur Vol* 2017;42:281-5.
36. Colonna MR, Pino D, Battiston B, d'Alcontres FS, Natsis K, et al. Distal nerve transfer from the median nerve lumbrical fibers to the distal ulnar nerve motor branches in the palm: an anatomical cadaveric study. *Microsurgery* 2019;39:434-40.
37. Bertelli JA, Soldado F, Rodríguez-Baeza A, Ghizoni MF. Transferring the motor branch of the opponens pollicis to the terminal division of the deep branch of the ulnar nerve for pinch reconstruction. *J Hand Surg Am* 2019;44:9-17.
38. Ebied AM, Kemp GJ, Frostick SP. The role of cutaneous sensation in the motor function of the hand. *J Orthop Res* 2004;22:862-6.
39. Nath RK, Mackinnon SE, Shenaq SM. New nerve transfers following peripheral nerve injuries. *Operat Tech Plast Reconstr Surg* 1997;4:2-11.
40. Imbriglia JE, Hagberg WC, Baratz ME. Median nerve reconstruction. In: Peimer CA, editor. *Surgery of the hand and upper extremity*, vol II. New York: McGraw-Hill; 1996. p. 1381.
41. García-López A, Sebastian P, Martinez F, Perea D. Transfer of the nerve to the brachioradialis muscle to the anterior interosseous nerve for treatment for lower brachial plexus lesions: case report. *J Hand Surg Am* 2011;36:394-7.
42. Rapp E, Lallemand S, Ehrler S, Buch N, Foucher G. Restoration of sensation over the contact surfaces of the thumb-index pinch grip using the terminal branches of the superficial branch of the radial nerve. *Chir Main* 1999;18:179-83.
43. Bertelli JA, Ghizoni MF. Very distal sensory nerve transfers in high median nerve lesions. *J Hand Surg Am* 2011;36:387-93.
44. Ross D, Mackinnon SE, Chang YL. Intraneural anatomy of the median nerve provides "third web space" donor nerve graft. *J Reconstr Microsurg* 1992;8:225-32.
45. Cheng J. Nerve transfers for digital sensation. In: Slutsky DJ, editor. *Master skills in nerve repair: tips and techniques*. Rosemont (IL): ASSH; 2008.
46. Mennen U. End-to-side nerve suture in clinical practice *Hand Surg* 2003;8:33-42.
47. Pienaar C, Swan MC, De Jager W, Solomons M. Clinical experience with end-to-side nerve transfer. *J Hand Surg* 2004;29:438-43.
48. Brenner MJ, Dvali L, Hunter DA, Myckatyn TM, Mackinnon SE. Motor neuron regeneration through end-to-side repairs is a function of donor nerve axotomy. *Plast Reconstr Surg* 2007;120:215-23.
49. Amr SM, Moharram AN. Repair of brachial plexus lesions by end-to-side side-to-side grafting neuroorrhaphy: experience based on 11 cases. *Microsurgery* 2005;25:126-46.
50. Tarasidis G, Watanabe O, Mackinnon SE, Strasberg SR, Haughey BH, et al. End-to-side neuroorrhaphy: a long-term study of neural regeneration in a rat model. *Otolaryngol Head Neck Surg* 1998;119:337-41.
51. Tarasidis G, Watanabe O, Mackinnon SE, Strasberg SR, Haughey BH, et al. End-to-side neuroorrhaphy resulting in limited sensory axonal regeneration in a rat model. *Ann Otol Rhinol Laryngol* 1997;106:506-12.
52. Somsak L, Kittipod N, Kanchai M, Chairaj U, Kiat W, et al. End-to-side radial sensory to median nerve transfer to restore sensation and relieve pain in C5 and C6 nerve root avulsion. *J Hand Surg* 2011;36A:209-15.
53. Anderson GA. Ulnar nerve palsy. In: Green DP, Hotchkiss RN, Pederson WC, editors. *Green's operative hand surgery*. 5th edition. Philadelphia: Elsevier; 2005. p. 1162.
54. Brown JM, Yee A, Mackinnon SE. Distal median to ulnar nerve transfers to restore ulnar motor and sensory function within the hand: technical nuances. *Neurosurgery* 2009;65:966-78.
55. Flores LP. Distal anterior interosseous nerve transfer to the deep ulnar nerve and end-to-side suture of the superficial ulnar nerve to the third common palmar digital nerve for treatment of high ulnar nerve injuries: experience in five cases. *Arq Neuropsiquiatr* 2011;69:519-24.
56. Oberlin C, Teboul F, Severin S, Beaulieu JY. Transfer of the lateral cutaneous nerve of the forearm to the dorsal branch of the ulnar nerve, for providing sensation on the ulnar aspect of the hand. *Plast Reconstr Surg* 2003;112:1498-500.
57. Ruchelsman DE, Price AE, Valencia H, Ramos LE, Grossman JA. Sensory restoration by lateral antebrachial cutaneous to ulnar nerve transfer in children with global brachial plexus injuries. *Hand (N Y)* 2010;5:370-3.
58. Sassu P, Libberecht K, Nilsson A. Nerve transfers of the forearm and hand: a review of current indications. *Plast Aesthet Res* 2015;2:195-201.

Review

Open Access



# Established and experimental techniques to improve phalloplasty outcomes/optimization of a hypercomplex surgery

Erin E. Carter<sup>1</sup>, Curtis N. Crane<sup>2</sup>, Richard A. Santucci<sup>2</sup>

<sup>1</sup>Department of Urology, Boston University School of Medicine, Boston, MA 02119, USA.

<sup>2</sup>The Crane Center for Transgender Surgery, Austin, TX 78746, USA.

**Correspondence to:** Dr. Richard A. Santucci, Senior Surgeon, The Crane Center for Transgender Surgery, 5656 Bee Cave Rd Suite J201, Austin, TX 78746, USA. E-mail: richard@cranectx.com

**How to cite this article:** Carter EE, Crane CN, Santucci RA. Established and experimental techniques to improve phalloplasty outcomes/optimization of a hypercomplex surgery. *Plast Aesthet Res* 2020;7:33. <http://dx.doi.org/10.20517/2347-9264.2020.81>

**Received:** 15 Apr 2020 **First Decision:** 18 May 2020 **Revised:** 27 May 2020 **Accepted:** 19 Jun 2020 **Published:** 30 Jun 2020

**Science Editors:** Marlon E. Buncamper, Stan J. Monstrey **Copy Editor:** Cai-Hong Wang **Production Editor:** Tian Zhang

## Abstract

An increasing number of transgender and gender non-conforming patients are seeking genital gender affirming surgeries in order to better align their physical characteristics with their innate gender identity and treat gender dysphoria. Phalloplasty is the most complex of these surgeries, and this complexity creates a wide range of potential complications. Some of the most common complications and therefore, targets for improvement in outcomes, concern neourethral fistula/stricture, efficacy of reinnervation of the phalloplasty flap, postoperative flap monitoring, and donor site morbidity. In the setting of no established "gold standard", this review seeks to describe the components and staging of phalloplasty, with an emphasis on established and experimental solutions to the most common and vexing problems.

**Keywords:** Phalloplasty, transgender, female to male, transmasculine, surgical complications, radial forearm flap, anterolateral thigh flap, genital gender confirmation

## INTRODUCTION

Gender dysphoria is the distress resulting from a marked incongruence between a patient's natal sex and their innate gender identity. Treatment of gender dysphoria in transgender (TG), non-binary, and gender non-conforming patients (TGNC) may include both medical and surgical interventions using shared decision making to customize treatment plans according to individual patient goals. A significant and growing population are seeking out genital gender-affirming surgery (gGAS) and an increasing number of surgical centers are available to meet that need<sup>[1,2]</sup>. For such patients, these procedures improve quality of life,



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** Excellent aesthetic results following phalloplasty and placement of erectile and testicular prostheses

relieve gender dysphoria and decrease suicidality<sup>[1-5]</sup>. Masculinizing gGAS usually involves some combination of removing natal female genital structures and creating functional natal male-type genitourinary structures. Phalloplasty (with or without simultaneous vaginectomy, urethral lengthening and scrotoplasty) is one such procedure that seeks to create an aesthetic and functional neophallus.

Phalloplasty uses existing analogous structures that share a common embryologic origin, as well as carefully harvested fasciocutaneous allograft(s), to create a neophallus. The goals are to create an aesthetic neophallus that has both tactile and erogenous sensation that can be directed for standing micturition, and has sufficient girth to accommodate penile prosthesis<sup>[6-9]</sup>. Erection and penetrative intercourse may be achieved, usually following penile prosthetic placement<sup>[6,7]</sup>.

Phalloplasty is an exceptionally complex reconstructive surgery. For instance, a single-stage phalloplasty (including vaginectomy, urethral lengthening and scrotoplasty) represents 200 relative value units (RVUs) of surgery (RVUs are a measure of value used in the United States which rank on a common scale the resources used to provide physician services). As a comparison, a craniotomy for removal of a glioblastoma multiforme brain tumor is 85 RVUs. Though this ultracomplex surgery may be daunting, excellent aesthetic and functional results with high patient satisfaction are achievable [Figure 1].

Surgical techniques used by gGAS surgeons for each component varies widely and are largely nonstandardized<sup>[4]</sup>. This article seeks to describe the components of phalloplasty as well as common complications and potential technical improvements that may be used to lower complications. Some targets for improvement in outcomes concern neourethral fistula/stricture, efficacy of reinnervation of the phalloplasty flap, postoperative flap monitoring, and donor site morbidity. Although several improvements in technique have occurred in recent years, ongoing innovation in surgical technique is necessary to further improve patient outcomes.

## THE QUESTION OF STAGING

The components of phalloplasty include the creation of a penile shaft, penile urethroplasty, urethral lengthening (perineal urethroplasty), scrotoplasty, glansplasty, vaginectomy, hysterectomy, salpingoophorectomy (if desired), testicular implants, and erectile device implant<sup>[6,9]</sup>. The inclusion, staging, and order of these procedures may be altered to align with each patient's treatment goals as well as the surgeon's assessment of best practices. Each individual component, especially penile shaft creation and penile urethroplasty, have multiple surgical approaches.

The ideal phalloplasty would be a single-stage and reproducible surgery with minimal complications. However, because of the range, severity, and prevalence of complications of the surgery, some have elected to

divide the procedure into multiple stages<sup>[10]</sup>. There is no consensus or “gold standard” among gGAS surgeons regarding the optimal number of stages or sequencing of reconstructive steps. Of note, our high-volume center generally does not stage phalloplasties except in unusual situations.

Centers like ours that usually perform phalloplasty, vaginectomy, scrotoplasty and urethral lengthening in a single stage have shown decreased rates of flap-related complications and increased patient satisfaction<sup>[10]</sup>. A major advantage to the single-stage approach is that most patients (up to 60% at our center) will require no further surgery until the time of penile prosthesis placement. We use a team approach to efficiently use operating room time such that a single-stage phalloplasty routinely takes about 6 h, and seldom as much as 8 h. Other centers favor a staged approach, citing the potential for fewer neourethral complications and more straightforward management of complications, even at the cost of more operations for these patients<sup>[4,9,10]</sup>. However, not all researchers have found that staging phalloplasties will result in fewer complications<sup>[4]</sup>. Even if staging ultimately proves to not be effective at decreasing complications, multiple staged procedures allow for sophisticated surgical planning. For example, hysterectomy may be consolidated into one of the phalloplasty stages. Regardless of staging choice, it is agreed that any prosthetic placement, including penile prosthesis and testicular prostheses should be performed in a later stage after complete healing has occurred and some sensation restored<sup>[6,8,11]</sup>.

## FLAP SELECTION, PREPARATION AND DONOR SITE HEALING

When selecting a donor site for creation of the penile shaft and penile neourethra, it is important to consider the benefits and drawbacks of each potential site, as well as patient goals. All flaps must have sufficient innervation and vascularity to allow for microsurgical creation of the neophallus. The radial forearm flaps (RFF) are the most commonly used and most widely studied option<sup>[6,12]</sup>, followed by pedicled anterolateral thigh flaps (ALT)<sup>[3,11]</sup>. RFF provides a robust, well vascularized and appropriately-sized neophallus in most cases, at the cost of requiring a microvascular anastomosis that may clot suddenly (< 1% of cases). The RFF tends to be thinner (particularly important in patients with increased adiposity) and more pliable, and may be more similar to natal genital tissue<sup>[3,11]</sup>. The major downside of the RFF is removal of skin from an exposed and functionally important location on the body<sup>[13]</sup>. Some patients may have concerns about the visibility and potential recognizability of the resultant scar, and thus may want to avoid using a RFF flap<sup>[1,5,6]</sup>.

The ALT has the noted benefit of not usually requiring a microvascular anastomosis, and may have less risk of significant vascular occlusion emergencies as a result. However, the microcirculation of ALT flaps is less robust than RFF flaps, and a moderately higher chance of partial flap loss is well described with ALT flap use<sup>[9,12]</sup>. It is said that in some cases, the inherent rigidity of the ALT flap may allow for sufficient rigidity for penetrative intercourse without the need for an additional prosthetic device, although most of our patients still require the addition of a penile prosthesis for penetrative intercourse<sup>[3,6]</sup>. The resultant scarring at the donor site may be less distressing and easier to hide for ALT flaps.

Less common flap donor sites include abdominal flaps, modified latissimus dorsi flaps (MLD), tibial free flaps, and superficial circumflex artery perforator flaps<sup>[3]</sup>. Of these, only the MLD is frequently chosen today.

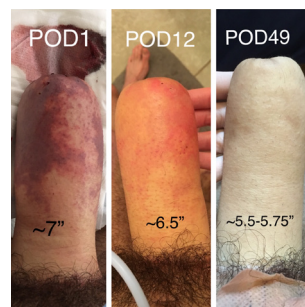
## Preoperative hair removal

The consensus is that donor flaps should be hairless, either innately or following removal methods such as laser hair removal or electrolysis. However, the efficacy and long-term permanence of these methods is poorly studied. While preoperative hair removal likely decreases the number and thickness of hairs, it is unlikely that the results of even the most thorough hair removal protocol are ever truly permanent<sup>[3,14]</sup>. Many phalloplasty patients have some hair regrowth in the neourethra<sup>[9,14,15]</sup>. Most patients are unaffected by urethral hair, but complications including infection, calculi, and trichobezoars/hairballs can occur, although uncommon<sup>[15,16]</sup>. Urinary obstruction from neourethral hair [Figure 2] can happen despite thorough





**Figure 2.** Cystoscopic appearance of abundant hair in the proximal penile urethra, which became calcified by urinary solutes and created an obstructive intraurethral trichobezoar/hairball



**Figure 3.** Comparison of penile girth after liposuction by postoperative day (POD), showing a 20% decrease in girth over time. Of note, there is prominent ecchymosis and swelling immediately after the procedure, which subsides with time

preoperative depilation<sup>[14]</sup>. While we continue to recommend thorough preoperative depilation, especially of the neourethral portion of the future flap, the optimal hair removal protocol has yet to be devised.

### Implications of donor site adiposity

Donor site individualization based on patient preference is our practice. However, certain body habitus types may have more favorable outcomes by choosing one flap type over another. For instance, particularly thin individuals should avoid the RFF because the resultant neophallus may be disproportionately thin. Conversely, these thin individuals tend to do especially well with ALT flaps, as the donor site will not be too thick for creating an aesthetic and proportionate neophallus. Individuals with increased adiposity of the anterior thigh should avoid ALT flaps if possible and may do particularly well with a RFF flap instead<sup>[3]</sup>. We advise our patients of these considerations before they finalize their choice of donor site.

Especially thick ALT flaps create several problems. The first is that the resultant neophallus may be disproportionately large in circumference. Contouring these very large neophalluses down to an aesthetic, biologically-appropriate size may be difficult or even impossible. Most ALT flap patients will require one liposuction session to reduce penile girth [Figure 3]. However, some particularly large ALT neophalluses may require multiple liposuction procedures, open surgical reduction of ventral penile skin to decrease girth of the shaft, and contouring of the base of the neophallus to create a more proportional outcome. Even with multiple revision surgeries, excellent aesthetic results may never be achievable.

The second major problem with too-thick ALT flaps is the possibility that the neourethra cannot be made in the first stage. Using the tube-within-a-tube (TWT) technique, it may be impossible to close a particularly



**Figure 4.** Delayed anterolateral thigh flap ready for use for phalloplasty, 6 months after flap creation. Note that the superior contour of the flap has not been incised in order to decrease flap edema. Nonviable flap edges (not present in this patient) can be identified and removed if present at this stage

thick flap to create the neophallus if the flap thickness is too great<sup>[3]</sup>. In that case, we do not tubularize the flap to create the neourethra in the first stage and instead, perform penile urethroplasty in a subsequent stage. Staged penile urethroplasty is a long, invasive procedure that may have a higher rate of dehiscence than primary urethroplasty<sup>[6,10]</sup>. We counsel and caution our patients when pinched anterior thigh skin/fat thickness is  $> \frac{1}{2}$  inch (1.3 cm) and strongly advise against the ALT flap in those with a pinched thigh skin/fat thickness  $> 1$  inch (2.5 cm).

The ideal management of the too-thick ALT donor site, other than avoidance of this flap, has not been established. We have created an experimental protocol for patients with thick ALT flap donor sites who request ALT phalloplasty and will not/cannot consider RFF phalloplasty. The surgery is staged, allowing for two opportunities to thin the flap while protecting the blood supply in the tissue to be used to make the neourethra and shaft. We perform a simultaneous subtotal vaginectomy and delay of the flap in the first stage. The flap is dissected in the usual fashion, except the tissue around its perforators is left unoperated. Fat deep to Scarpa's fascia is removed, which moderately reduces phallic girth. The superior incision line is not created at this time in order to decrease subsequent lymphedema of the flap [Figure 4]. After 6 months, there is compensatory hypertrophy of the perforators, any tissue loss at the edge of the flap will have occurred and is discarded, and a second moderate thinning of the flap possible. We do not thin the flap during the typical single-stage ALT phalloplasty because of the increased risk of devascularizing the flap.

### Optimizing the donor template

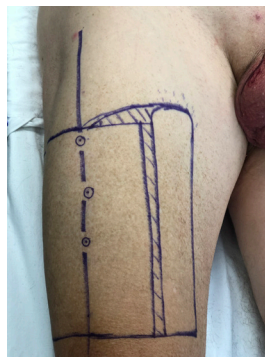
Donor site dimensions are important when considering the final length and girth of the neophallus, including sufficient girth for the insertion of a penile prosthetic device. Depending on the anticipated surgical technique, the flap design may include the penile shaft only, two separate flaps for the penile shaft and penile urethra, or the TWT flap design. The TWT flap style is a well-established technique and is almost exclusively used in our high-volume center when urethral extension is desired. The ideal donor site configuration is unknown, but we have introduced several small modifications that appear to improve outcomes.

(1) Create an “as short-as-possible” urethral extension. This allows the neourethra to protrude from the shaft, simplifying urethral anastomosis. This urethral extension can theoretically impair blood flow to this portion of the flap, so we create the shortest possible extension that will allow for tensionless anastomosis.

(2) In order to maximize the proximal urethral blood supply, we spare the dermis medial to the urethral extension as well as a 2-3 cm zone of subdermal fat superior to the urethral extension.



**Figure 5.** Intraoperative display of small anterolateral thigh flap perforators, measuring < 1 mm in diameter



**Figure 6.** Preoperative marking of an anterolateral thigh flap donor site showing (1) as short-as-possible urethral extension; (2) de-epithelialization of the proximolateral dermis next to the urethral extension; (3) sparing of the fat around the urethral extension. Circled dots represent the location of three flap perforators identified with a handheld Doppler

(3) The vascularity of an ALT flap relies on the patient's anatomy, specifically the perforators arising from the descending branch of the lateral circumflex femoral artery<sup>[3,9]</sup>. It is advantageous to include the largest and as many perforators as possible when designing this particularly large flap<sup>[9]</sup>. When using the ALT flap, we use a handheld Doppler to mark the patient's dominant perforators while the patient is awake in the preoperative holding area. An example of particularly small ALT perforators is shown in [Figure 5](#).

(4) When using the ALT flap, we advise the patient to stop injecting testosterone into the donor thigh. Common formulations of depo testosterone contain benzyl benzoate, benzyl alcohol, and a large amount of cottonseed oil (736/100 mg of testosterone), creating a nidus of scar, which is best avoided in flap donor sites. In fact, we advocate that standard instructions for intramuscular testosterone injections should be modified from "switch thigh injection sites from side to side with each injection" to "pick a side and stick to it" in a patient who thinks they may ever be interested in ALT phalloplasty.

[Figure 6](#) illustrates the preoperative marking of an ALT donor site from our center highlighting these configuration modifications.

We are interested in using imaging technology, perhaps CT angiogram, as some other groups have reported, to select the thigh with the most robust perforator blood supply in the future<sup>[6,9]</sup>. However, this has not currently been shown to be effective in improving outcomes<sup>[17]</sup>.

#### RFF donor site coverage

Once a fasciocutaneous RFF flap is removed, the underlying tendons, muscles, and nerve are exposed. Historically, the RFF donor site has been managed with a single layer split thickness skin graft (STSG)<sup>[18]</sup>.



**Figure 7.** Six-week postoperative appearance of radial forearm flap donor site covered with thin Integra®, 18/1000-inch thick split thickness skin graft, and negative pressure wound therapy for 8 days. Note excellent take of the skin graft, minimal step-off, and plump, nonadherent configuration of the graft

However, using an STSG alone can be associated with significant donor site morbidity<sup>[11,18]</sup>. STSGs may adhere to the exposed tendons and reduce the strength and range of motion, lead to contractures, and is frequently distressing to the patient<sup>[13,18]</sup>.

Recently, we and others have been using artificial dermal substitutes such as Integra® Wound Matrix (Thin) (Integra LifeSciences; Plainsboro, NJ) to improve RFF donor site healing and cosmesis<sup>[6,13,18]</sup>. Integra® is a synthetic acellular dermal regeneration template that is composed of a bilaminate sheet of cross-linked bovine tendon collagen coated with shark glycosaminoglycans (chondroitin-6-sulfate)<sup>[19]</sup>. Originally developed for the coverage of burn wounds, Integra® provides a scaffolding for revascularization and growth of the neodermis while creating a gliding surface against the underlying nerve, muscle, and tendon<sup>[19]</sup>. We place the thin Integra® over the wound, followed by an 18/1000-inch unmeshed STSG, and then apply negative pressure wound therapy with a V.A.C™ device (KCI; San Antonio, TX) for at least 9 days postoperatively, and sometimes up to 12 days. We have found that this method of single-stage Integra® and immediate STSG application has equivalent graft take results as the staged approach. Though a staged approach is the most common, single-stage Integra® application has been described by others in a variety of applications previously<sup>[20-23]</sup>.

We have found that using Integra® under the usual STSG in this way results in improved cosmesis with a noticeably thicker result and less step-off. It also minimizes the risk of tight adhesion of the STSG to the underlying deep arm structures<sup>[13,18]</sup>. Other groups have also documented improvement in skin elasticity, motor function, range of movement, wound contractures and hypertrophic scar formation with Integra®<sup>[13,18]</sup>. [Figure 7](#) displays one of our patient's RFF donor site managed with thin Integra®, STSG, and negative pressure wound therapy at six weeks after surgery.

## URETHRAL LENGTHENING COMPLICATIONS

The most common complications of phalloplasty involve the urethral lengthening portion of the procedure. In recent years, improved surgical technique has greatly decreased the rate of neourethral complications, but all-cause urinary complications from phalloplasty are still reported to be between 35%-58%<sup>[4,8]</sup>. The rate of complications is so high that patients should decide if urinating from the tip of the neophallus is imperative for their treatment goals, as maintaining the external urethral orifice in the native position and forgoing standing voiding forever greatly reduces the risk of urinary complications<sup>[6]</sup>.

In general, urinary strictures tend to have significant morbidity, and open surgical intervention is required in most (94%-96%) stricture patients<sup>[24]</sup>. The management options of neourethral stricture after phalloplasty

are the same as those for native male urethral repair<sup>[11,24]</sup>. Unfortunately, these repairs have a high predicted failure rate, reported as high as 50% in this population<sup>[24]</sup>. In some cases, especially those requiring multiple surgical interventions, a temporary or permanent perineal urethrostomy may be necessary<sup>[24]</sup>. Patients generally are at risk for stricture formation for at least 1 year, although most strictures present within the first 6 months after phalloplasty<sup>[11]</sup>.

Fistulas have overall lower morbidity than strictures. 17%-35% of fistulas may heal spontaneously within 3 months without the need for surgical intervention<sup>[24]</sup>. If spontaneous recovery does not occur, surgical repair has a high expected success rate<sup>[24]</sup>. Small (< 5 mm) fistulas are good candidates for a primary repair, whereas larger fistulas (> 5 mm) may require a graft to cover the defect<sup>[11]</sup>. Patients who do not elect to have vaginectomy have an especially high (up to 60%) risk for urethrocutaneous fistulas<sup>[11,25]</sup>.

Asymptomatic pseudodiverticula are very common at the point between the native urethra and the pars fixa. They can be more prominent in those with high pressure voiding from a more distal anastomotic stricture, but almost always decompress when the distal obstruction is treated. Uncommonly, these pseudodiverticula may suffer urinary tract infections from trapped urine, or cause significant post-void dribbling that may require surgical management<sup>[6]</sup>.

Meatal stenosis is a short narrowing of the most distal portion of the penile urethra and is thought to be caused by local ischemia of a watershed region, leading to contracture of the skin at the meatus<sup>[8]</sup>. This complication is generally straightforward to treat through meatoplasty with good outcomes<sup>[24]</sup>. Meatal stenosis generally does not have significant long-term urinary consequences when treated<sup>[24]</sup>.

Multiple urethral complications may occur concurrently, and many patients with urethral complications may present with numerous simultaneous urologic findings<sup>[7]</sup>. For example, in RFF phalloplasty, fistulas are often located immediately proximal to a concurrent stricture or urethral trichobezoar/hairball<sup>[7,14]</sup>.

There are several methods of creating the neourethra, but we currently use the TWT almost exclusively. Prelamination of the RFF or ALT with buccal mucosal graft or even vaginal mucosa harvested from vaginectomy has been proposed, but requires an additional staged surgery without significant improvement in outcomes<sup>[8,10]</sup>. Additionally, these grafts heal by scarring into the tissue over which they are placed, and do not seem to be an improvement over the healthy, unscarred, well-vascularized arm/leg tissue they are meant to replace. Creating an ALT neourethra out of a second free flap harvested from the forearm has also been proposed, but we and others are concerned that the addition of a second free flap (especially to the ALT surgery which only requires a pedicled flap) invites a potentially dangerous second microvascular anastomosis and may not provide any advantage to the patient<sup>[6,10]</sup>.

### Improvements in pars fixa creation

A critical improvement in phalloplasty came from using bulbospongiosus muscle flaps as a second layer of the proximal pars fixa. It greatly decreased the rate of fistulas and strictures in this area<sup>[26]</sup>. This is an essential step in our urethral lengthening technique when vaginectomy is simultaneously performed. Additionally, we cover the more distal pars fixa with a second layer of labial minora tissue<sup>[26]</sup>. These additional layers are thought to reinforce the vascular supply of the pars fixa, and have further reduced the rate of fistulas and strictures in this area dramatically.

### Improvements in distal urethral anastomosis

The anastomosis of the pars fixa with the pars pendulans is the most common site for urethral strictures and fistulas after phalloplasty<sup>[7,8]</sup>. The higher prevalence of anastomotic strictures and fistulas here is most often attributed to local ischemia in this “double watershed” zone where the edge of the neourethra portion of the flap has the poorest blood flow, and the edge of the pars fixa urethra flaps have their poorest blood supply<sup>[6,8]</sup>.



It has been challenging to surgically create the anastomosis of the pars fixa and the pars pendulans of the neourethra in a way that maintains sufficient vascular supply, promotes healing, and minimizes these complications. It is recommended that all patients undergoing phalloplasty have the placement of a suprapubic catheter for urinary flow diversion during the initial healing stages to prevent complications<sup>[8]</sup>. This usually stays in place for 2-3 weeks postoperatively<sup>[11]</sup>. Techniques used to maximize vascularity in this troublesome area begin with donor flap choice. The RFF flaps are generally the most well-vascularized option<sup>[3,6]</sup>. Additionally, careful dissection of the flap to preserve its blood supply is also imperative; dissection of an additional 2 cm rim of nearby fatty tissue that might usually be discarded and sparing the nearby dermis can help further preserve blood supply.

When attaching the pars fixa to the pars pendulans, it is important to create a tensionless closure. Methods to reduce tension in this area include creating a urethral extension from the donor flap. The anastomotic suture line can be further protected by the addition of a second well-vascularized layer over the anastomotic section of urethra using a variety of tissues. We have studied the use of the dorsal clitoris dartos tissue, which is dissected free and moved ventrally to cover the urethral suture line, with indeterminate results so far. Overlay of the urethra with the gracilis flap seems to decrease the rate of stricture and fistula formation at the cost of significant additional surgery, but has not yet been widely adopted<sup>[27,28]</sup>. Some manuscripts mention the use of pedicled rectus flaps as an additional well-vascularized layer, but we were unable to find any scientific reports describing its use<sup>[7]</sup>. While coverage with the labia majora (Martius) flap has also been suggested, we hesitate to disrupt the delicate labial majora flaps that are already used to create a neoscrotum in most patients<sup>[6-8]</sup>.

Our group has also studied using tissue engineered technology, particularly a bioactive tissue matrix allograft composed of dehydrated human amnion/chorion membrane (dHACM) (Amniofix<sup>TM</sup>; MiMedx, Marietta, GA) to promote healing of the anastomotic urethra. dHACM is a scaffold that contains hundreds of functioning growth factors and cytokines, including platelet derived growth factor AA (PDGF-AA), transforming growth factor b1 (TGFb1), vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF-2), interleukin (IL)-4, 6, 8, and 10, and tissue inhibitor of metalloproteinase 1 and 2 in high concentrations and physiologic ratios<sup>[29]</sup>. This material has been shown to promote fibroblast and endothelial cell proliferation and support angiogenesis of the surrounding tissue<sup>[29]</sup>. In order to decrease scarring and improve healing in this vulnerable area, we have covered the neourethral anastomosis with a 12 cm by 2 cm piece of Amniofix<sup>TM</sup>, with uncertain benefit to date.

## HEMOSTATIC AGENTS IN SCROTOPLASTY

Dissection of the labia majora to create the neoscrotum must maximally preserve the blood supply to these flaps. These flaps have a large surface area, loose underlying connective tissue, and are closed into a spherical geometric shape that tamponades bleeding poorly. Ongoing bleeding after scrotoplasty can be clinically troublesome and result in flap necrosis, scrotal dehiscence, or urethral fistula<sup>[9]</sup>. Rarely, some patients may require anticoagulation after microsurgery (e.g., vessel thrombosis), which increases the risk of hematoma formation. Because of this, we routinely use a thrombin-gelatin hemostatic matrix FloSeal<sup>TM</sup> (Baxter Healthcare; Deerfield, IL) to eliminate the need for scrotal drains and have limited scrotal hematoma, with good effect.

## NERVE REGENERATION

Attainment of both tactile and erogenous sensation in the neophallus is one of the principal goals of phalloplasty. With widespread adoption of microvascular techniques, a sensate neophallus is now achievable via microanastomosis of one or more flap nerves to the dorsal clitoral nerve of the clitoris<sup>[8]</sup>. The donor nerve from the flap (i.e., medial/lateral antebrachial cutaneous nerve from a RFF flap, or femoral cutaneous nerve from an ALT flap) is microanastomosed to one of the two dominant dorsal clitoral nerve branches.

Additional nerves that are present in the flap may also be anastomosed to the ilioinguinal nerve, further improving light touch sensation to the neophallus<sup>[11,12]</sup>.

The technique of microsuturing the epineurium has imperfect functional outcomes<sup>[30]</sup>. The axons from the distal natal nerve must traverse the anastomosis and reach the proximal segment of the donor nerve. Any anastomotic site scarring or fibrosis may create a mechanical impediment to the axons and limit reinnervation outcomes<sup>[30]</sup>. In addition to joining the aponeuroses with several sutures, we augment the nerve repair with a fibrin sealant (Tisseel®; Baxter Healthcare; Deerfield, IL or an equivalent product). This serves to mechanically protect the nerve repair during the remaining surgery, and from pulling during patient movement<sup>[31,32]</sup>. Our patients have had favorable nerve regeneration outcomes after phalloplasty; about 80% achieve erogenous sensation in the neophallus, and up to 95% achieve light touch sensation in the neophallus. A notable 5% have no sensation to the neophallus whatsoever.

In order to further increase the speed and outcome of nerve regeneration, we have studied the use of nerve conduit wraps intended for peripheral nerve repair, such as the Axoguard™ Nerve Protector Wrap (Axogen; Alachua, FL) to augment the standard end-to-end surgical technique. The ideal nerve wrap is non-immunogenic, strong enough to resist degradation and compression, and prevents both scarring and nerve adhesion formation<sup>[33]</sup>. The Axoguard™ implant is a sheath made of porcine extracellular matrix (ECM) from small intestinal submucosa<sup>[33,34]</sup>. Its acellular matrix is composed of collagen, fibronectin, growth factors, glycosaminoglycans, proteoglycans, and glycoproteins, all which may promote neural tissue revascularization through the induction of cellular proliferation/differentiation as well as the deposition of host ECM components<sup>[33,34]</sup>. It provides a physical barrier around the anastomosis, potentially protecting it from cellular infiltration and allowing the nerve to glide normally during movement<sup>[33]</sup>. With other surgical indications, it has been shown to decrease nerve scarring during healing, with favorable functional outcomes<sup>[33,34]</sup>. We will continue to investigate the role of these ECM nerve connection sheaths to see if they will improve the efficiency of nerve regeneration over fibrin sealant alone.

## POSTOPERATIVE FLAP MONITORING & FLAP COMPLICATIONS

The immediate postoperative healing period is crucial to phalloplasty flap survival. Notable potential complications include infection, hematoma, wound dehiscence, urethral loss, partial phallic loss, and full phallic loss<sup>[9]</sup>. These complications are most frequently due to vascular compromise, often venous congestion leading to potentially irreversible tissue injury<sup>[35-37]</sup>. The flaps used in phalloplasty pose an additional challenge: they are much larger than most free flaps used for other tissue coverage indications, are freely mobile, are anchored only at the base, are in a dependent position, and are positioned close to a natural flexion point of the body<sup>[6,9]</sup>. Partial and total phallic loss are devastating complications for both the patient and surgeon.

An estimated 1%-9% of free flaps across all indications are lost due to vascular compromise<sup>[4,35-38]</sup> with an estimated 95% of losses occurring during the first 24-72 h after surgery<sup>[37,39]</sup>. At our center, the rate of flap compromise requiring reoperation after RFF phalloplasty is less than 1%, and much lower after ALT phalloplasty. We currently have a 0% rate of acute, total neophallus loss. Even in those rare patients with arterial/venous thrombosis in the early postoperative period, in our cohort of > 750 phalloplasty patients we have not lost any flaps to acute graft thrombosis. Similarly, clinically significant partial flap loss is rare at our center, although instances of partial flap compromise may contribute to urethral stricture and/or fistula development (20% and 22% at our center, respectively). This is likely because of several factors: our use of continuous flap oxygen monitoring, attentive expert bedside nursing, an on-call team of two surgeons available at all times for emergency revision surgery, and the optimization of techniques made possible by a large volume experience.

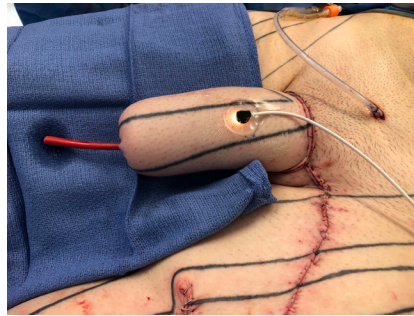


**Figure 8.** We use the T-Stat™ VLS Tissue Oximeter (Spectros; Portola Valley, CA) for postoperative flap monitoring in all patients, and sometimes for intraoperative decision-making. It provides continuous quantitative measurements of total hemoglobin concentration [Hgb] and hemoglobin oxygen saturation

Early identification of vascular compromise is directly correlated with successful flap salvage rates<sup>[35-37,39]</sup>. Typical postoperative flap monitoring includes bedside physical exam in combination with handheld Doppler monitoring of the arterial signal<sup>[35,37,39]</sup>. The clinical exam includes flap skin color, capillary refill, and surface temperature<sup>[11,37]</sup>. If necessary, pinprick bleeding may be used, although we do not routinely use this at our center. Cutaneous handheld Dopplers are used to detect intravascular arterial and venous blood flow<sup>[37]</sup>. These checks occur every 1-2 h at the bedside for the first few days postoperatively. Flaps that are concerning for incipient vascular compromise are monitored even more frequently. While clinical evaluation and Doppler alone are generally effective and have negligible additional cost<sup>[37]</sup>, there are drawbacks to this method. Clinical examination can vary between observers, require trained staff, and may not be able to detect vascular compromise until relatively late physical findings become apparent<sup>[36]</sup>. At that time, damage to the flap microvasculature may be irreversible. Additionally, while Doppler is sensitive to arterial compromise, it is less sensitive to venous compromise<sup>[35,36]</sup>. This is problematic given that venous congestion is the most common cause of flap failure<sup>[35-37]</sup>. Furthermore, the strength of the arterial Doppler signal does not correlate with sufficient oxygen delivery to the flap and loss of the Doppler signal can be a relatively late finding<sup>[35,38]</sup>. Neither clinical examination nor handheld Dopplers are able to continuously monitor the integrity of the flap, which could delay detection of vascular compromise<sup>[35,36]</sup>.

The ideal flap monitoring system is noninvasive, continuous, reliable, sensitive, and easy to interpret<sup>[37,39]</sup>. Our group uses the T-Stat™ VLS Tissue Oximeter (Spectros; Portola Valley, CA) to augment routine bedside clinical examinations and handheld Doppler checks [Figure 8]. The T-Stat™ oximeter uses visible light spectroscopy (VLS) to continuously monitor total hemoglobin concentration [Hgb] as well as hemoglobin saturation (StO<sub>2</sub>) of tissue at the capillary level in real time<sup>[35,36]</sup>. [Hgb] approximates the total blood volume in the flap, and an increased value indicates obstruction of venous drainage<sup>[36]</sup>. StO<sub>2</sub> measurements correlate directly with arterial tissue perfusion, and a decreased value indicates diminished arterial inflow from an arterial thrombus or is a later finding in the case of venous drainage obstruction<sup>[36]</sup>. In this way, VLS monitoring is able to continuously quantify the arterial inflow, venous outflow, and perfusion status of the flap<sup>[36]</sup>.

Multiple groups have demonstrated an initial gradual increase in StO<sub>2</sub> detected with VLS in the first ~8 h after surgery before the value stabilizes, attributable to mild ischemia-reperfusion injury<sup>[35,36]</sup>. The mean



**Figure 9.** Placement of the T-Stat™ VLS Tissue Oximeter (Spectros; Portola Valley, CA) probe for monitoring of the vascular integrity of the phalloplasty flap in the immediate postoperative period

StO<sub>2</sub> has been reported as 49%-57% with a range of 40%-75% regardless of flap type<sup>[35,36,39]</sup>. There are no established criteria for oximeter detection of flap compromise, although one group proposed using either a 20-point drop in StO<sub>2</sub> within a 1-h period or an absolute reading less than 30% to indicate vascular compromise<sup>[38]</sup>. Following the trends in [Hgb] and StO<sub>2</sub> over time is more valuable than the absolute readings due to the heterogeneity of vessels in the flap<sup>[38]</sup>. Some advantages of the T-Stat™ oximeter is the ability to set an alarm when user-specified criteria are met, and the ability to mirror quantitative outputs to the surgeon's smartphone app in real time for remote monitoring<sup>[36,38]</sup>. Previous groups have found that VLS continuous monitoring resulted in earlier detection of flap vascular compromise 1-3 h or more before clinical examination or Doppler findings were apparent<sup>[36,39]</sup>.

We use the T-Stat™ oximeter in all of our patients and consider it to be a nonnegotiable requirement at our center for safe flap surgery. The sensor probe is placed on the neophallus by the surgeon at the conclusion of the operation and remains in place until the patient is discharged [Figure 9].

Ultimately, flap monitoring and protective measures continue outside of the immediate postoperative period. After discharge, patients are instructed to minimize bending at the waist of more than 20° for 4 weeks to avoid compression of the vascular supply to the neophallus, limit walking for 4 weeks, avoid prolonged sitting, maintain excellent genital hygiene, and to keep the neophallus positioned at 90° to the body to minimize flap-related complications<sup>[9]</sup>.

## CLINICAL PATHWAYS

Clinical pathways (CPWs) are tools to translate evidence-based medicine to a clinical setting for a specific clinical situation. The main goal of a CPW is to provide high quality care through aligning clinical practice with guideline recommendations. At the same time, a CPW also seeks to minimize economic measures, such as healthcare costs, resource allocation, and length of stay<sup>[40]</sup>. CPWs have been used in clinical practice internationally since the 1980s, and an estimated 80% of hospitals in the US have implemented CPWs as of 2003<sup>[40,41]</sup>. A 2012 Cochrane Review showed that CPW implementation consistently reduced in-hospital complications and improved documentation without increasing patients' length of stay or healthcare costs<sup>[40]</sup>. The majority of studies reported that CPWs decreased in-hospital complications for surgical procedures<sup>[40]</sup>.

We use a robust, standardized, 5-day inpatient CPW for all phalloplasty patients. The pathway covers day-by-day flap monitoring requirements, clinical staffing level (ICU, med-surg step-down, or general med-surg nursing care), scheduled and as-needed (PRN) medications, activity level, diet, and more. In this way, order sets are standardized between patients, decreasing the potential for errors and avoiding *ad hoc* orders placed for each individual. Nursing expectations for daily progress is also standardized. Patients who are not meeting the expected milestones are identified early and receive extra diagnostic/therapeutic attention.

Finally, our patients' understanding of the day-by-day hospital course is greatly improved. Patients are given individualized treatments, especially when encountering complications, but our CPW allows patient, physician and nursing to be aligned as to each day's expectations and treatments. Because of the predictable safety profile provided by our post phalloplasty CPW, most phalloplasty patients can be safely discharged 5 days after the operation at our center.

## CONCLUSION

We have described some of the more common postoperative complications and have summarized some of the established surgical techniques in the literature to date to address these complications. We have also described several more experimental techniques, many of which include promising new technology to further optimize the results of phalloplasty. Each of these techniques represents a modest but important improvement towards the goal of a safe and reproducible surgery with optimal results and minimized risk of complications.

We and others endeavor to constantly evaluate and improve the steps of this surgery and investigate new concepts and materials in this field in an ongoing effort to further improve outcomes. We have had good success with these techniques at our high-volume center, although more thorough investigation and quantification of these patient outcomes is needed. Ultimately, with continued innovation and sharing of improved surgical techniques, it may be possible to better standardize care and improve the aesthetic and functional outcomes of this incredibly complex and increasingly common surgery.

## DECLARATIONS

### Authors' contributions

Made substantial contribution to the conception and design of the study and performed data analysis and interpretation: Carter EE, Crane CN, Santucci RA

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

An informed consent to participate was obtained from the patients.

### Consent for publication

A written informed consent for publication was obtained.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Frey JD, Poudrier G, Chiodo MV, Hazen A. An update on genital reconstruction options for the female-to-male transgender patient: a review of the literature. *Plast Reconstr Surg* 2017;139:728-37.
2. Özer M, Pigot GLS, Bouman MB, van de Grift TC, Elfering L, et al. Development of a decision aid for genital gender-affirming surgery in transmen. *J Sex Med* 2018;15:1041-8.



3. Hadj-Moussa M, Agarwal S, Ohl DA, Kuzon WM Jr. Masculinizing genital gender confirmation surgery. *Sex Med Rev* 2019;7:141-55.
4. Remington AC, Morrison SD, Massie JP, Crowe CS, Shakir A, et al. Outcomes after phalloplasty: do transgender patients and multiple urethral procedures carry a higher rate of complication? *Plast Reconstr Surg* 2018;141:220e-9.
5. Ascha M, Massie JP, Morrison SD, Crane CN, Chen ML. Outcomes of single stage phalloplasty by pedicled anterolateral thigh flap versus radial forearm free flap in gender confirming surgery. *J Urol* 2018;199:206-14.
6. Heston AL, Esmonde NO, Dugi DD 3rd, Berli JU. Phalloplasty: techniques and outcomes. *Transl Androl Urol* 2019;8:254-65.
7. Dy GW, Granieri MA, Fu BC, Vanni AJ, Voelzke B, et al. Presenting complications to a reconstructive urologist after masculinizing genital reconstructive surgery. *Urology* 2019;132:202-6.
8. Dy GW, Sun J, Granieri MA, Zhao LC. Reconstructive management pearls for the transgender patient. *Curr Urol Rep* 2018;19:36.
9. Esmonde N, Bluebond-Langner R, Berli JU. Phalloplasty flap-related complication. *Clin Plast Surg* 2018;45:415-24.
10. Danker S, Esmonde N, Berli JU. "Staging" in phalloplasty. *Urol Clin North Am* 2019;46:581-90.
11. Chen ML, Reyblat P, Poh MM, Chi AC. Overview of surgical techniques in gender-affirming genital surgery. *Transl Androl Urol* 2019;8:191-208.
12. Morrison SD, Shakir A, Vyas KS, Kirby J, Crane CN, et al. Phalloplasty: a review of techniques and outcomes. *Plast Reconstr Surg* 2016;138:594-615.
13. Gravvanis AI, Tsoutsos DA, Iconomou T, Gremoutis G. The use of integra artificial dermis to minimize donor-site morbidity after suprafascial dissection of the radial forearm flap. *Microsurgery* 2007;27:583-7.
14. Pigot GLS, Belboukhaddaoui S, Bouman MB, Meuleman EJH, de Boer EM, et al. Effectiveness of preoperative depilation of the urethral donor site for phalloplasty: neourethral hair growth and its effects on voiding. *Eur Urol Focus* 2020;6:770-5.
15. Bryson C, Honig SC. Genitourinary complications of gender-affirming surgery. *Curr Urol Rep* 2019;20:31.
16. Gao Y, Maurer T, Mirmirani P. Understanding and addressing hair disorders in transgender individuals. *Am J Clin Dermatol* 2018;19:517-27.
17. Carney MJ, Samra F, Momeni A, Bauder AR, Weissler JM, et al. Anastomotic technique and preoperative imaging in microsurgical lower-extremity reconstruction: a single-surgeon experience. *Ann Plast Surg* 2020;84:425-30.
18. Wirthmann A, Finke JC, Giovanoli P, Lindenblatt N. Long-term follow-up of donor site morbidity after defect coverage with Integra following radial forearm flap elevation. *Eur J Plast Surg* 2014;37:159-66.
19. Chang DK, Louis MR, Gimenez A, Reece EM. The basics of integra dermal regeneration template and its expanding clinical applications. *Semin Plast Surg* 2019;33:185-9.
20. Rudnicki PA, Purt B, True D, Siordia H, Lohmeier S, et al. Single-stage composite skin reconstruction using a dermal regeneration template. *Plast Reconstr Surg Glob Open* 2020;8:e2622.
21. Demiri E, Papaconstantinou A, Dionysiou D, Dionysopoulos A, Kaidoglou K, et al. Reconstruction of skin avulsion injuries of the upper extremity with integra® dermal regeneration template and skin grafts in a single-stage procedure. *Arch Orthop Trauma Surg* 2013;133:1521-6.
22. Koenen W, Felcht M, Vockenroth K, Sassmann G, Goerd S, et al. One-stage reconstruction of deep facial defects with a single layer dermal regeneration template. *J Eur Acad Dermatol Venereol* 2011;25:788-93.
23. Papa G, Pangos M, Renzi N, Ramella V, Panizzo N, et al. Five years of experience using a dermal substitute: indications, histologic studies, and first results using a new single-layer tool. *Dermatol Surg* 2011;37:1631-7.
24. Santucci RA. Urethral complications after transgender phalloplasty: strategies to treat them and minimize their occurrence. *Clin Anat* 2018;31:187-90.
25. Al-Tamimi M, Pigot GL, van der Sluis WB, van de Grift TC, Mullender MG, et al. Colpectomy significantly reduces the risk of urethral fistula formation after urethral lengthening in transgender men undergoing genital gender affirming surgery. *J Urol* 2018;200:1315-22.
26. Massie JP, Morrison SD, Wilson SC, Crane CN, Chen ML. Phalloplasty with urethral lengthening: addition of a vascularized bulbospongiosus flap from vaginectomy reduces postoperative urethral complications. *Plast Reconstr Surg* 2017;140:551e-8.
27. Gilbert DA, Winslow BH, Gilbert DM, Jordan GH, Horton CE. Transsexual surgery in the genetic female. *Clin Plast Surg* 1988;15:471-87.
28. Salgado CJ, Nugent AG, Moody AM, Chim H, Paz AM, et al. Immediate pedicled gracilis flap in radial forearm flap phalloplasty for transgender male patients to reduce urinary fistula. *J Plast Reconstr Aesthet Surg* 2016;69:1551-7.
29. Maan ZN, Rennert RC, Koob TJ, Januszyk M, Li WW, et al. Cell recruitment by amnion chorion grafts promotes neovascularization. *J Surg Res* 2015;193:953-62.
30. Sarhane KA, Ibrahim Z, Martin R, Krick K, Cashman CR, et al. Macroporous nanofiber wraps promote axonal regeneration and functional recovery in nerve repair by limiting fibrosis. *Acta Biomater* 2019;88:332-45.
31. Narakas A. The use of fibrin glue in repair of peripheral nerves. *Orthop Clin North Am* 1988;19:187-99.
32. Childe JR, Regal S, Schimoler P, Kharlamov A, Miller MC, et al. Fibrin glue increases the tensile strength of conduit-assisted primary digital nerve repair. *Hand (N Y)* 2018;13:45-9.
33. Siemionow M, Uygur S, Ozturk C, Siemionow K. Techniques and materials for enhancement of peripheral nerve regeneration: a literature review. *Microsurgery* 2013;33:318-28.
34. Kehoe S, Zhang XF, Boyd D. FDA approved guidance conduits and wraps for peripheral nerve injury: a review of materials and efficacy. *Injury* 2012;43:553-72.
35. Fox PM, Zeidler K, Carey J, Lee GK. White light spectroscopy for free flap monitoring. *Microsurgery* 2013;33:198-202.
36. Mericli AF, Wren J, Garvey PB, Liu J, Butler CE, et al. A prospective clinical trial comparing visible light spectroscopy to handheld

- doppler for postoperative free tissue transfer monitoring. *Plast Reconstr Surg* 2017;140:604-13.
37. Kohlert S, Quimby AE, Saman M, Ducic Y. Postoperative free-flap monitoring techniques. *Semin Plast Surg* 2019;33:13-6.
  38. Chao AH, Meyerson J, Povoski SP, Kocak E. A review of devices used in the monitoring of microvascular free tissue transfers. *Expert Rev Med Devices* 2013;10:649-60.
  39. Cornejo A, Rodriguez T, Steigelman M, Stephenson S, Sahar D, et al. The use of visible light spectroscopy to measure tissue oxygenation in free flap reconstruction. *J Reconstr Microsurg* 2011;27:397-402.
  40. Rotter T, Kinsman L, James E, Machotta A, Willis J, et al. The effects of clinical pathways on professional practice, patient outcomes, length of stay, and hospital costs: Cochrane systematic review and meta-analysis. *Eval Health Prof* 2012;35:3-27.
  41. Müller MK, Dedes KJ, Dindo D, Steiner S, Hahnloser D, et al. Impact of clinical pathways in surgery. *Langenbecks Arch Surg* 2009;394:31-9.

Opinion

Open Access



# Psychological stress enhances keloid development via stress hormone-induced abnormal cytokine profiles and inflammatory responses

Ya-Ting Yang, Xiao-Li Wu<sup>#</sup>, Wei Liu<sup>#</sup>

Department of Plastic and Reconstructive Surgery, Shanghai Tissue Engineering Key Laboratory, Shanghai Research Institute of Plastic and Reconstructive Surgery, Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200011, China.

<sup>#</sup>These authors contributed equally to this work.

**Correspondence to:** Dr. Wei Liu and Dr. Xiao-Li Wu, Department of Plastic and Reconstructive Surgery, Shanghai Tissue Engineering Key Laboratory, Shanghai Research Institute of Plastic and Reconstructive Surgery, Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine, 639 Zhi Zao Ju Rd, Shanghai 200011, China.  
E-mail: liuwei\_2000@yahoo.com; wuxiaoli528@icloud.com

**How to cite this article:** Yang YT, Wu XL, Liu W. Psychological stress enhances keloid development via stress hormone-induced abnormal cytokine profiles and inflammatory responses. *Plast Aesthet Res* 2020;7:34.  
<http://dx.doi.org/10.20517/2347-9264.2020.24>

**Received:** 17 Feb 2020 **First Decision:** 27 Apr 2020 **Revised:** 17 Jun 2020 **Accepted:** 20 Jun 2020 **Available online:** 12 Jul 2020

**Academic Editor:** Alexis Desmoulière, Jérôme Laloze **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Keloid is a fibroproliferative disorder resulting from the abnormal wound healing process, and it causes both cosmetic concerns and functional disabilities. Genetic predisposition, wound trauma, foreign body reaction, mechanical stretch, and immune dysfunction are common risk factors, but there remain mechanisms unclarified, leaving challenges in addressing the clinical concerns of recurrence and resistance. However, similar patterns of growth and metabolism between keloids and cancers provide a unique insight into the future exploration of keloid pathogenesis. Psychological stress has been demonstrated to be involved in the development and drug resistance of multiple cancers, but this aspect remains less-explored in keloids. Clinical observations and published investigations have noticed that persistent stress is common among keloid patients and their symptoms tend to deteriorate under stressful conditions. Following a thorough review of the published literature, we have identified three signaling pathways that might imply how stress hormones are likely to influence the keloid pathogenesis via activating adrenergic receptors and dysregulating the immune system. Thus, we hypothesized that psychological stress would be a key risk factor for keloid development via stimulating fibrosis, aggravating local hypoxia, and inflammation.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Keywords:** Keloids, psychological stress, adrenergic receptors, dysregulated immune system

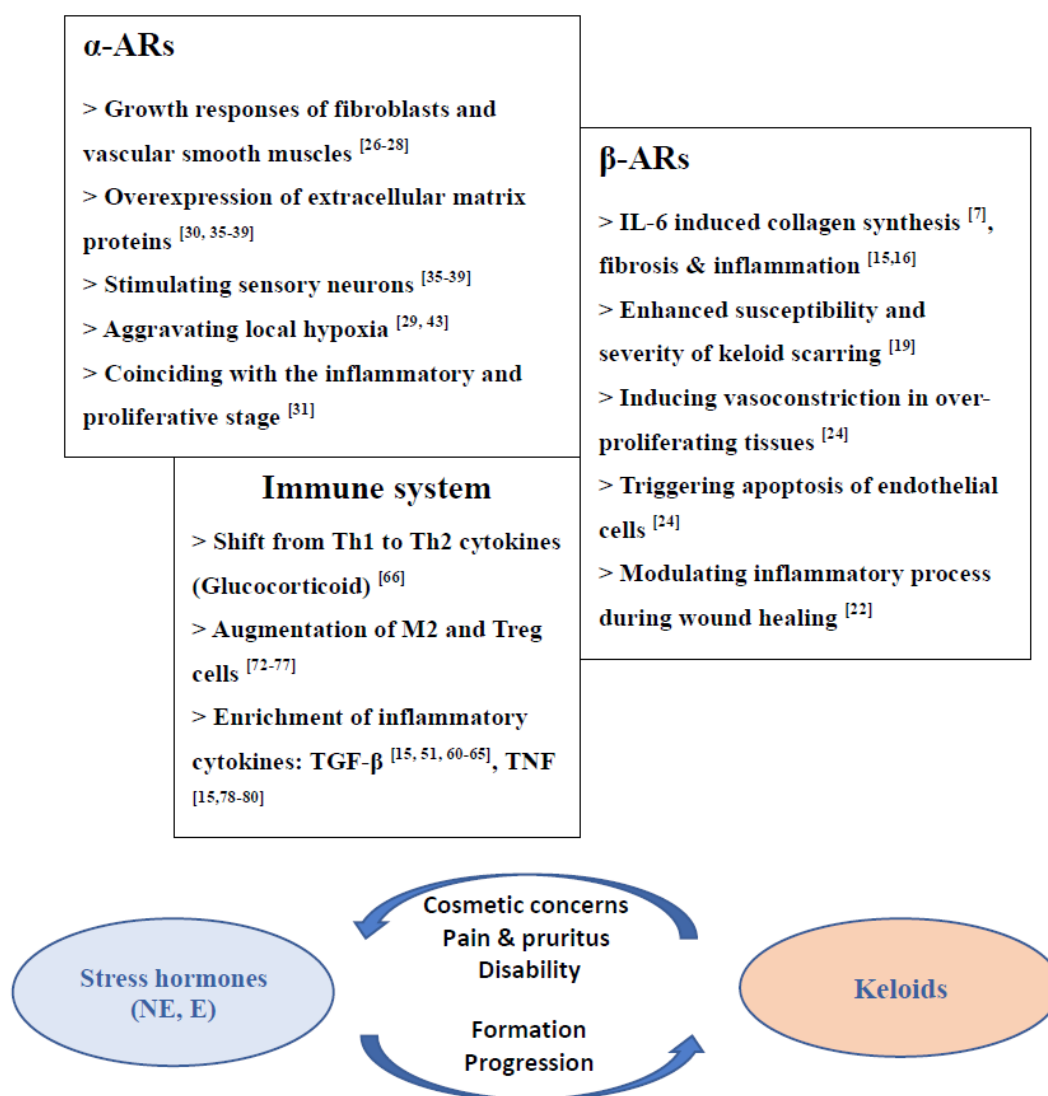
## INTRODUCTION

Keloids give rise to both cosmetic concerns and functional disabilities as a result of fibroproliferative disorder and excessive collagen deposition after abnormal wound healing. Furthermore, subjective symptoms derived from this disease such as pain and pruritus could dramatically affect patients' quality of life by causing significant psychological stress. Previous studies have revealed that the genetic background, wound trauma, foreign body reaction, mechanical stretch, and immune dysfunction are critical risk factors for keloid development, but the exact mechanism of keloid formation could be more complicated than what has been found and other risk factors are also likely to be involved, posing challenges to clinical treatment.

Described as “the non-specific response of the body”<sup>[1]</sup>, psychological stress has a complicated and profound influence on the functional state of affected human bodies by secreting various stress hormones. These primarily include glucocorticoids through the activation of the hypothalamic-pituitary-adrenal axis and catecholamines through the sympathetic nervous system<sup>[2]</sup>. Glucocorticoids can significantly affect cell metabolism and immune functions in the long term, while the effects of catecholamines [norepinephrine (NE), epinephrine (E)] are mediated via binding to  $\alpha$ -adrenergic receptors ( $\alpha$ -AR) or  $\beta$ -adrenergic receptors ( $\beta$ -ARs) facilitating the human body to react to all kinds of stressors. However, if the stressful situation becomes overwhelming, the combined action of stress stimulators remains persistent the physiologically maintained balance maybe disrupted leading to enhancement of pathophysiological processes for multiple diseases.

Psychological stress has been indicated for contributing to cancer development for decades. For example, in patients with lung cancers, stress has become an established predictor of mortality<sup>[3]</sup>. Stress hormones (NE, E) can also promote resistance to tyrosine kinase inhibitors (TKIs) of epidermal growth factor receptor (EGFR) in non-small cell lung cancer (NSCLC)<sup>[4]</sup> and increase tumor-derived interleukin-6 (IL-6) overexpression in ovarian cancer cells<sup>[5]</sup>. Featured with uncontrollable proliferation, invasiveness, and glycolysis-dominant metabolic pattern<sup>[6]</sup>, keloids are regarded as benign skin tumors and the accumulating literature evidence suggests that certain pathogenic signaling pathways might be shared between keloids and tumors. As an example, the elevation of IL-6 level, which was determined as a promoting agent in NSCLC resistance and its poor prognosis, has also been identified to contribute to keloid formation<sup>[7]</sup>. Therefore, it would be reasonable to investigate whether psychological stress influences keloid pathogenesis and explore the potential of stress hormones (NE, E) as therapeutic targets.

The psychological and mental impacts of pathological scars have been studied in clinical settings and been reported in an investigation among a black African population. Of this, 48.9% of keloid patients thought they were stigmatized and 35.8% complained about their limited social interactions<sup>[8]</sup>. Furtado *et al.*<sup>[9]</sup> from Brazil have reported psychological stress as a risk factor for postoperative keloid recurrence in a clinical study and proposed a novel psycho-neuro-immune-endocrine etiology where they pictured a macroscope of the “brain-skin connection” in keloid pathogenesis without mentioning detailed pathways<sup>[10]</sup>. For quite a long time, the potential association between stress hormones and keloid pathogenesis has been neglected according to Pubmed and Embase database searched with key words: keloid AND stress, keloid AND psychological stress, keloid AND mood disorders. By analyzing the published evidence with regards to bio-active molecules in keloid tissues and the effects of stress hormones (NE, E) on skin fibroblasts and immune cells (macrophages) *in vitro* and immune cytokine profiles both *in vitro* and *in vivo*, we outline in this manuscript three possible signaling pathways that might explain these phenomena.



**Figure 1.** Three possible pathways that may explain how stress hormones (NE, E) could induce abnormal cytokine profiles and inflammatory responses. TGF: transforming growth factor; TNF: tumor necrosis factor; AR: adrenergic receptors; IL: interleukin; NE: norepinephrine; E: epinephrine

## HYPOTHESIS

The psychological and mental state of keloid patients has been investigated and observed in clinical practice, but unfortunately, the association between the psychological stress-induced pathological alterations in keloids has been neglected. Based on the published literature, we propose the hypothesis that psychological stress can be a risk factor of keloid development as stress hormones (NE, E) might contribute to keloid pathogenesis [Figure 1]. Therefore, attenuating the AR-receptor function(s) may enhance the efficacy of traditional keloid treatments and reduce the therapeutic resistance. The following sections provide a detailed description of the hypothesis with related supporting evidence from the literature.

## EVALUATIONS OF THE HYPOTHESIS: THE PROMOTING EFFECT OF PSYCHOLOGICAL STRESS ON KELOID PATHOGENESIS

### Stress hormones could increase IL-6 expression to enhance fibrosis via activating β-ARs

As a critical mediator of fibrosis [11-14] and inflammation [15], elevated IL-6 level has been identified in both keloid tissues and psychologically stressed population. Other studies revealed that the stress hormones



(NE, E) increased IL-6 expression and increased resistance in NSCLC patients<sup>[4]</sup>. Therefore, we hypothesize that the psychological stress (NE, E) might stimulate keloid pathogenesis by enhancing IL-6 expression via activating  $\beta$ -ARs, and the use of  $\beta$ -blockers such as propranolol might facilitate the efficacy of current keloid treatments.

IL-6 is one of the Th1 type cytokines related to pro-fibrosis and inflammation<sup>[16,17]</sup>, which has been regarded as a marker of keloid progression. A significant increase of IL-6 and IL-6 signaling elements was observed in keloid fibroblasts (KFs) compared to normal fibroblasts (NFs)<sup>[7]</sup>. Moreover, the induction of IL-6 by IL-6 peptide in NF cultures or inhibition of IL-6 or IL-6Ra by their corresponding antibodies in KF cultures rendered a dose-dependent increase or decrease in the synthesis of collagen type I<sup>[7]</sup>. This was possibly the result of the suppression of matrix metalloproteinases (MMPs) at mRNA level and pro-matrix MMPs at the protein level<sup>[18]</sup>. Besides, a Japanese population-based study involving IL6R genotypic and allelic analyses among 239 normal and 376 keloid patients revealed that the IL-6 572G/C polymorphism is associated with susceptibility to keloid formation and the severity of keloid scarring<sup>[19]</sup>.

Multiple stress models have confirmed that a higher plasma level of IL-6 was also observed in people with depressive symptoms or at a stressed state (for example, angry couples after a domestic conflict or vaccination with an influenza virus vaccine)<sup>[20,21]</sup>, suggesting a stress hormone (NE, E)-mediated IL-6 augmentation.

The importance of psycho-physiological interactions has gained increasing attention recently, and NE-induced IL-6 elevation has been taken as a predictor of treatment resistance and poor outcomes in certain cancers. For example, researchers found that by binding to  $\beta$ 2-ARs, stress hormones (NE, E) can subsequently induce IL-6 expression via suppressing liver kinase B1 and activating cAMP-responsive element-binding protein. Therefore, Combinational treatments with propranolol ( $\beta$ -AR inhibitor) could effectively lower the IL-6 concentration and prolong the progression-free survival in EGFR TKIs resistant patients<sup>[4]</sup>. Apart from lung cancers, it was also observed that the NE/E induced activation of ARs also resulted in a similar increase of IL-6 in ovarian cancer cells<sup>[5]</sup>.

The  $\beta$ -ARs have been suggested as potential pharmacologic targets of catecholamine actions that influence numerous physiological and metabolic activities systemically in human bodies<sup>[22,23]</sup>. Both *in vitro* and *in vivo* studies of  $\beta$ -ARs carried out over the past decades focused mainly on their effects on cardiac function, whereas reported studies on non-cardiac  $\beta$ -blocker effects focused on their roles in the wound healing process<sup>[22]</sup>. Propranolol is a representative nonselective  $\beta$ -adrenergic blockade agent with promising efficacy in rhythm disturbances and hypertension. It was found that in propranolol-treated animals, wound contraction and the formation of the neo-epidermis and granulation tissue were delayed<sup>[22]</sup>. de Mesquita<sup>[24]</sup> hypothesized that systemic or intralesional injection of propranolol could serve as a novel cure of keloids because of its potential to induce vasoconstriction in over-proliferating tissues, trigger apoptosis of endothelial cells, and modulate inflammatory process during wound healing. Moreover, one single-institution case-control study in 2017 also observed better scar formation in post-surgery patients who were administrated with  $\beta$  blockers<sup>[25]</sup>. It is noteworthy that patients with abnormal scar histories or family tendency are excluded in this study, and the administration of other hypertension drugs such as calcium channel blockers showed no association with the scar quality, a phenomenon that indirectly supports our hypothesis that adrenergic activation might be an independent risk factor for the pathogenesis of keloid and hypertrophic scars<sup>[25]</sup>. Noticeably, yearlong administration of oxandrolone and propranolol successfully reduced scar severity and pliability in the -post-burn hypertrophic scar patients and their emotional health state was also improved<sup>[26]</sup>. Encouraged by the findings of the propranolol-based study showing the reduction in NE-induced IL-6 elevation with an altered prognosis of NSCLC patients and the studies outlined above, we strongly propose that it might be promising to use propranolol for targeting  $\beta$ -ARs on keloid cells to disrupt IL-6 mediated keloid pathogenesis.

### **Stress hormones could activate $\alpha$ -ARs to promote keloid formation by enhancing growth-related responses and aggravating local hypoxia environment**

The  $\alpha$ -ARs are another group of adrenergic receptors that were found to be increased in keloid tissues and peripheral sensory neurons of scarred skin. Stress hormones could activate  $\alpha$ -ARs to promote keloid formation by enhancing growth-related responses and aggravating the local hypoxia environment. Furthermore, their enhanced expression was associated with cell proliferation, inflammation, and uncomfortable symptoms of pain and pruritus in hypertrophic scars.

Activated  $\alpha$ -ARs can evoke growth-related responses after bonding to stress hormones (NE, E). For example, the  $\alpha$ -1B subtype stimulates cell proliferation<sup>[27-29]</sup> and the activation of the  $\alpha$ -1A subtype evokes protein biosynthesis and cell hypertrophy<sup>[30]</sup>. Tissue biopsy and immunohistochemistry detected an increase of  $\alpha$ -ARs in keloid scars compared to burn scars and unscarred skins in the regenerated epidermis, dense bands of cells in the upper dermis and collagen fibers in the deep dermis, coinciding with the inflammatory and proliferative stage<sup>[31]</sup>. Since it was reported that injury-induced growth-related responses are  $\alpha$ 1-AR subtype-dependent<sup>[32-34]</sup>, over-expression of  $\alpha$ 1-ARs might result in both hyperplasia and hypertrophy of fibroblasts and vascular smooth muscles in keloid tissues. Moreover, the adrenergic activation of fibroblasts could increase the production of extracellular matrix proteins (such as collagen and fibronectin) and the expression of  $\alpha$ 1-ARs in peripheral sensory neurons was in line with enhanced sensitivity to adrenergic agents in injured tissues, suggesting that the up-regulation of  $\alpha$ 1-ARs might not only be involved in the inflammation and wound healing processes, but also be a significant source of pain, itching, and hyperaesthesia<sup>[31]</sup>.

Apart from  $\alpha$ 1-AR-induced growth-related responses that have been discussed, the vasoconstriction caused by activated  $\alpha$ 1-ARs in vascular smooth muscles is another important aspect that could aggravate the local hypoxia of keloid microenvironment and trigger hypoxia-related pathogenesis. Hypoxia is a common environmental stress factor associated with various physiological and pathological conditions, including angiogenesis, cell proliferation, glucose metabolism, pH regulation, and migration<sup>[35,36]</sup>. Accumulating evidence suggested an anoxic microenvironment is crucial in keloid pathogenesis because of abnormal hypoxia-associated occluded microvessels, which is also partially responsible for keloid resistance to radiation therapy<sup>[37]</sup>. It was observed that the central area of keloid is severely ischemic, exhibiting higher hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) expression and lower vascular density than their marginal areas and normal skin borders<sup>[38,39]</sup>. The HIF-1 $\alpha$  is also involved in the inflammatory process by regulating angiogenesis and inflammatory cell functions<sup>[40-42]</sup>. Blocking HIF-1 signal pathways by either 2ME2 or HIF-1 $\alpha$  siRNA has been shown to successfully increase the radiation-induced apoptosis in keloid fibroblasts<sup>[37]</sup>. Hypoxia can also drive the transition of human dermal fibroblasts to a myofibroblast-like phenotype via the transforming growth factor- $\beta$ 1/SMAD3 pathway<sup>[36]</sup>, and increase the expression of vascular endothelial growth factor (VEGF) in keloids<sup>[35,38]</sup>. Glycolysis, the major glucose metabolic pattern for keloid tissues, could also interact with hypoxia and promote the lactate accumulation, resulting in excessive collagen production and fibrogenic activities<sup>[43]</sup>.

### **Stress hormones might influence keloid formation by dysregulating the immune system and inflammation**

The classical model of wound healing involves three distinct but overlapping phases that chronologically occur as the inflammatory, the proliferative, and the remodeling phases. Disturbance of these processes, especially the prolonged and excessive inflammatory reactions could lead to an increase of fibroblast activities and excessive extracellular matrix (ECM) production<sup>[44]</sup>. The available evidence indicates that malfunction of the immune system and inflammation might be involved in keloid formation. Keloid tissues are highly infiltrated with various immune cells, immunoglobulins and complements<sup>[45]</sup>, as well as growth factors, cytokines and proteases, such as IL-6, tumor necrosis factor (TNF), transforming growth

factor- $\beta$  (TGF- $\beta$ ), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF)<sup>[46,47]</sup>, which are critical for the migration, proliferation and collagen synthesis of fibroblasts. Moreover, previous studies have demonstrated that the expression of several immune-related genes was also dysregulated in keloid tissues<sup>[48,49]</sup>.

The elevated C-reactive protein (CRP, a marker of inflammation) plasma concentration in caregivers of Alzheimer's patients<sup>[50,51]</sup> also implied that psychological stress was a potent inducer of the chronic inflammation state. Analysis of stress models revealed that stress hormones (NE, E, glucocorticoid) have detrimental effects on immune functions as well as the inflammation process from various aspects<sup>[52]</sup>. Here are some keloid-associated inflammatory molecules or mechanisms that have been identified dysregulated in stressed conditions.

### TGF- $\beta$

TGF- $\beta$  is probably the most fibrogenic factor associated with keloid formation by acting as a strong chemotactic agent for fibroblasts<sup>[53]</sup> and increasing cell rigidity through TGF- $\beta$ 1 receptor-smooth muscle actin axis<sup>[54]</sup>. It is also known as a regulatory resolution factor that can induce remodeling within sites of damaged tissues upon mood disorder-associated inflammatory processes<sup>[15]</sup>.

In the inflammatory and proliferative phase, degranulation of platelets releases and activates several fibrogenic growth factors and chemotactic agents including TGF- $\beta$ 1 and TGF- $\beta$ 2<sup>[44]</sup>, to increase the corresponding receptors and responsiveness compared to fibroblasts from normal tissues<sup>[55-58]</sup>. Although there is no direct evidence that stress hormones can enhance the efficacy of TGF- $\beta$  in keloids, it is nevertheless clear that TGF- $\beta$  plays a pivotal role in keloid formation and stress-derived inflammatory conditions.

### The cells and cytokines

CD4 T cells express T helper lymphocyte (Th)1 or Th2 responses, while glucocorticoids are thought to cause a shift from Th1 to Th2 cytokines by downregulating Th1 cytokines and upregulating Th2 cytokines<sup>[59]</sup>. Th1 responses produce interferons and IL-12 and are thought to be related to the attenuation of fibrogenesis, whereas Th2 responses (IL-4, IL-5, IL-10 and IL-13, IL-1, and IL-6) are generally related to fibrogenesis, among which IL-4, IL-5, IL-6, and IL-13 are thought to be essential for promoting fibroblast recruitment and proliferation, ECM deposition, angiogenesis and re-epithelialization<sup>[16,17,47]</sup> (except for IL-10, which are mainly related to anti-fibrosis<sup>[60-62]</sup>). In a published report, stress was associated with a decrease in IL-2 receptor (IL-2R) mRNA levels and the protein expression in peripheral blood leukocytes compared to the baseline<sup>[63]</sup>. In a longitudinal study over 6 years, caregivers and former caregiver's (a kind of stress model) showed elevated plasma IL-6 levels that increased at a rate four times faster than those of age-matched controls<sup>[50]</sup>. Elevation of serum IL-6 (a marker of inflammation) levels have been previously described in both chronically stressed older adults<sup>[64]</sup> and keloid patients. Since a clear NE-IL-6 pathway has been identified in NSCLCs and ovarian cancer<sup>[4,5]</sup>, we presumed that a similar NE-induced IL-6 elevation might exist in keloid. Stress hormones and related receptors could thus serve as feasible therapeutic targets.

### Macrophages and treg cells

Macrophages are divided into two subsets, the IL-12- and inducible nitric oxide synthase (iNOS)-expressing M1 type and the IL-10- and TGF- $\beta$ -expressing M2 type<sup>[65]</sup>. The classically-activated (M1) cells that secrete pro-inflammatory cytokines, whereas alternatively-activated (M2) cells that foster tissue repair and regeneration<sup>[66,67]</sup>. It was found that M1-associated genes, including iNOS and IL-12, were less elevated in keloid tissues than M2-associated genes, including IL-10 and TGF- $\beta$ <sup>[68]</sup>, suggesting that macrophages in keloids were shifted toward the M2 polarization.

The Foxp3<sup>+</sup>CD4<sup>+</sup> regulatory T (Treg) cells represent a critical T cell subset, the dysfunction of which was implicated in multiple inflammatory diseases<sup>[69]</sup>. While the normal skin displays a relative lack of CD3<sup>+</sup> T cells, the levels of Foxp3<sup>+</sup> Treg were significantly higher in keloid tissues (range of 25.5%-72.5%) in contrast to those in the circulation of keloid patients (4%-10.5%)<sup>[68]</sup>. Additionally, it was also observed in the same research that incubating circulating CD3<sup>+</sup> T cells with keloid macrophages could significantly raise the expression of Foxp3, suggesting that these keloid macrophages could promote Treg differentiation by upregulating Foxp3 expression.

Even though no research on the levels of macrophages and depression in patients with skin conditions has been reported thus far, other previous studies did show that the activation and polarization of microglia (central nervous system-resident macrophages) could modulate the production and secretion of pro-inflammatory cytokines, implicating the involvement of macrophages in the etiology of major depressive disorder, which was referred as the “macrophage theory of depression”<sup>[70]</sup>. In light of this, it could be argued that an abundance of pro-inflammatory cytokines induced by the altered profile of macrophages inside keloids might exist, and further investigations, therefore, need to be performed in keloid patients with diagnosed depression.

### The tumor necrosis factor

TNF produced by monocytes and macrophages during the inflammatory phase has been known to induce collagen degranulation and minimize excessive scarring possibly by increasing the MMP1/TIMP3, MMP2/TIMP3 ratios<sup>[71]</sup>.

Various animal and patient-based clinical studies have demonstrated the associations between the concentration of pro-inflammatory cytokines, specifically IL-1 $\beta$ , IL-6, TNF, and depressive symptoms<sup>[15]</sup>. They also showed a general normalization (decline) of IL-6 and TNF concentration after antidepressant treatment<sup>[72,73]</sup>.

## CONCLUSION

As a benign skin tumor outgrowing the original wound boundary or growing spontaneously on the normal skin, keloid can bring great pain and inconvenience to patients. Although risk factors such as genes and infection have been noticed, the pathologic mechanisms remain unclear, leading to challenges for treatment resistance and keloid recurrence, including 9%-50% recurrent rate of the corticosteroid injection, 45%-100% recurrence rate of the surgical removal and 9.59% relapse rate of the radiotherapy<sup>[44]</sup>.

Psychological stress evoked by traumatic events and depressive conditions has huge impacts on the overall health state. Through stress hormones (NE, E, glucocorticoids) and their respective receptors, tissue-specific responses are triggered as well as the general modulation of the immune system and inflammation. Studies have confirmed that stress hormones are critical for the initiation and development of multiple diseases<sup>[2,3,52]</sup>, but the impact of psychological stress on keloid pathogenesis has been neglected.

As for keloid patients, the original trauma, uncomfortable feelings, together with the cosmetic concerns are all potent and constant underlying stressors, which make them very likely to be trapped in a stress-intensive state. Therefore, psychological stress is a pivotal and inevitable element that should be taken into consideration in formulating optimal treatment regimens. In view of the reviewed literature, especially with regards to stress hormone-induced cellular and physiological changes observed during psychological stress, we advance the hypothesis that stress hormones (NE, E) may participate in the keloid formation by: (1) increasing the expression of keloid-associated IL-6 via activating  $\beta$ -ARs; (2) triggering growth responses of fibroblasts and symptoms (pain and pruritus) in scar tissues by directly activating  $\alpha$ -ARs; (3) exacerbating

the local hypoxia conditions; and (4) dysregulating immune systems to provide an inflammatory microenvironment that is in favor of keloid formation, thus promoting keloid pathogenesis.

Although the impact of psychological stress on the pathogenesis of diseases, such as cancers, has been known for decades, its relevance and impact concerning keloid pathogenesis and therapy have barely been studied. Although the stressed state in keloid patients has been well-observed during clinical practice and demonstrated by investigations in an African population, more direct evidence are required in the future. For example, current studies have detected elevated adrenergic receptors, but the concentration and distribution of catecholamines in keloid tissues remain uninvestigated, so are specific cytokines and immune cells in the targeted population. Clinically, large-scale evaluations of psychological stress among keloid patients should also be undertaken as a follow up to “as a proof of principle” pilot studies. Moreover, studies combining  $\alpha$ ,  $\beta$ -receptor antagonists and anti-depressant medicines with conventional keloid therapies could be explored in future clinical trials to realize better treatment outcomes.

## DECLARATIONS

### Author's contributions

Made substantial contributions to the conception of the hypothesis: Yang YT, Wu XL, Liu W

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

This work is financially supported by the National Natural Science Foundation (81671921).

### Conflict of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

- 1 Selye H. Stress without distress. Le stress sans detresse. *Brux Med* 1976;56:205-10.
- 2 Kyrou I, Tsigos C. Stress hormones: physiological stress and regulation of metabolism. *Curr Opin Pharmacol* 2009;9:787-93.
- 3 Hamer M, Chida Y, Molloy GJ. Psychological distress and cancer mortality. *J Psychosom Res* 2009;66:255-8.
- 4 Nilsson MB, Sun H, Diao L, Tong P, Liu D, et al. Stress hormones promote EGFR inhibitor resistance in NSCLC: implications for combinations with  $\beta$ -blockers. *Sci Transl Med* 2017;9:eaao4307.
- 5 Nilsson MB, Armaiz-Pena G, Takahashi R, Lin YG, Trevino J, et al. Stress hormones regulate interleukin-6 expression by human ovarian carcinoma cells through a Src-dependent mechanism. *J Biol Chem* 2007;282:29919-26.
- 6 Vincent AS, Phan TT, Mukhopadhyay A, Lim HY, Halliwell B, et al. Human skin keloid fibroblasts display bioenergetics of cancer cells. *J Invest Dermatol* 2008;128:702-9.
- 7 Ghazizadeh M, Tosa M, Shimizu H, Hyakusoku H, Kawanami O. Functional implications of the IL-6 signaling pathway in keloid pathogenesis. *J Invest Dermatol* 2007;127:98-105.
- 8 Olaitan PB. Keloids: assessment of effects and psychosocial-impacts on subjects in a black African population. *Indian J Dermatol Venereol Leprol* 2009;75:368-72.
- 9 Furtado F, Hochman B, Farber PL, Muller MC, Hayashi LF, et al. Psychological stress as a risk factor for postoperative keloid recurrence.



- J Psychosom Res 2012;72:282-87.
10. Hochman B, Isoldi FC, Furtado F, Ferreira LM. New approach to the understanding of keloid: psychoneuroimmune-endocrine aspects. *Clin Cosmet Investig Dermatol* 2015;8:67-73.
  11. Tan PL, Farmiloe S, Yeoman S, Watson JD. Expression of the interleukin 6 gene in rheumatoid synovial fibroblasts. *J Rheumatol* 1990;17:1608-12.
  12. Feghali CA, Bost KL, Boulware DW, Levy LS. Control of IL-6 expression and response in fibroblasts from patients with systemic sclerosis. *Autoimmunity* 1994;17:309-18.
  13. Gurram M, Pahwa S, Frieri M. Augmented interleukin-6 secretion in collagen-stimulated peripheral blood mononuclear cells from patients with systemic sclerosis. *Ann Allergy* 1994;73:493-96.
  14. Shahar I, Fireman E, Topilsky M, Grief J, Kivity S, et al. Effect of IL-6 on alveolar fibroblast proliferation in interstitial lung diseases. *Clin Immunol Immunopathol* 1996;79:244-51.
  15. Chistyakov DV, Astakhova AA, Sergeeva MG. Resolution of inflammation and mood disorders. *Exp Mol Pathol* 2018;105:190-201.
  16. Doucet C, Brouty-Boye D, Pottin-Clemenceau C, Canonica GW, Jasmin C, et al. Interleukin (IL) 4 and IL-13 act on human lung fibroblasts. Implication in asthma. *J Clin Invest* 1998;101:2129-39.
  17. Wynn TA. Fibrotic disease and the T(H)1/T(H)2 paradigm. *Nat Rev Immunol* 2004;4:583-94.
  18. Dasu MR, Hawkins HK, Barrow RE, Xue H, Herndon DN. Gene expression profiles from hypertrophic scar fibroblasts before and after IL-6 stimulation. *J Pathol* 2004;202:476-85.
  19. Tosa M, Watanabe A, Ghazizadeh M. IL-6 polymorphism and susceptibility to keloid formation in a Japanese population. *J Invest Dermatol* 2016;136:1069-72.
  20. Kiecolt-Glaser JK, Loving TJ, Stowell JR, Malarkey WB, Lemeshow S, et al. Hostile marital interactions, proinflammatory cytokine production, and wound healing. *Arch Gen Psychiatry* 2005;62:1377-84.
  21. Glaser R, Robles TF, Sheridan J, Malarkey WB, Kiecolt-Glaser JK. Mild depressive symptoms are associated with amplified and prolonged inflammatory responses after influenza virus vaccination in older adults. *Arch Gen Psychiatry* 2003;60:1009-14.
  22. Souza BR, Santos JS, Costa AM. Blockade of beta1- and beta2-adrenoceptors delays wound contraction and re-epithelialization in rats. *Clin Exp Pharmacol Physiol* 2006;33:421-30.
  23. Pelat M, Verwaerde P, Galitzky J, Lafontan M, Berlan M, et al. High isoproterenol doses are required to activate beta3-adrenoceptor-mediated functions in dogs. *J Pharmacol Exp Ther* 2003;304:246-53.
  24. de Mesquita CJG. About strawberry, crab claws, and the Sir James Black's invention. Hypothesis: can we battle keloids with propranolol? *Med Hypotheses* 2010;74:353-9.
  25. Enoshiri T, Naitoh M, Yamawaki S, Kawaguchi A, Aya R, et al.  $\beta$ -adrenergic receptor blockers reduce the occurrence of keloids and hypertrophic scars after cardiac device implantation: a single-institution case-control study. *Plast Reconstr Surg* 2017;139:1248-56.
  26. Herndon D, Capek KD, Ross E, Jay JW, Prasai A, et al. Reduced postburn hypertrophic scarring and improved physical recovery with yearlong administration of oxandrolone and propranolol. *Ann Surg* 2018;268:431-41.
  27. Gonzalez-Cabrera PJ, Shi T, Yun J, McCune DF, Rorabaugh BR, et al. Differential regulation of the cell cycle by alpha1-adrenergic receptor subtypes. *Endocrinology* 2004;145:5157-67.
  28. Lei B, Schwinn DA, Morris DP. Stimulation of alpha1a adrenergic receptors induces cellular proliferation or antiproliferative hypertrophy dependent solely on agonist concentration. *PLoS One* 2013;8:e72430.
  29. Sterin-Borda L, Furlan C, Orman B, Borda E. Differential regulation on human skin fibroblast by alpha1 adrenergic receptor subtypes. *Biochem Pharmacol* 2007;74:1401-12.
  30. Waldrop BA, Mastalerz D, Piascik MT, Post GR. Alpha(1B)- and alpha(1D)-Adrenergic receptors exhibit different requirements for agonist and mitogen-activated protein kinase activation to regulate growth responses in rat 1 fibroblasts. *J Pharmacol Exp Ther* 2002;300:83-90.
  31. Drummond PD, Dawson LF, Wood FM, Fear MW. Up-regulation of alpha1-adrenoceptors in burn and keloid scars. *Burns* 2018;44:582-8.
  32. Zhang H, Faber JE. Trophic effect of norepinephrine on arterial intima-media and adventitia is augmented by injury and mediated by different alpha1-adrenoceptor subtypes. *Circ Res* 2001;89:815-22.
  33. Faber JE, Szymeczek CL, Cotecchia S, Thomas SA, Tanoue A, et al. Alpha1-adrenoceptor-dependent vascular hypertrophy and remodeling in murine hypoxic pulmonary hypertension. *Am J Physiol Heart Circ Physiol* 2007;292:H2316-23.
  34. Pang X, Sun NL. Calcineurin-NFAT signaling is involved in phenylephrine-induced vascular smooth muscle cell proliferation. *Acta Pharmacol Sin* 2009;30:537-44.
  35. Zhang Q, Oh CK, Messadi DV, Duong HS, Kelly AP, et al. Hypoxia-induced HIF-1 alpha accumulation is augmented in a co-culture of keloid fibroblasts and human mast cells: involvement of ERK1/2 and PI-3K/Akt. *Exp Cell Res* 2006;312:145-55.
  36. Zhao B, Guan H, Liu JQ, Zheng Z, Zhou Q, et al. Hypoxia drives the transition of human dermal fibroblasts to a myofibroblast-like phenotype via the TGF-beta1/Smad3 pathway. *Int J Mol Med* 2017;39:153-9.
  37. Long F, Si L, Long X, Yang B, Wang XJ, et al. 2ME2 increase radiation-induced apoptosis of keloid fibroblasts by targeting HIF-1alpha in vitro. *Australas J Dermatol* 2016;57:e32-8.
  38. Touchi R, Ueda K, Kurokawa N, Tsuji M. Central regions of keloids are severely ischaemic. *J Plast Reconstr Aesthet Surg* 2016;69:e35-41.
  39. Zhang Z, Nie F, Kang C, Chen B, Qin Z, et al. Increased periostin expression affects the proliferation, collagen synthesis, migration and invasion of keloid fibroblasts under hypoxic conditions. *Int J Mol Med* 2014;34:253-61.
  40. Jung YJ, Isaacs JS, Lee S, Trepel J, Neckers L. IL-1beta-mediated up-regulation of HIF-1alpha via an NFkappaB/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis. *FASEB J* 2003;17:2115-7.

41. Karhausen J, Haase VH, Colgan SP. Inflammatory hypoxia: role of hypoxia-inducible factor. *Cell Cycle* 2005;4:256-8.
42. Cramer T, Johnson RS. A novel role for the hypoxia inducible transcription factor HIF-1 $\alpha$ : critical regulation of inflammatory cell function. *Cell Cycle* 2003;2:192-3.
43. Okuno R, Ito Y, Eid N, Otsuki Y, Kondo Y, et al. Upregulation of autophagy and glycolysis markers in keloid hypoxic-zone fibroblasts: morphological characteristics and implications. *Histol Histopathol* 2018;33:1075-87.
44. Lee HJ, Jang YJ. Recent understandings of biology, prophylaxis and treatment strategies for hypertrophic scars and keloids. *Int J Mol Sci* 2018;19:711.
45. Jiao H, Fan J, Cai J, Pan B, Yan L, et al. Analysis of characteristics similar to autoimmune disease in keloid patients. *Aesthetic Plast Surg* 2015;39:818-25.
46. Gauglitz GG, Korting HC, Pavicic T, Ruzicka T, Jeschke MG. Hypertrophic scarring and keloids: pathomechanisms and current and emerging treatment strategies. *Mol Med* 2011;17:113-25.
47. Tredget EE, Nedelec B, Scott PG, Ghahary A. Hypertrophic scars, keloids, and contractures. The cellular and molecular basis for therapy. *Surg Clin North Am* 1997;77:701-30.
48. Chike-Obi CJ, Cole PD, Brissett AE. Keloids: pathogenesis, clinical features, and management. *Semin Plast Surg* 2009;23:178-84.
49. Ogawa R. Keloid and hypertrophic scars are the result of chronic inflammation in the reticular dermis. *Int J Mol Sci* 2017;18:606.
50. Kiecolt-Glaser JK, Preacher KJ, MacCallum RC, Atkinson C, Malarkey WB, et al. Chronic stress and age-related increases in the proinflammatory cytokine IL-6. *Proc Natl Acad Sci U S A* 2003;100:9090-5.
51. Graham JE, Robles TF, Kiecolt-Glaser JK, Malarkey WB, Bissell MG, et al. Hostility and pain are related to inflammation in older adults. *Brain Behav Immun* 2006;20:389-400.
52. Webster Marketon JI, Glaser R. Stress hormones and immune function. *Cell Immunol* 2008;252:16-26.
53. Liu W, Wang DR, Cao YL. TGF- $\beta$ : a fibrotic factor in wound scarring and a potential target for anti-scarring gene therapy. *Curr Gene Ther* 2004;4:123-36.
54. Lee CH, Hong CH, Chen YT, Chen YC, Shen MR. TGF- $\beta$ 1 increases cell rigidity by enhancing expression of smooth muscle actin: keloid-derived fibroblasts as a model for cellular mechanics. *J Dermatol Sci* 2012;67:173-80.
55. Tuan TL, Nichter LS. The molecular basis of keloid and hypertrophic scar formation. *Mol Med Today* 1998;4:19-24.
56. Ishihara H, Yoshimoto H, Fujioka M, Murakami R, Hirano A, et al. Keloid fibroblasts resist ceramide-induced apoptosis by overexpression of insulin-like growth factor I receptor. *J Invest Dermatol* 2000;115:1065-71.
57. Butler PD, Longaker MT, Yang GP. Current progress in keloid research and treatment. *J Am Coll Surg* 2008;206:731-41.
58. Ladak A, Tredget EE. Pathophysiology and management of the burn scar. *Clin Plast Surg* 2009;36:661-74.
59. Calcagni E, Elenkov I. Stress system activity, innate and T helper cytokines, and susceptibility to immune-related diseases. *Ann N Y Acad Sci* 2006;1069:62-76.
60. van den Broek LJ, van der Veer WM, de Jong EH, Gibbs S, Niessen FB. Suppressed inflammatory gene expression during human hypertrophic scar compared to normotrophic scar formation. *Exp Dermatol* 2015;24:623-9.
61. Namazi MR, Fallahzadeh MK, Schwartz RA. Strategies for prevention of scars: what can we learn from fetal skin? *Int J Dermatol* 2011;50:85-93.
62. Liechty KW, Kim HB, Adzick NS, Crombleholme TM. Fetal wound repair results in scar formation in interleukin-10-deficient mice in a syngeneic murine model of scarless fetal wound repair. *J Pediatr Surg* 2000;35:866-73.
63. Glaser R, Kennedy S, Lafuse WP, Bonneau RH, Speicher C, et al. Psychological stress-induced modulation of interleukin 2 receptor gene expression and interleukin 2 production in peripheral blood leukocytes. *Arch Gen Psychiatry* 1990;47:707-12.
64. Glaser R, MacCallum RC, Laskowski BF, Malarkey WB, Sheridan JF, et al. Evidence for a shift in the Th-1 to Th-2 cytokine response associated with chronic stress and aging. *J Gerontol A Biol Sci Med Sci* 2001;56:M477-82.
65. Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, et al. The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 2004;25:677-86.
66. Italiani P, Boraschi D. From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation. *Front Immunol* 2014;5:514.
67. Mills CD, Ley K. M1 and M2 macrophages: the chicken and the egg of immunity. *J Innate Immun* 2014;6:716-26.
68. Jin Q, Gui L, Niu F, Yu B, Lauda N, et al. Macrophages in keloid are potent at promoting the differentiation and function of regulatory T cells. *Exp Cell Res* 2018;362:472-6.
69. Schmidt A, Oberle N, Krammer PH. Molecular mechanisms of treg-mediated T cell suppression. *Front Immunol* 2012;3:51.
70. Dey A, Hankey Giblin PA. Insights into macrophage heterogeneity and cytokine-induced neuroinflammation in major depressive disorder. *Pharmaceuticals (Basel)* 2018;11:64.
71. Chen X, Thibeault SL. Role of tumor necrosis factor- $\alpha$  in wound repair in human vocal fold fibroblasts. *Laryngoscope* 2010;120:1819-25.
72. Haapakoski R, Mathieu J, Ebmeier KP, Alenius H, Kivimäki M. Cumulative meta-analysis of interleukins 6 and 1 $\beta$ , tumour necrosis factor  $\alpha$  and C-reactive protein in patients with major depressive disorder. *Brain Behav Immun* 2015;49:206-15.
73. Kohler CA, Freitas TH, Stubbs B, Maes M, Solmi M, et al. Peripheral alterations in cytokine and chemokine levels after antidepressant drug treatment for major depressive disorder: systematic review and meta-analysis. *Mol Neurobiol* 2018;55:4195-206.

Review

Open Access



# Equality in cleft and craniofacial care

Nicholas D. Sharratt<sup>1</sup>, Jean Calleja Agius<sup>2</sup>, Gareth Davies<sup>3</sup>, Felicity V. Mehendale<sup>4</sup>, Peter Hagell<sup>5</sup>, Martin Persson<sup>5</sup>

<sup>1</sup>Centre for Appearance Research, University of the West of England, Bristol BS16 1QY, UK.

<sup>2</sup>Department of Anatomy, Faculty of Medicine and Surgery, University of Malta, Msida MSD2080, Malta.

<sup>3</sup>European Cleft Organisation, Rijswijk ZH 2288 EL, The Netherlands.

<sup>4</sup>Global Cleft Lip and Palate Research Programme, Global Health Research Centre, Usher Institute, University of Edinburgh, Edinburgh EH8 9AG, UK.

<sup>5</sup>Faculty of Health Sciences, Kristianstad University, Kristianstad SE-291 88, Sweden.

**Correspondence to:** Prof. Martin Persson, Faculty of Health Sciences, Kristianstad University, Kristianstad SE-291 88, Sweden. E-mail: martin.j.persson@hkr.se

**How to cite this article:** Sharratt ND, Calleja Agius J, Davies G, Mehendale FV, Hagell P, Persson M. Equality in cleft and craniofacial care. *Plast Aesthet Res* 2020;7:35. <http://dx.doi.org/10.20517/2347-9264.2020.99>

**Received:** 29 Apr 2019 **First Decision:** 12 Jun 2020 **Revised:** 19 Jun 2020 **Accepted:** 29 Jun 2020 **Published:** 12 Jul 2020

**Academic Editor:** Carroll Ann Trotman **Copy Editor:** Cai-Hong Wang **Production Editor:** Tian Zhang

## Abstract

This review examines the issue of equality of care amongst those with cleft lip and/or palate in the European Union (EU) and beyond. Issues of equality both between and within national populations are considered, and it is argued that those from countries with smaller healthcare expenditure and who are from marginalised groups are at the greatest risk of, and affected most severely by, healthcare inequalities. The socioeconomic impact of inequality is also discussed. Having reviewed these topics, the goals and activities of the European Cleft and Craniofacial Initiative for Equality in Care Action, formed pursuant to an award from the EU's European Cooperation in Science and Technology, are introduced. Constituted of an open network of clinicians and researchers committed to exploring and reducing such inequalities, the ongoing Action is formed of multiple working groups examining these issues within the EU and has organised training schools, conferences and short-term scientific missions concerned with these issues. These activities are discussed along with the future directions of the Action, the impact it has had to date and the benefits of the European Cooperation in Science and Technology award.

**Keywords:** Cleft, craniofacial, equality, care, healthcare, Europe

## INTRODUCTION

The European Union (EU) currently consists of 27 countries with a combined population of 446 million inhabitants<sup>[1]</sup>. There are approximately 637,000-743,000 individuals living with a cleft lip and/or palate (for convenience referred to hereafter as “cleft”) in Europe and many others with other craniofacial conditions.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Throughout their childhood and into their adult life, these individuals may benefit from the expertise of and care from a variety of specialists including surgeons (who may perform primary cleft surgery, revision surgery and further aesthetic surgeries) but also dentists, orthodontists, geneticists, nurses, paediatricians, psychologists, speech and language therapists, but their access to healthcare can vary greatly. For the general population in Europe, there is a significant variance in access to healthcare. When looking at the health expenditure per inhabitant in 2016, the countries with the lowest health expenditure were Romania (€400), Bulgaria (€600) and Poland (€700). In contrast, the countries with the highest expenditures were Luxembourg (€5600), Sweden (€5100) and Denmark (€5000)<sup>[2]</sup>. The difference in resources can have an impact on the provision of care for cleft and craniofacial conditions and can therefore be important in determining health and social outcomes. In other words, the health and social outcomes of an individual with a cleft or craniofacial condition are dependent on the country in which they were born.

It is established that access to effective treatment for cleft and other craniofacial conditions varies widely throughout Europe, meaning that many children born with these conditions are never given the opportunity to realise their full potential. The concept of a comprehensive multi-specialist-team approach to care is not universal. Furthermore, infants with clefts are still institutionalised in some EU countries. A survey carried out for UNICEF in Bulgaria in 2011 showed that, in up to 40 per cent of cases where babies were born with a cleft, the parents were advised (in most cases by health professionals) to leave their baby in an orphanage. Via a fruitful collaboration, the European Cleft Organisation (ECO) and The Association of Children with Facial Anomalies and their Parents in Bulgaria (ALA) has helped reduce these numbers to 28 per cent through educating and training “front line” healthcare providers and families with children with facial anomalies. The work is ongoing<sup>[3]</sup>.

Clinicians and patients across Europe, especially in those regions that have limited healthcare expenditure, state that access to the necessary care pathways during the treatment span (from the moment of diagnosis to adulthood) is fragmented or in some cases non-existent in their countries. The importance of the early and adequate provision of information and treatment of clefts has been emphasised by the 2015 report of the European Committee for Standardisation (CEN)-Early Care Services for Babies Born with Cleft Lip and/or Palate. This involved input from 12 European countries and detailed the first agreed set of European guidelines in early cleft care<sup>[4]</sup>. The minimum standards of care and best practice models presented in this document led to a European Science Foundation funded conference held in Bucharest in October 2015, attended by nurses working in the field of cleft in 17 European countries. Key outcomes of the meeting were the urgent demand for a Europe-wide programme of implementation of the guidelines together with protocols on how to evaluate progress, especially in Eastern Europe. In these respects, the outcomes mirrored those of the earlier Eurocleft project, involving 30 countries and 201 centres, which recommended the adoption of a common set of policy statements governing clinical practice for European cleft teams and practice guidelines detailing minimum standards of care<sup>[5]</sup>. These recommendations, however, were never implemented on a consistent, Europe-wide basis.

Further evidence of the urgent need to improve cleft and craniofacial care in these countries was presented at a subsequent European Science Foundation funded meeting at CEN in Brussels in March 2016 attended by user groups, politicians and doctors from 13 European countries. It was emphasised that there is a need to adhere to evidence-based guidelines in order to develop national protocols. It was noted that there is still a lack of coordinated general measures in cleft care at a national level in many European countries including prenatal diagnosis, genetic advice, national registry development, equal access to treatment and transparent rules for treatment. There is a lack of coordinated multidisciplinary team protocols, deficiencies in information provision, both for professionals and parents, and deficiencies in long-term management. For example, it has been shown that the intervention of multidisciplinary team management in cases of women whose foetuses were diagnosed with cleft lip/palate on prenatal ultrasound screening was associated

with these women being more likely to decide to continue their pregnancy than would have been expected if such intervention was not received<sup>[6]</sup>.

At the same time, the European Pillar of Social Rights states that “Everyone has the right to timely access to affordable, preventive and curative healthcare of good quality” p.21<sup>[7]</sup>. There have been significant improvements in health in Europe due to advancing healthcare systems; however, access to healthcare remains uneven across countries and social groups, according to an individual’s socioeconomic status, place of residence, ethnic group and gender<sup>[8]</sup>. Furthermore, it has been established that the lack of coverage and provision of certain types of care, such as cleft and craniofacial care, means that significant inequality remains in Europe. It is extremely important to acknowledge, however, that this inequality is not just between countries, but also within a country.

Inequalities are more present in socially marginalised groups; they are, for example (but not limited to), individuals who are unemployed, experience mental health problems, migrants or refugees and have disabilities. Belonging to a socially marginalised group also increases the risks of health inequalities, poverty and social exclusion. In 2017, 22.4 per cent of the European population (112.8 million people) lived in households at risk of poverty or social exclusion. In Bulgaria, Romania and Greece, more than a third of the population was at risk<sup>[9]</sup>. When it comes to children, figures for 2018 show they are the group at highest risk of poverty or social exclusion in Europe<sup>[10]</sup>.

A fundamental factor that explains inequalities in healthcare is that the lower the income of an individual or family is, the more self-reported unmet healthcare needs they have. A descriptive example is that in Greece those who are in the lowest income quintile report 34.3 per cent unmet healthcare needs while those in the highest quintile report only 0.4 per cent<sup>[8]</sup>. Currently, there exists evidence that large income differences result in poorer health and more negative social consequences<sup>[11]</sup> and health inequities are increasing in many countries<sup>[12]</sup>. One of the factors that contributes to belonging to a group that is at risk of being in poverty and social exclusion is an individual’s level of education<sup>[13]</sup>, since higher educational attainment often correlates with higher employment rates and higher earnings<sup>[14]</sup>. It also depends on which region an individual resides, since the 2018 unemployment rates in EU regions vary from 1.3% to 35.1%<sup>[15]</sup>.

At the same time, research shows that individuals with cleft are at an elevated risk of poor educational outcomes in comparison to their peers<sup>[16-19]</sup>. This carries the potential to influence the long-term outcomes for individuals with cleft, since they are then hypothetically at a higher risk of poverty and/or social exclusion if they do not succeed in their education.

Experiencing inequality, poverty or social exclusion is also associated with mental health problems. Currently, in Europe, more than one in six people experience mental health problems<sup>[20]</sup>, and, when it comes to mental health problems for individuals with cleft, there are population-based studies from Denmark and Sweden that show a significantly increased risk for having psychiatric and neurodevelopmental disorders<sup>[21,22]</sup> as well as increased risk for being prescribed psychotropic medication<sup>[23,24]</sup>.

When individuals with a cleft or craniofacial conditions are seen by their healthcare providers, based on EU data for the general population, approximately 22 per cent are at risk for poverty or social exclusion<sup>[25]</sup>. However, the real figures are probably higher since the research introduced above shows that some individuals with a cleft are at significant risk of not doing well academically and at risk of mental health problems, which are associated with a higher risk of experiencing inequality, poverty and/or social exclusion. The impact is also influenced by the access to healthcare, as well as the economic situation in the country in which an individual with a cleft or craniofacial condition resides.



The European Cleft and Craniofacial Initiative for Equality in Care (ECCE) is a pan-European multidisciplinary network with participants from hospitals and research institutions located in 27 European countries and beyond. ECCE is a 4-year project funded by the European Cooperation in Science and Technology (COST), promoting research and the development of innovation networks by providing support for arranging meetings, training schools and short-term scientific missions (STSMs) within the focus of the ECCE Action.

The ECCE Action is focusing on sharing scientific knowledge on treatment, research methods and organisational implementation in the different disciplinary groups in cleft and craniofacial care between the participants in the Action. The Action is made up of working groups to provide a structured environment within which mentoring and guidance will be offered to all the participants, with a particular focus on the early career investigators (ECIs) and researchers hailing from Inclusiveness Target Countries (ITC). This is aimed to facilitate the gaining of experience of leadership by participating in working group meetings and plenary sessions. In addition, as part of the COST framework, the Action organises conferences, short-term scientific missions and training schools targeting different aspects of equality in cleft and craniofacial care.

## THE EUROPEAN CLEFT AND CRANIOFACIAL INITIATIVE FOR EQUALITY IN CARE

The activities that have been and are being undertaken as part of the ECCE Action are now introduced in order to consider how they contribute to the goals of the Action.

### Working groups

To achieve a European perspective, the following areas are addressed by different working groups in the Action.

1. The primary level: the patient as the beneficiary
2. The level of the organisational context: the multidisciplinary team
3. The level of funding and policies: administration and resources

These areas are integrated with the imperative focus on the inequality of care in relation to healthcare resources, together with the impact for socially marginalised groups with cleft or craniofacial conditions. To address these objectives, the members of the Action have developed the following questionnaires during working group meetings in the first year of the Action.

1. Access to care: Asking questions about the ability to provide appropriate care for individuals with a cleft or craniofacial condition, based upon the respondents' experience where they work
2. Provision of care: Asking questions about the allocation of staff in the different specialities, available resources, number of new cases per year and funding modalities at their hospital
3. Patient organisations: Asking questions about perceptions of access to care amongst patient representative groups (e.g. whose members are primarily parents of children and young people with cleft or craniofacial conditions)

The analysis of the data is due to begin in the coming months.

Another activity undertaken in order to consider issues connected to equality of care was the use of Group Concept Mapping (GCM). GCM is a mixed qualitative and quantitative participant-driven method that aims to facilitate the understanding of complex phenomena, reveal their structures and discover new meaning<sup>[26,27]</sup>. The methodology comprises the generation of ideas (statements/items) through focus group brainstorming guided by a study-specific prompt that explicitly targets the focus or objective of the study. The ideas generated by the focus groups are then reviewed and edited (for clarity, redundancy, etc.) into a list of statements. This is followed by conceptual sorting and importance rating of the statements (alternative and additional ratings may be used). That is, participants individually sort the statements

according to how they think they relate to one another, and then rate each statement's importance (with respect to the focus prompt) relative to all other statements. Sorted data are then analysed quantitatively (using multidimensional scaling) to map out relationships among individual statements, and cluster analysis is used to identify clusters of statements representing common aspects of the studied area. Finally, the map and its clusters are interpreted qualitatively together with rating data as a means to aid their use in, e.g., evaluation, planning and development. GCM has been used in a large variety of settings and purposes, including educational ones. For example, we have found GCM to be a valuable and very appropriate approach for evaluating and planning educational activities in higher education<sup>[28]</sup> and for assessing the value of educational interventions for healthcare professionals<sup>[29]</sup>.

For ECCE, the first phase of the GCM involved the generation of statements and short sentences in relation to this question: "An important thing to help improve equality of care is..." These statements and short sentences were generated by healthcare professionals and members of the ECCE Action attending the ECCE meeting in Kristianstad, Sweden, on 14 December 2019. The second phase, which involves the conceptual sorting and importance rating of the statements generated in the first phase, is ongoing.

In the final year of the Action, a comprehensive plan for a minimum standard of multidisciplinary cleft and craniofacial care will be developed based on the outcomes from the areas mentioned above as well as the COST Action members' feedback. The creation of this standard will take into account the following aspects of health system goals.

1. Health: Improving the health condition for all individuals affected by cleft and craniofacial conditions
2. Responsiveness: In this context, all affected individuals and their families should have the same right to comprehensive treatment in Europe; hence, it is paramount to include differences concerning economic, social, demographic and other factors
3. Fair financing: All affected individuals should have access to comprehensive care and should not become impoverished or pay a disproportionate share of their income in obtaining needed healthcare or forfeit it because they cannot afford it

The envisioned benefits from scientific and socioeconomic perspectives are:

#### *Scientific perspective*

1. The ECCE Action will establish a sustainable network of European scientists and clinicians dedicated to research on how to provide and deliver the best care for families affected by cleft and craniofacial conditions.
2. The ECCE Action will agree on study designs in the different specialities for cleft and craniofacial research. This will enable studies to be undertaken with comparable models and comparable assessment tools.
3. The ECCE Action will facilitate enhanced research in this area across Europe, especially in those countries that have low healthcare expenditure. This has the potential to improve the provision of care for the families affected by cleft and craniofacial conditions.
4. The ECCE Action will increase the knowledge and skills of researchers and clinicians through their participation in the Action's activities.
5. The ECCE Action will enable a direct comparison of cleft and craniofacial care in Europe via the participants involved in the Action.

#### *Socioeconomic perspective*

1. The ECCE Action will facilitate the exchange and dissemination of knowledge on the effects of having an adequate provision of care for families with cleft and craniofacial conditions. This will help clinicians in each of the multidisciplinary team mentioned specialities to implement appropriate care, informed by evidence-based practice.

2. The ECCE Action complements the European strategy of developing sustainable actions<sup>[30]</sup>.
3. The ECCE Action will develop and share knowledge on what works for whom and will facilitate improvements in cleft and craniofacial care, increase the chances of equal access to care.
4. The ECCE Action will actively facilitate interaction between clinicians, scientists and policymakers in seeking ways of ensuring that all affected families have the right to receive the minimum standard of care in all of the participating COST countries.
5. The ECCE Action could bring about substantial cost savings in relation to joint research activities across Europe.
6. The ECCE Action will increase the capacity for research by generating awareness amongst policymakers of the need for research into the cleft and craniofacial care and healthcare integration.
7. Since congenital anomalies do not discriminate against anyone, they also affect socially disadvantaged target groups, such as immigrants, the unemployed and the Roma population; thus, the ECCE Action addresses key areas of European focus such as disability<sup>[31]</sup>, children's rights<sup>[32]</sup> and social exclusion<sup>[33]</sup>.

### Training schools

An integral part of the ECCE Action is the organisation of training schools. Four training schools are planned, and they relate to the objectives of the different working groups. To date, one training school has been held, with the second one postponed in view of the COVID-19 pandemic.

The first training school was held in March 2019 in collaboration with the University of Malta, Valletta. This training school focused on the clinical and research-oriented approaches of integrated healthcare in relation to the decision-making process related to the diagnosis and treatment of patients. Since the patient is the primary beneficiary, this school trained the participants to facilitate patient empowerment programmes to make the patient feel he/she is an equal partner in decisions made around his/her care and have the confidence to pursue his/her own life goals unhindered by the stigma of his/her condition<sup>[34]</sup>.

The second training school is planned for October 2020 and will be held in Estonia. The focus will be on equality of care within the context of multidisciplinary teamwork, organisational structures and the implementation of change and will include training in the Health Innovation, Implementation and Impact (HI3) concept<sup>[35]</sup>. Further details of both these training schools are included within [Supplementary Materials 1](#). The final two training schools will be held during the final year of the Action.

### Conferences

The ECCE Action conferences are free to attend and are vital to the Action's activities. Three major conference events are scheduled during the life of the Action, which bring together a rich and diverse network of researchers and stakeholders: user groups (beneficiaries); clinicians; health service providers (hospitals and health system coordinators); experts in public health medicine; representatives from health ministries; health economists; NGO's; and political lobbyists and activists. These events enable knowledge sharing at all levels, which is key to the Action's outputs and integral to promoting and facilitating dissemination. They are attended by local healthcare professionals, trainees, researchers and students in order to increase general awareness of and engagement with the issues discussed. They help cement research relationships between the COST participating countries leading to new connections and relationships, which enhance future research funding opportunities, sustaining the long-term effectiveness of treatment for all patients with orofacial clefts and other craniofacial conditions.

To date (June 2020), two conferences have been held. The first action conference was held in Niš in Serbia in 2018 and the second in Kristianstad, Sweden in late 2019. Further details are included in [Supplementary Materials 2](#). The final Action summit will take place in Bucharest, Romania in September 2021, with a focus on the Action's outputs and recommendations and, importantly, promote the sustainability of the network(s) beyond the life of the Action.

### Short-term scientific missions

One of the benefits of COST Actions is the availability of STSMs, described by COST as a networking tool that encompasses a cross-border visit or exchange from one member of an Action to another. Typically, the subject matter of an STSM is connected to the Action or to some facet of the Action and, in turn, this facilitates future collaboration and the mutual sharing of techniques or ideas that might not otherwise be available or apparent.

The ECCE Action has the resources to offer approximately six STSMs per year of the Action, each of at least five working days duration. In tandem with the focus of this Action upon equality, the intention was that at least 75 per cent of these STSMs would be undertaken by ECIs or members of the Action based in ITCs and that there would be an equal gender split amongst the successful applicants.

To undertake an STSM, applicants (the “visitor”) develop a proposal in collaboration with their proposed host and submit this to the Action’s core group, who have been delegated the responsibility of considering and approving applications. To date (April, 2020), despite having to postpone one STSM as a result of the COVID-19 pandemic, the Action has supported 12 STSMs. Of these, 10 have been undertaken by members who satisfy the criteria of being an ECI and/or who are based in an ITC. There has also been an equal gender split, reflecting the balanced composition of the membership of the ECCE Action.

As a result of the applied nature and focus of the ECCE Action, a high proportion of its membership is clinically active. This has meant that the Action’s STSMs have been well placed to benefit both research into cleft and craniofacial conditions as well as clinical and practical implications and applications. Example STSMs are described within Supplementary Materials 3.

It is also worth noting that the core group of the ECCE Action believed that any STSM that may have the potential to improve cleft care or our understanding of the condition was potentially within the remit of the Action. This is especially pertinent as cleft care is complex, can involve a large number of inter-connected disciplines, whether or not professionals are formally organised into multidisciplinary cleft teams, and can therefore be difficult for an individual to navigate, with their socioeconomic status and health literacy likely to be important factors in determining their ability to do so.

As the examples provided in Supplementary Materials 3 illustrate, the STSMs supported by the ECCE Action have covered a diverse array of topics, all undertaken with the desire to improve our understanding of and care for cleft and craniofacial conditions; to allow applicants and hosts to learn from one another and take that learning back to their local environment, share it and apply it; to foster collaborations and relationships that will endure beyond the life of the specific Action; and, ultimately, to improve and equalise access to care for cleft and craniofacial conditions. The members of the Action remain excited to follow the ongoing outcomes from the STSMs that have been performed to date, to discover what proposals will be submitted in the future and to develop the relationships built as part of the Action, at least in part attributable to the availability of STSMs.

### CONCLUSION

It is well established that disparities and inequalities exist within healthcare in the EU and subsist within cleft and craniofacial care. These extend to health expenditure, access to effective and multidisciplinary healthcare following a clear treatment pathway or protocol, the provision of long-term case management and the operation of national data registries. Such disparities exist between countries and within subsets of a national population. In turn, this may prejudice patients’ social and economic opportunities and, in cleft care, compound the impact of their condition on educational attainment and socioeconomic status.

Whilst the activities of the ECCE Action are ongoing, it has already enabled over 135 researchers and clinicians from 30 countries to attend one or more of the events facilitated by the Action, including training schools, short-term scientific missions and conferences, all related to the topic of equality in care. It is believed by the authors that this exposure to the experiences and healthcare cultures of such a diverse group of researchers and clinicians can only serve to increase awareness of this topic amongst those researchers, clinicians and trainees involved in the Action. In addition to facilitating individual awareness and, potentially, enabling those working clinically to carry this awareness into their practice, those involved in the Action are committed to sharing their learning with colleagues and, gradually, instigating organisational change at a local level in order to combat healthcare inequalities where possible.

The activities of the working groups, including the surveys and questionnaires and GCM exercises being undertaken, the STSMs and the long-term collaborations forged in performing this activity together as well as during conferences and training schools, will also lead to contributions to the literature on this topic. This collaboration between participants in the ECCE Action has resulted in four ongoing research or pilot endeavours concerning the access to and provision of care in cleft and craniofacial conditions and has generated six further EU grants comprising a total of 16 different country partners and a combined grant sum of 1,372,000 Euros. In turn, it is hoped that the immediate outputs of the ECCE Action as well as those stemming from partnerships it has helped create will increase awareness and encourage reflection, action and application within the clinical, research and healthcare policy spheres. These outputs will also be important in establishing equality of access to cleft and craniofacial care as a critical issue going forward and maintaining a focus on the importance of equality of care.

These achievements should be understood against the background of it being a significant challenge to attract funding to enhance knowledge of relatively rare medical conditions such as cleft and other craniofacial anomalies. In this respect, COST provides networking opportunities for researchers and innovators to strengthen Europe's capacity to address scientific, technological and societal challenges. The principal areas of activity are promoting and spreading excellence, fostering interdisciplinary research for breakthrough science and empowering and retaining young researchers and innovators. COST implements its mission by funding bottom-up, excellence-driven, open and inclusive networks in all areas of science and technology. Whilst COST does not fund research time, it provides support for networking activities carried out within Actions and, as has materialised with the ECCE Action, increases the possibility of the topic under consideration being the subject of further applications for research funding.

The fact that COST puts significant emphasis on helping early career researchers and clinicians grow professionally is both unique and very important for topics such as equality of care. This is because there exists an opportunity to influence and shape future approaches to cleft and craniofacial care and research in Europe. For these groups, the training schools and short-term scientific missions have been, and will continue to be, powerful tools that can act as an introduction to cross-border collaboration and highlight future possibilities. Furthermore, COST also prioritises clinicians and researchers from less research-intensive countries. This has been hugely beneficial in bringing together researchers, especially in those parts of Europe that have hitherto not had the opportunity to exchange knowledge and ideas with counterparts in other countries due to limited resources. Indeed, funding is not only open to Action members but also a limited number of colleagues and researchers in countries represented in the Action. Grants to attend the conferences and training schools and take part in STSMs are usually sufficient to cover most, if not all, of the costs of travel, accommodation and subsistence, which ensure networking opportunities are genuinely open to everyone eligible. In this sense, the ECCE Action has been able to encourage and foster opportunities and equality within its own members and its own activities, a critical requirement given the focus of this Action upon equality.



A vital component of the Action is to generate awareness about the inequalities of care that exist between and within countries in Europe and beyond. In addition to the activities detailed, members of the Action are participating in dissemination activities, including key conferences around the world and with the European Parliament. The ECCE Action network is, however, not closed but remains open. Interested clinicians and researchers, from the EU and beyond, who wish to learn more and/or collaborate on this project or on future projects are invited to contact the corresponding author and discuss these possibilities.

## DECLARATIONS

### Authors' contributions

Substantially contributed to drafting and reviewing the manuscript: Sharratt ND, Calleja Agius J, Davies G, Mehendale FV, Hagell P, Persson M

Substantially contributed to the conception of this project and preparation of the funding application: Davies G, Persson M

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

This project is supported by funding awarded by the EU's European Cooperation in Science and Technology programme, Action CA16234. The funding awarded supports the costs and expenses associated with the activities of the Action and the management of the Action. The time commitment required of members of the Action is supported by each individual member's employer and/or the dedication of their personal time to the Action. The employment of the first author is supported by a donation made to the University of the West of England by the Vocational Training Charitable Trust Foundation.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. European Union. EU in figures: Living in the EU: 2020. Available from: [https://europa.eu/european-union/about-eu/figures/living\\_en](https://europa.eu/european-union/about-eu/figures/living_en) [Last accessed on 29 Apr 2020]
2. Eurostat. Healthcare expenditure in the EU: 2018. Available from: <https://ec.europa.eu/eurostat/web/products-eurostat-news/-/DDN-20181129-2> [Last accessed on 3 Jul 2020]
3. European Cleft Organisation – 10 years in Bulgaria: 2019. Available from: [http://europeancleft.org/wp-content/uploads/2020/04/ECO-in-Bulgaria-FINAL\\_1.pdf](http://europeancleft.org/wp-content/uploads/2020/04/ECO-in-Bulgaria-FINAL_1.pdf) [Last accessed on 19 Jun 2020]
4. The European Committee for Standardisation. CEN/TR 16824: 2015 Early care services for babies born with cleft lip and/or palate. Available from: <https://standards.iteh.ai/catalog/tc/cen/d0bb9258-df47-411a-a9e1-cd383fcb5b9/cen-tc-424> [Last accessed on 8 Jul 2020]
5. Shaw WC, Semb G, Nelson P, Brattström V, Mølsted K, et al. The Eurocleft project 1996-2000: overview. *J Craniomaxillofac Surg* 2001;29:131-40.
6. Han HH, Choi EJ, Kim JM, Shin JC, Rhie JW. The importance of multidisciplinary management during prenatal care for cleft lip and palate. *Arch Plast Surg* 2016;43:153-9.
7. European Parliament, Council of the European Union, and European Commission. European Pillar of Social Rights. Available from:

- [https://ec.europa.eu/commission/sites/beta-political/files/social-summit-european-pillar-social-rights-booklet\\_en.pdf](https://ec.europa.eu/commission/sites/beta-political/files/social-summit-european-pillar-social-rights-booklet_en.pdf) [Last accessed on 3 Jul 2020]
8. Baeten R, Spasova S, Vanhercke B, Coster S. Inequalities in access to healthcare. A study of national policies, European Social Policy Network (ESPN). Brussels: European Commission; 2018.
  9. Eurostat. Archive: People at risk of poverty or social exclusion. 2017. Available from: [https://ec.europa.eu/eurostat/statistics-explained/index.php/People\\_at\\_risk\\_of\\_poverty\\_or\\_social\\_exclusion#Number\\_of\\_people\\_at\\_risk\\_of\\_poverty\\_or\\_social\\_exclusion](https://ec.europa.eu/eurostat/statistics-explained/index.php/People_at_risk_of_poverty_or_social_exclusion#Number_of_people_at_risk_of_poverty_or_social_exclusion) [Last accessed on 6 Apr 2020]
  10. Eurostat. Children at risk of poverty or social exclusion. Available from: [https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Children\\_at\\_risk\\_of\\_poverty\\_or\\_social\\_exclusion#The\\_highest\\_risk\\_of\\_poverty\\_or\\_social\\_exclusion](https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Children_at_risk_of_poverty_or_social_exclusion#The_highest_risk_of_poverty_or_social_exclusion) [Last accessed on 6 Apr 2020]
  11. Pickett KE, Wilkinson RG. Income inequality and health: a causal review. *Soc Sci Med* 2015;128:316-26.
  12. Marmot M. Review of social determinants and the health divide in the WHO European Region: final report. Copenhagen:WHO Regional Office for Europe; 2014.
  13. Keeley B. Human Capital: How what you know shapes your life. Paris: OECD Publishing; 2007.
  14. U.S. Bureau of Labor Statistics. Unemployment rates and earnings by educational attainment. Available from: <https://www.bls.gov/emp/chart-unemployment-earnings-education.htm> [Last accessed on 8 Apr 2020]
  15. Eurostat. Unemployment statistics at regional level. Available from: [https://ec.europa.eu/eurostat/statistics-explained/index.php/Unemployment\\_statistics\\_at\\_regional\\_level#Regional\\_unemployment\\_rates\\_and\\_the\\_EU\\_average](https://ec.europa.eu/eurostat/statistics-explained/index.php/Unemployment_statistics_at_regional_level#Regional_unemployment_rates_and_the_EU_average) [Last accessed on 8 Apr 2020]
  16. Watkins SE, Meyer RE, Aylsworth AS, Marcus JR, Allori AC, et al. Academic achievement among children with nonsyndromic orofacial clefts: a population-based study. *Cleft Palate Craniofac J* 2018;55:12-20.
  17. Fitzsimons KJ, Copley LP, Setakis E, Charman SC, Deacon SA, et al. Early academic achievement in children with isolated clefts: a population-based study in England. *Arch Dis Child* 2018;103:356-62.
  18. Wehby GL, Collet B, Barron S, Romitti PA, Ansley TN, et al. Academic achievement of children and adolescents with oral clefts. *Pediatrics* 2014;133:785-92.
  19. Persson M, Becker M, Svensson H. Academic achievement in individuals with cleft: a population-based register study. *Cleft Palate Craniofac J* 2012;49:153-9.
  20. OECD. "More than one in six people in EU countries have a mental health problem" in *Health at a Glance: Europe 2018: State of Health in the EU Cycle*. Paris: OECD Publishing; 2018.
  21. Pedersen DA, Wehby GL, Murray JC, Christensen K. Psychiatric diagnoses in individuals with non-syndromic oral clefts: a danish population-based cohort study. *PLoS One* 2016;11:e0156261.
  22. Tillman KK, Hakelius M, Højjer J, Ramklint M, Ekselius L, et al. Increased risk for neurodevelopmental disorders in children with orofacial clefts. *J Am Acad Child Adolesc Psychiatry* 2018;57:876-83.
  23. Nilsson S, Merlo J, Lyberg-Ahlander V, Psouni E. Psychotropic drug use in adolescents born with an orofacial cleft: a population-based study. *BMJ Open* 2015;5:e005306.
  24. Pedersen DA, Hageman I, Wehby GL, Christensen K. Use of psychotropic medications and visits to psychiatrists and psychologists among individuals with nonsyndromic oral clefts: a population-based cohort study. *Birth Defects Res* 2017;109:824-35.
  25. Eurostat. Archive: People at risk of poverty or social exclusion. 2017. Available from: [https://ec.europa.eu/eurostat/statistics-explained/index.php/People\\_at\\_risk\\_of\\_poverty\\_or\\_social\\_exclusion#Number\\_of\\_people\\_at\\_risk\\_of\\_poverty\\_or\\_social\\_exclusion](https://ec.europa.eu/eurostat/statistics-explained/index.php/People_at_risk_of_poverty_or_social_exclusion#Number_of_people_at_risk_of_poverty_or_social_exclusion) [Last accessed on 06 April 2020]
  26. Kane M, Trochim WMK. Concept mapping for planning and evaluation. Thousand Oaks: Sage Publications, Inc; 2007.
  27. Trochim WMK. An introduction to concept mapping for planning and evaluation. *Eval Program Plann* 1989;12:1-16.
  28. Hagell P, Edfors E, Hedin G, Westergren A, Hammarlund CS. Group concept mapping for evaluation and development in nursing education. *Nurse Educ Pract* 2016;20:147-53.
  29. Westergren A, Edfors E, Norberg E, Stubbendorff A, Hedin G, et al. Computer-based training in eating and nutrition facilitates person-centered hospital care: a group concept mapping study. *Comput Inform Nurs* 2018;36:199-207.
  30. European Commission Communication. EUROPE 2020 A strategy for smart, sustainable and inclusive growth. Brussels European Commission 2010. Available from: <https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A52010DC2020> [Last accessed on 8 Jul 2020]
  31. European Commission Communication. European Disability Strategy 2010-2020: A Renewed Commitment to a Barrier-Free Europe. Brussels: European Commission; 2010.
  32. European Commission Communication. Early Childhood Education and Care: Providing all our children with the best start for the world of tomorrow. Brussels European Commission 2011. Available from: <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=CELEX%3A52011DC0066> [Last accessed on 8 Jul 2020]
  33. European Commission Communication. The European Platform against Poverty and Social Exclusion: A European framework for social and territorial cohesion. Brussels European Commission 2010. Available from: <https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM%3A2010%3A0758%3AFIN%3AEN%3APDF> [Last accessed on 8 Jul 2020]
  34. ECCE. Training School Malta. The European Cleft and Craniofacial Initiative for Equality in Care 2019. Available from: <https://ecce.nu/Malta-2019> [Last accessed on 8 Jul 2020]
  35. HI3. Health Innovation, Implementation and Impact - A functional training program on how to implement sustainable change in the healthcare system on a clinical level. Available from: <http://health-innovation.nu/> [Last accessed 15 Jun 2020]

Review

Open Access



# The interaction between hyaluronidase and hyaluronic acid gel fillers - a review of the literature and comparative analysis

Michael K. Paap<sup>1</sup>, Rona Z. Silkiss<sup>2</sup>

<sup>1</sup>University of California, San Diego School of Medicine, La Jolla, CA 92093, USA.

<sup>2</sup>Division of Oculofacial Plastic Surgery, California Pacific Medical Center, San Francisco, CA 94102, USA.

**Correspondence to:** Dr. Rona Z. Silkiss, Division of Oculofacial Plastic Surgery, California Pacific Medical Center, 711 Van Ness Ave, Ste 340, San Francisco, CA 94102, USA. E-mail: drsilkiss@silkisseyesurgery.com

**How to cite this article:** Paap MK, Silkiss RZ. The interaction between hyaluronidase and hyaluronic acid gel fillers - a review of the literature and comparative analysis. *Plast Aesthet Res* 2020;7:36. <http://dx.doi.org/10.20517/2347-9264.2020.121>

**Received:** 22 May 2020 **First Decision:** 19 Jun 2020 **Revised:** 21 Jun 2020 **Accepted:** 28 Jun 2020 **Published:** 12 Jul 2020

**Academic Editor:** Wen-Guo Cui **Copy Editor:** Cai-Hong Wang **Production Editor:** Tian Zhang

## Abstract

Hyaluronic acid (HA) is the most common component of aesthetic fillers. Many formulations exist, each exhibiting properties that are manifestations of individual molecular modifications. The enzyme hyaluronidase degrades hyaluronic acid and can therefore be injected into soft tissue to reduce suboptimally placed HA fillers or to reverse local ischemic complications. The clinically available varieties of hyaluronidase may be derived from crude animal extracts or genetically engineered from recombinant human DNA. Different HA fillers are not uniformly dissolved by a single source hyaluronidase, and hyaluronidase from different sources may have varying efficacy in the degradation of HA. Previous studies of subsets of HA fillers and hyaluronidases have provided limited and often conflicting data regarding these differences, and a more comprehensive scientific study is needed. In this review, the authors describe commonly available formulations of HA and hyaluronidase and review all studies of HA-hyaluronidase interaction available via a PubMed and Google Scholar search from 2005 to present, exploring trends in the data. Factors determined to confer increased resistance to degradation included higher concentration of HA, higher crosslinking density, and status as monophasic versus biphasic. Fillers of the Juvéderm family were generally found to be more resistant to degradation than members of the Restylane family. Results are less consistent for Belotero Balance. No variety of hyaluronidase was consistently superior at dissolving any variety of HA filler. More research is needed to clarify these clinically relevant relationships.

**Keywords:** Hyaluronic acid, hyaluronic acid gel, hyaluronidase, dermal fillers, enzymatic degradation, filler complications



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



## INTRODUCTION

Hyaluronic acid (HA) in various configurations of density and crosslinking is commonly used as a substrate for dermal fillers. These widely available and popular fillers augment volume and smooth contour irregularities in cosmetic and age-related changes of the face, hands and other anatomic areas<sup>[1]</sup>. Though many filler materials, including collagen, autologous fat, calcium hydroxyapatite, and poly-L-lactic acid are used in facial rejuvenation, HA accounts for more injections than all other filler varieties combined<sup>[2]</sup>.

One of the main advantages of HA fillers is their easy reversibility. This quality is useful as a means of addressing patient dissatisfaction from superficial or inappropriate placement following filler injection<sup>[3]</sup>. Additionally and most importantly, these injections have been associated with rare but serious ischemic complications including local soft tissue necrosis as well as blindness from possible embolic occlusion of the central retinal artery<sup>[3]</sup>. Hyaluronidase is a naturally occurring enzyme that catalyzes the degradation of HA by hydrolyzing the bond between *N*-acetylglucosamine and glucuronic acid<sup>[4,5]</sup>. In addition to its off-label use as a subcutaneous adjuvant that increases the dispersion of drugs, it has been shown to be effective in both reversing the volumetric effects of HA filler injections and treating local ischemic complications<sup>[6-9]</sup>, although the reversibility of HA filler associated blindness with hyaluronidase has not been established<sup>[10,11]</sup>. Understanding the interactions between the various fillers and the enzymes available for dissolution might optimize outcomes in the event of a filler misplacement or occlusive event.

Since the first FDA approval in 2003 of the HA-containing filler, Restylane, additional HA formulations have become commercially available. Aiming to increase stability and longevity, manufacturers of HA fillers chemically modify the molecules by crosslinking them into larger conjugated derivatives<sup>[12]</sup>. The different HA fillers vary according to the method and extent of crosslinking, concentration of HA, particulate size, HA source, and status as monophasic or biphasic<sup>[13]</sup>. This influences the properties of each gel filler, including hydrophilia, cohesivity, hardness, and viscosity<sup>[2]</sup>. Clinically, knowledge of these differences can be used to select the optimal HA filler for a given application<sup>[6]</sup>. Additionally, the structural differences between HA fillers are known to alter their response to hyaluronidase<sup>[2,6,12,14-18]</sup>.

Hyaluronidase, similarly, is available in several formulations, broadly categorized as purified crude extracts from ovine or bovine testicular tissue, and products of recombinant technology from human DNA<sup>[19]</sup>. Though all major formulations are dosed equivalently, each type has its own optimal pH and is considered therapeutically distinct by the FDA<sup>[19]</sup>.

In the clinical setting, it has been reported that some fillers are more or less responsive to enzymatic degradation as compared to others<sup>[16]</sup>. Thus, the interaction between different HA fillers and the varieties of hyaluronidase is an area of research with important implications, especially for the choice of reversal agent in treating suboptimal outcomes or complications from filler injections. Although multiple studies have explored the effect of hyaluronidase on different types of HA fillers, few have examined more than a single type of hyaluronidase<sup>[6,16]</sup>. A comprehensive analysis of the full range of commercially available HA fillers and hyaluronidases is needed. Unfortunately, the literature to date concerning this topic has been noted to exhibit significant and unresolved heterogeneity in both experimentation and results<sup>[20]</sup>. To address the incompletely answered question of how different HA fillers respond to different types of hyaluronidase, the authors first summarize the known literature available on PubMed and Google Scholar of HA-hyaluronidase interactions. The authors then attempt to clarify, across studies, the relationships between commercially available HA-based fillers and ovine-, bovine-, and recombinant-sourced hyaluronidase. Results from experiments using different fillers and hyaluronidases are cross-referenced and information describing how different HA fillers respond to hyaluronidase will be presented. Additionally, possible explanations for discrepancies found across studies are explored. Through this review, we attempt to provide a summary of foundational knowledge and highlight the need for future clarifying studies.

**Table 1. Properties of the Three Major Families of HA Filler**

Family name	Crosslinking agent	Monophasic/Biphasic	Crosslinking technology	Sub varieties	Concentration (mg/mL)	Percent crosslinking
Restylane	BDDE	Biphasic	NASHA	Restylane	20	1%
				Restylane-L	20	1%
				Restylane Lyft	20	1%
				Restylane Silk	20	1%
				XpresHAN	20	6%
				Restylane Refyne	20	8%
Juvéderm	BDDE	Monophasic	Hylacross	Restylane Defyne	20	8%
				Juvéderm Ultra	24	6%-9%
			Vycross	Juvéderm Ultra Plus	24	8%-11%
				Juvéderm Voluma	20	Unreported
				Juvéderm Vollure	17.5	Unreported
				Juvéderm Volbella	15	Unreported
Belotero	N/A	Monophasic	CPM	Juvéderm Volite	12	Unreported
				Belotero Balance	22.5	Polydensified gel
				Belotero Soft	20	Polydensified gel
				Belotero Intense	25.5	Polydensified gel
				Belotero Volume	26	Polydensified gel
			Uncrosslinked	Belotero Hydro	18	N/A

BDDE: 1,4-butanediol diglycidyl ether

## REVIEWING THE LITERATURE

A literature search of published reports from 2005 to April 2020 was performed on the interactions between different types of hyaluronic acid fillers and hyaluronidases. The databases of PubMed, Ovid MEDLINE, and Google Scholar were searched using keywords including: “hyaluronic acid fillers and hyaluronidase,” “degradation of hyaluronic acid,” “hyaluronic acid and hyaluronidase interactions,” “hyaluronic acid filler comparison,” and “hyaluronic acid filler sensitivity.” The search was limited to the literature in English. In addition, references in the identified articles were reviewed to identify additional reports, if any.

## LITERATURE FINDINGS

More than one hundred experiments, reviews, and reports regarding hyaluronic acid-hyaluronidase relationships and relevant topics were identified and reviewed. Eight relevant studies were identified and analyzed in detail. The authors attempted to include all relevant data in this report.

### Classification of hyaluronic acid dermal fillers and hyaluronidases

#### *Varieties of hyaluronic acid filler*

At the time of this writing, 18 individual formulations of HA have been approved for use as dermal fillers by the FDA<sup>[21]</sup>. The subtypes and properties of three major filler families, Restylane, Juvéderm, and Belotero Balance, are described below and summarized in Table 1.

Restylane is manufactured by Q-Med AB (Uppsala, Sweden) and produced from HA generated via fermentation<sup>[22]</sup>. Produced entirely from non-animal sources, it is classified as a non-animal stabilized hyaluronic acid (NASHA) and is stabilized through a crosslinking process to the compound 1,4-butanediol diglycidyl ether (BDDE)<sup>[22,23]</sup>. It has a HA concentration of 20 mg/mL and a biphasic formulation with a gel particulate size of 330-430 micrometers<sup>[22,24]</sup>. The degree of crosslinking is relatively low at 1%<sup>[24]</sup>. Subsequently introduced members of the Restylane family include Restylane Lyft (formerly known as Perlane), which contains fewer, larger gel particles per milliliter (8,000 per mL vs. 100,000 per mL); Restylane-L, which contains lidocaine; and Restylane Silk, which contains lidocaine, is less viscous, and was formulated specifically for lip augmentation<sup>[22,24]</sup>. Restylane Refyne, Restylane Defyne, and Restylane Kysse are manufactured with XpresHAN Technology, which varies the degree of crosslinking and gel particle size to create softer gels<sup>[25,26]</sup>.



Juvéderm, initially produced by Lea Derm (Paris, France) and subsequently manufactured and distributed by Allergan (Irvine, CA), is also a BDDE-crosslinked NASHA produced from equine streptococci<sup>[24]</sup>. In contrast to Restylane, it is a monophasic gel without distinct particles, originally produced through Hylacross technology that allows for a high concentration of crosslinking. First approved by the FDA in 2006, the Juvéderm family of fillers has expanded to many formulations that vary in concentration of HA and crosslinking density. The two main varieties available for facial wrinkles and folds in the US market are Juvéderm Ultra (Juvéderm 24 HV) and Juvéderm Ultra Plus (Juvéderm 30 HV), which both have a concentration of 24 mg/mL but differ in their crosslinking percentages (9% and 11%, respectively)<sup>[27]</sup>.

The more recent formulations of Juvéderm are manufactured using Vycross technology, which combines low- and high-weight HA molecules to improve crosslinking efficiency<sup>[24]</sup>. Juvéderm Voluma XC (20 mg/mL) is a formulation with high cohesivity and viscosity used for deep injections and cheek augmentation. Juvéderm Vollure XC (17.5 mg/mL) is slightly less concentrated and designed for volumizing nasolabial folds<sup>[28]</sup>. Even denser crosslinking is present in Juvéderm Volbella XC (15 mg/mL), which is manufactured with a higher proportion of low molecular weight HA and designed for lip augmentation and perioral rhytids<sup>[29]</sup>. Juvéderm Volite, the least concentrated variety at 12 mg/mL, is for superficial cutaneous depressions and fine lines<sup>[30]</sup>.

Members of the Belotero family, in contrast to the above monodensified varieties, are polydensified compounds that contain continuously crosslinked HA in a single phase<sup>[31]</sup>. Manufactured by Anteis S.A. (Geneva, Switzerland), fillers of the Belotero family are made with a cohesive polydensified matrix (CPM) technology that creates a gel with nonuniform crosslinking<sup>[15]</sup>. This creates a low-viscosity filler that exhibits homogenous intradermal distribution as compared to other fillers, theoretically allowing for increased injection precision<sup>[24,32]</sup>. Like Juvéderm, the Belotero family has expanded into many different formulations that vary in concentration<sup>[31]</sup>. However, the only one currently available in the US is Belotero Balance, which has a concentration of 22.5 mg/mL and is approved for facial wrinkles and folds<sup>[31]</sup>.

Several other filler varieties, including RHA 2, RHA 3, RHA 4 (Teoxane SA), Revanesse Versa, and Revanesse Versa Plus, are also available in the US, but are less commonly studied and used clinically. Hydrelle (previously known as Elevee) is manufactured by Anika Therapeutics (Bedford, MA) and marketed through Coapt Systems (Palo Alto, California). Though also sourced from equine streptococci, it is crosslinked with p-phenylene bisethyl carbodiimide (BCDI)<sup>[22]</sup>. With a concentration of 28 mg/mL, it has amongst the highest available content of HA<sup>[22]</sup>. Prevelle Silk, the second generation of now-unavailable Captique, is manufactured by Genzyme Corporation (Cambridge, MA) and marketed by Johnson & Johnson (Skillman, NJ); it has a concentration ranging from 4.5-6 mg/mL and is cross-linked with divinyl sulfone<sup>[22]</sup>. Hylaform (manufactured by Genzyme Biosurgery in Ridgefield, NJ), was an avian-sourced formulation with concentration ranging from 4.5-6 mg/mL of HA. Notably, it is no longer available on the market in the US<sup>[21,27]</sup>.

#### *Varieties of hyaluronidase*

There are four varieties of hyaluronidase currently available in the United States: two derived from purified bovine testicular hyaluronidase, one derived from purified ovine testicular hyaluronidase, and one manufactured from recombinant human DNA<sup>[19]</sup>.

Vitrase, manufactured by STA Pharmaceutical (Irvine, CA), is derived from purified ovine testicular hyaluronidase. Amphadase, manufactured by Amphastar Pharmaceuticals, Inc. (Rancho Cucamonga, CA), and Hydase, manufactured by PrimaPharm, Inc. (San Diego, CA), are derived from purified bovine testicular hyaluronidase. Hylenex, manufactured by Halozyme Therapeutics (San Diego, CA), is made from recombinant human DNA<sup>[33]</sup>. Though one experimental study has suggested these varieties are

**Table 2. Summary of reviewed experiments of HA-hyaluronidase interactions**

Study	Type	Measurement	Hyaluronidase(s) used	Findings: x > y (x dissolves more than y)
Rao <i>et al.</i> <sup>[6]</sup>	<i>In vitro</i>	Visual comparison	Recombinant Ovine	Res > Juv Voluma > Juv Ultra > Belo Res > Juv Voluma > Juv Ultra > Belo
Jones <i>et al.</i> <sup>[2]</sup>	<i>In vitro</i>	Chromatography	Ovine	Hylenex > Res > Juv Ultra
Flynn <i>et al.</i> <sup>[15]</sup>	<i>In vitro</i>	Chromatography	Ovine	Res > Juv Ultra > Belo
Sall <i>et al.</i> <sup>[12]</sup>	<i>In vitro</i>	Absorbance measurement	Bovine	Res > Res Lyft >> Surg 18 > Juv 30 > Juv 24 > Juv 30 HV > Juv 24 HV > Surg 30 XP > Surg 24 XP > Surg 30
Cavallini <i>et al.</i> <sup>[14]</sup>	<i>In vitro</i>	Visual comparison	Bovine	Juv Volite > Teosyal RHA > Teosyal Ultra = Juv Voluma > Macrolane = Res
Buhren <i>et al.</i> <sup>[17]</sup>	<i>In vitro</i>	Fluorescence Measurement	Bovine	Belo > Res >>> Juv (didn't degrade)
Shumate <i>et al.</i> <sup>[16]</sup>	<i>In vivo</i> - Rat model	3D Image Quantification	Recombinant Ovine	Res-L > Juv Ultra = Juv Voluma Res-L = Juv Ultra > Juv Voluma
Juhasz <i>et al.</i> <sup>[18]</sup>	<i>In vivo</i> - Human subjects	5-Point Palpation Score	Ovine	Belo >> Juv Ultra > Res Lyft > Juv Ultra Plus > Res-L > Res Silk > Juv Voluma

Res: Restylane; Juv: Juvéderm; Belo: Belotero Balance; Surg: Surgiderm

equally potent and can be used interchangeably<sup>[6]</sup>, subsequent tests and prevailing clinical perceptions have questioned this assumption<sup>[16,34]</sup>. Clinically, it is noteworthy that animal-derived enzymes, though less expensive and more readily available, generally have a less favorable immunogenic profile than those that are recombinantly produced<sup>[34]</sup>.

### Interactions between different types of hyaluronic acid filler and hyaluronidase

Prior studies examining the relationship between different HA fillers and hyaluronidases vary broadly with respect to the products tested and methodology. Experimental designs have included qualitative *in vitro* studies<sup>[6,14]</sup>, quantitative *in vitro* studies<sup>[2,12,15,17]</sup>, an animal model<sup>[16]</sup>, and clinical testing of human subjects<sup>[18]</sup>. Twenty-one individual formulations of HA-containing fillers have been examined, with over 75% being members of the families Restylane, Juvéderm, or Belotero Balance<sup>[2,6,12,14-18]</sup>. We focused our analysis on these three product families, excluding exploration of fillers such as Surgiderm and Teosyal that have not been studied widely or comparatively. A summary of the relevant literature, including type(s) of HA filler and hyaluronidase tested, can be found in Table 2. Although there are some inconsistencies in the reported susceptibility of specific HA fillers to dissolution by hyaluronidase, these studies collectively suggest several guiding principles of HA filler dissolution by hyaluronidase.

#### *Greater concentrations of hyaluronic acid are associated with greater resistance to degradation*

The concentration of HA in currently available fillers range from 4.5 mg/mL to 30 mg/mL, with the most commonly injected varieties falling between 20-24 mg/mL<sup>[13]</sup>. A higher concentration of HA generally corresponds to increased stiffness and longevity<sup>[27]</sup>. In nearly every study of HA-hyaluronidase interaction, filler varieties with a lower concentration of HA tended to dissolve more quickly than fillers with higher HA concentrations. For example, in comparing three filler varieties with different HA concentrations (24 mg/mL, 20 mg/mL, and 5.5 mg/mL), Jones *et al.*<sup>[2]</sup> measured the generation of soluble HA to demonstrate that a lower concentration of HA correlated with increased susceptibility to dissolution by ovine hyaluronidase. Rao *et al.*<sup>[6]</sup> observed similar findings in an *in vitro* study with ovine-derived hyaluronidase and validated this finding for recombinant hyaluronidase. Cavallini *et al.*<sup>[14]</sup> studied the response of six fillers to bovine-derived hyaluronidase and observed the most rapid and homogenous dissolution in the two varieties with the lowest concentrations of HA, Juvederm Volite (12 mg/mL) and Teosyal RHA 1 (Teoxane Laboratories, Geneva, Switzerland; monophasic; 15 mg/mL). *In vivo*, using a rat model, Shumate *et al.*<sup>[16]</sup> demonstrated faster degradation by ovine hyaluronidase of Restylane-L (20 mg/mL) when compared to two filler varieties from the Juvederm family (20-24 mg/mL). Altogether, the improved dissolution of lower concentration HA by hyaluronidase was validated across all major HA filler varieties and with all three sources of hyaluronidase, in both *in vivo* and *in vitro* studies.

### *Greater degree of crosslinking is associated with greater resistance to degradation*

HA is stabilized by crosslinking individual particles to each other with covalent bonds in all HA-containing fillers<sup>[12]</sup>. Across all studies of HA-hyaluronidase interactions, a higher degree of crosslinking generally correlated with greater resistance to degradation by hyaluronidase. Sall *et al.*<sup>[12]</sup> examined the response of 11 individual formulations of HA filler to bovine hyaluronidase and found that the slowest to dissolve, Surgiderm 30 (Allergan, Irvine, California; monophasic; 24 mg/mL) had the greatest degree of crosslinking. The fastest to dissolve, Restylane and Perlane, had the lowest degree of crosslinking. These findings were further validated by comparisons of fillers within the same family, which eliminated possible confounders such as the monophasic/biphasic status that differ between filler classes such as in Surgiderm and Restylane. Within the Juvéderm family, for example, less-crosslinked fillers such as Juvéderm 18 dissolved more quickly than their more-crosslinked counterparts.

These findings were validated in additional *in vitro* experiments of bovine hyaluronidase<sup>[14,17]</sup>, ovine hyaluronidase<sup>[2,6,15]</sup>, and recombinant hyaluronidase<sup>[6]</sup>. *In vivo*, these findings were validated in human subjects by Juhasz *et al.*<sup>[18]</sup>, who found less dissolution of Juvéderm Ultra Plus than Juvéderm Ultra. Although this difference was small and the sample size was too small to achieve significance, these two filler varieties are ideal for comparing this variable as both have the same concentration of HA (24 mg/mL), but Juvéderm Ultra Plus has an increased density of crosslinking<sup>[27]</sup>. The decreased dissolution observed makes scientific sense, as a higher density of crosslinking decreases the access hyaluronidase has to its enzymatic substrate<sup>[12]</sup>.

### *Monophasic hyaluronic acid formulations are more resistant to degradation than biphasic formulations*

HA fillers are classified as monophasic (cohesive gels without distinct particles) or biphasic (particles suspended in gel)<sup>[35]</sup>. Because the individual particles of a biphasic filler create a greater surface area for enzymatic attack, these formulations, e.g., Restylane, have been predicted to exhibit increased susceptibility to degradation compared to their monophasic counterparts<sup>[12]</sup>. Although this and other principles may have origins in manufacturer claims or manufacturer-sponsored studies, this trend is upheld by the reviewed articles on HA-hyaluronidase interactions. Sall *et al.*<sup>[12]</sup> studied both major biphasic compounds (Restylane and Perlane) and found these fillers significantly more susceptible to degradation by bovine hyaluronidase than monophasic varieties of Juvéderm. In other studies, Restylane was routinely found to be more dissolvable than comparably concentrated monophasic fillers with all three varieties of hyaluronidase<sup>[2,6,15,17]</sup>, in both *in vitro* and *in vivo* experiments<sup>[16,18]</sup>.

### **Response of hyaluronic acid fillers to different types of hyaluronidase**

In the two studies that individually examined the effect of multiple types of hyaluronidase on different varieties of HA filler<sup>[6,16]</sup>, only Restylane-L demonstrated a source-dependent response to hyaluronidase. In their rat model, Shumate *et al.*<sup>[16]</sup> found that, at lower concentrations of hyaluronidase (10 U/0.1 mL), Restylane-L was degraded more by ovine hyaluronidase than by recombinant hyaluronidase. Notably, no difference in dissolution efficacy between hyaluronidases was observed for Juvéderm Voluma XC (which has the same HA content, 20 mg/mL, as Restylane-L) or Juvéderm Ultra Plus XC (24 mg/mL) at any concentration of hyaluronidase. However, it is also important to note that at higher doses of hyaluronidase (30 U/0.1 mL), all tested fillers exposed to each type of hyaluronidase were reduced to undetectable levels within 6 hours, leading the authors to conclude that any responsive differences due to HA filler structure or hyaluronidase type are clinically insignificant.

Rao *et al.*<sup>[6]</sup> also studied *in vitro* both ovine and recombinant hyaluronidase and found no hyaluronidase-based difference in the response of Restylane, Juvéderm, Juvéderm Voluma, or Belotero at any concentration. Interestingly, the ratio of ovine hyaluronidase to Restylane used by Rao *et al.*<sup>[6]</sup> (5 U/mg) was identical to that used by Shumate *et al.*<sup>[16]</sup> The differences in the experimental design of these two studies may suggest

that the activity of ovine hyaluronidase, when interacting with a biphasic HA filler, is measurably altered by the conditions of an *in vivo* system as compared to an *in vitro* system. More studies are needed for clarification.

### Trends observed in individual fillers and filler families

Across all studies considered together, it is difficult to compare the response of individual filler types to different types of hyaluronidase due to the variation in the amount of filler studied, quantity of hyaluronidase used, and the method of measuring degradation. However, when the same types of HA filler were used in different studies, it is possible to compare their response to different types of hyaluronidase relative to each other. Three filler families were studied in half or more of the experiments reviewed: Restylane, Juvéderm, and Belotero Balance.

#### Restylane

Restylane varieties were studied in all ten<sup>[1]</sup> (8 studies, 2 of which examined 2 different hyaluronidase types) reported experiments of HA-hyaluronidase interactions and were observed to be the most responsive to hyaluronidase in six studies and the least responsive to hyaluronidase in one. When compared directly to Belotero in the same experiment, Restylane was found to be more susceptible to degradation 60% of the time. When compared directly to any member of the Juvéderm family, it was found to be more susceptible to degradation 90% of the time.

A biphasic formulation with relatively little crosslinking, Restylane and its derivatives were unsurprisingly almost always found to be more susceptible to degradation than the Juvéderm family of fillers. In an outlier study, Cavallini *et al.*<sup>[14]</sup> qualitatively found Restylane (20 mg/mL) to be the least responsive of six fillers to bovine hyaluronidase. Using a ratio of 30 U hyaluronidase per 0.1 mL filler (15 U hyaluronidase per mg Restylane), which exceeds established recommendations of 5 U hyaluronidase per 0.1 mL Restylane for ischemic complications<sup>[34]</sup>, they noted that Restylane needed both additional time and enzyme to reach complete liquefaction, as compared to other fillers, including Juvéderm Volite (12 mg/mL), which dissolved instantly. The results of this study contrast with other studies of similar design. Rao *et al.*<sup>[6]</sup>, using a qualitative *in vitro* model, also found Restylane to be the most susceptible compound to ovine and recombinant hyaluronidase using lower ratios of 5 U per 1 mg and 3.75 U per 1 mg, respectively. In addition, other studies of *bovine* hyaluronidase also found that Restylane dissolved more rapidly than comparably concentrated varieties of Juvéderm<sup>[12,17]</sup>. The reason for Cavallini's experimental discrepancy is unclear, but may be attributable to subjective errors in qualitative interpretation of filler gel consistencies. Interestingly, Restylane is the only one of the six tested fillers to not be pictured in this study's photographic figure of its experimental results.

With regard to Restylane's response to different types of hyaluronidase, there is no evidence that any one enzyme is best at dissolution. Again, using Juvéderm as a comparison, Restylane was found to be more degradable in 2/2 studies of recombinant hyaluronidase, 5/5 studies of ovine hyaluronidase, and 2/3 studies of bovine hyaluronidase. While this might superficially suggest a relatively poor response to bovine hyaluronidase, this single anomaly (Cavallini *et al.*<sup>[14]</sup>) should be considered in the context outlined above. The slight predilection of Restylane for ovine hyaluronidase over recombinant hyaluronidase reported by Shumate *et al.*<sup>[16]</sup> is not clearly replicated across all studies considered together, though the small number of studies examining recombinant hyaluronidase limit the conclusions that may be drawn.

#### Juvéderm

Juvéderm varieties were also studied in all ten reported experiments and were observed to be the least responsive to hyaluronidase in five and the most responsive to hyaluronidase in one. When compared directly to Belotero, Juvéderm was found to be more resistant to degradation 40% of the time. When compared directly to Restylane, Juvéderm was found to be more resistant to degradation 90% of the time.

Overall, the Juvéderm family exhibits many of the qualities described to be associated with resistance to degradation by hyaluronidase: it is a monophasic compound and its formulations generally have higher concentrations of HA and more crosslinking than other HA fillers<sup>[22]</sup>. It is not unexpected then that it resists degradation more effectively than Restylane in a wide variety of experiments, including both *in vivo* and *in vitro* designs and with all three different sources of hyaluronidase.

In the single study in which a Juvéderm filler was observed to exhibit the most degradation of its studied subset<sup>[14]</sup>, the specific variety was Juvéderm Volite (12 mg/mL), a formulation with significantly lower HA concentration than the majority of the Juvéderm family of fillers. Juvéderm Voluma (20 mg/mL) demonstrated significantly more resistance to degradation in this experiment. No other studies examined the Volite member of the Juvéderm family.

When studies looking for differences in Juvéderm's response to different varieties of hyaluronidase are compared, no variety of hyaluronidase is clearly better at dissolving Juvéderm than others. In the recombinant hyaluronidase experiments where direct comparison is possible, Juvéderm fillers are universally more resistant than Restylane (2/2) and less resistant than Belotero (1/1). In ovine experiments, Juvéderm fillers are more resistant than Restylane (5/5) and again, generally less resistant than Belotero (2/3). In bovine experiments, Juvéderm fillers are generally more resistant to dissolution than Restylane (2/3) and, interestingly, more resistant than Belotero (1/1). Though this last result may appear to suggest that bovine hyaluronidase is less effective against Juvéderm (relative to Belotero), it is also notable that the one instance in which Juvéderm dissolved more than Restylane also occurred with bovine hyaluronidase, which would suggest the opposite.

When comparing studies that examined both Juvéderm and Belotero, the single *in vitro* study in which Juvéderm was less resistant utilized bovine hyaluronidase to compare the fillers<sup>[17]</sup>. However, no effective degradation at all was seen in Juvéderm Ultra 3 in this experiment, despite degradation of this formulation being observed with bovine hyaluronidase in other experiments<sup>[12]</sup>. This discrepancy makes it difficult to draw any specific conclusions about Juvéderm's response to bovine hyaluronidase, and no additional data from other studies is available as a point of comparison.

#### *Belotero balance*

Belotero balance was studied in 5 experiments and was observed to be the least responsive to hyaluronidase in 3 and the most responsive to hyaluronidase in 2. In 3 out of 4 *in vitro* experiments<sup>[6,15]</sup>, including 2 with ovine hyaluronidase and 1 with recombinant hyaluronidase, Belotero was observed to be the most resistant to degradation, likely in part due to its monophasic status, more extensive CPM crosslinking, and higher molecular weight than other common fillers like Juvéderm Ultra and Restylane<sup>[15]</sup>.

In an experiment on human subjects<sup>[18]</sup>, with ovine hyaluronidase, Belotero was observed to be the least resistant to degradation as compared to Restylane and Juvéderm Ultra Plus. This discrepancy might be attributable to factors introduced by the *in vivo* system or the experimental design of the study, in which HA degradation was measured clinically via a standardized palpation scale following hyaluronidase injection. Belotero, with its unique polydensified matrix structure, has a lower viscosity than other HA fillers and thus a greater homogenous intradermal distribution of injection<sup>[31]</sup>. Per the authors' own acknowledgement, these properties may have confounded measurements of Belotero relative to its more viscous counterparts.

Interestingly, the *in vitro* experiment in which Belotero was found to be the least resistant to degradation (Buhren et al.<sup>[17]</sup>) was the only experiment that studied Belotero (22.5 mg/mL) with bovine hyaluronidase. In this experiment of Belotero, Emervel (Restylane, 20 mg/mL) and Juvéderm Ultra 3 (24 mg/mL),



50 microliters of filler was combined with a fluorescent linker dye and exposed to 10 units (U/ml) of hyaluronidase, with degradation measured by recorded changes in fluorescence intensity. This can be contrasted with the work of Flynn *et al.*<sup>[15]</sup>, who used a different experimental design (chromatography) but the same ratio of hyaluronidase to filler (16 U per 0.08 mg) to show Belotero was more resistant to ovine hyaluronidase than Juvéderm Ultra 3. When compared directly, these studies may suggest that Belotero is more susceptible than Juvéderm to ovine hyaluronidase than bovine hyaluronidase.

However, Bühren *et al.*<sup>[17]</sup> notably observed no effective degradation of Juvéderm Ultra 3 by bovine hyaluronidase, even after 24 h. Sall *et al.*<sup>[12]</sup> did observe degradation of Juvéderm Ultra 3 at a lower concentration of bovine hyaluronidase than Burhen *et al.* Though Sall *et al.* did not study Belotero, this difference in results with the same Juvéderm variety using the same enzyme suggests that the Burhen experiment may have had some confounding feature either in measurement methodology or other experimental factors such as pH or temperature. More studies are needed to clarify whether Belotero is less responsive to bovine hyaluronidase than ovine hyaluronidase.

## CONCLUSION

Overall, the literature suggests that different varieties of hyaluronidase and HA fillers vary in their interactions. Factors associated with higher resistance to degradation include increased concentration of HA, increased crosslinking, and monophasic formulation. Unsurprisingly, filler varieties that are monophasic, highly concentrated, and exhibit a high density of crosslinking (such as Juvéderm Ultra Plus) are less responsive to hyaluronidase and require a larger quantity of enzyme to achieve complete dissolution.

Clinically, these differences should be considered when following established guidelines for the quantity of hyaluronidase required for filler dissolution. This amount needed depends on the nature of the complication. In cases of suboptimal injection placement or overcorrection, 5-10 U of hyaluronidase has been cited as the appropriate dose<sup>[36]</sup>. This recommendation is not specific to the volume of filler, which must be considered. Additionally, this recommendation established using Restylane, may not be appropriate for other fillers. In the treatment of ischemic complications as opposed to misplacement, higher doses of hyaluronidase are required, with 5 units suggested per 0.1 mL of Restylane and 10 units per 0.1 mL of Juvéderm<sup>[34]</sup>. Thus, for any given 1 mL of filler injected, the suggested starting dose of hyaluronidase varies from 5 to 100 U depending on the type and volume of filler injected and the nature of the complication. The general principles of degradation susceptibility outlined above in conjunction with clinical context and volume of filler injected should provide injectors with an estimated starting reversal dose.

In addition to presenting the accepted knowledge to date, this review highlights the information that remains uncertain and in need of further investigation. While members of the Restylane family appear to respond best to all varieties of hyaluronidase and members of the Juvéderm family appear to be the most resistant, experimental results are less consistent for Belotero Balance. There are no clear trends established across multiple studies to support the claim that any one type of hyaluronidase is better at dissolving a specific variety of HA filler.

More research is needed to substantiate these results and explore individual efficacy. Such experiments might include an *in vitro* study that examines all major HA fillers and all varieties of hyaluronidase under uniform volumetric, temperature, and time exposure conditions. Once a preliminary set of individual relationships between filler types and hyaluronidases is established, further studies in animals and human subjects can determine whether these trends remain valid *in vivo*. In definitively establishing these relationships, clarification of this issue will prepare injector clinicians to better address filler complications that arise from the use of a given HA product.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to the conception and design of the study; performed literature review, background research, and data analysis; drafted initial version of the work and contributed to revisions; drafted initial version of Table 1; edited and augmented Table 2; provided administrative support: Paap MK Made substantial contributions to the conception and design of the study; reviewed and substantially revised initial and subsequent drafts of manuscript; reviewed and analyzed relevant literature; contributed additional background research; drafted initial version of Table 2; edited and augmented Table 1: Silkiss RZ

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

Both authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

- Farolch-Prats L, Nome-Chamorro C. Facial contouring by using dermal fillers and botulinum toxin A: a practical approach. *Aesthetic Plast Surg* 2019;43:793-802.
- Jones D, Tezel A, Borrell M. In vitro resistance to degradation of hyaluronic acid dermal fillers by ovine testicular hyaluronidase. *Dermatol Surg* 2010;36:804-9.
- Urdiales-Galvez F, Delgado NE, Figueiredo V, Lajo-Plaza JV, Mira M, et al. Treatment of soft tissue filler complications: expert consensus recommendations. *Aesthetic Plast Surg* 2018;42:498-510.
- Cavallini M, Gazzola R, Metalla M, Vaienti L. The role of hyaluronidase in the treatment of complications from hyaluronic acid dermal fillers. *Aesthet Surg J* 2013;33:1167-74.
- Hylenex [Package insert]. San Diego, CA: Halozyne Therapeutics, Inc; 2012.
- Rao V, Chi S, Woodward J. Reversing facial fillers: interactions between hyaluronidase and commercially available hyaluronic-acid based fillers. *J Drugs Dermatol* 2014;13:1053-6.
- Lambros V. The use of hyaluronidase to reverse the effects of hyaluronic acid filler. *Plast Reconstr Surg* 2004;114:227.
- Surek CC, Said SA, Perry JD, Zins JE. Retrobulbar injection for hyaluronic acid gel filler-induced blindness: a review of efficacy and technique. *Aesthetic Plast Surg* 2019;43:1034-40.
- Hirsch RJ, Cohen JL, Carruthers JDA. Successful management of an unusual presentation of impending necrosis following a hyaluronic acid injection embolus and a proposed algorithm for management of hyaluronidase. *Dermatol Surg* 2007;33:357-60.
- Paap MK, Milman T, Ugradar S, Goldberg R, Silkiss RZ. Examining the role of retrobulbar hyaluronidase in reversing filler induced blindness: a systemic review. *Ophthalmic Plast Reconstr Surg* 2019;2020;36:231-8.
- Paap MK, Milman T, Ugradar S, Silkiss RZ. Assessing retrobulbar hyaluronidase as a treatment for filler-induced blindness in a cadaver model. *Plast Reconstr Surg* 2019;144:315-20.
- Sall I, Ferard G. Comparison of the sensitivity of 11 crosslinked hyaluronic acid gels to bovine testis hyaluronidase. *Polymer Degradation and Stability* 2007;92:915-9.
- Gold MH. Use of hyaluronic acid fillers for the treatment of the aging face. *Clinical Interventions in Aging* 2007;2:369-76.
- Cavallini M, Papagni M, Trocchi G. Sensitivity of hyaluronic acid fillers to hyaluronidase: an in vitro analysis. *J Clin Exp Dermatol Res* 2020;11:517.

15. Flynn TC, Thompson DH, Hyun SH. Molecular weight, analyses and enzymatic degradation profiles of the soft-tissue fillers Belotero Balance, Restylane, and Juvederm Ultra. *Plast Reconstr Surg* 2013;132:22S-32.
16. Shumate GT, Chopra R, Jones D, Messina DJ, Hee CK. In vivo degradation of crosslinked hyaluronic acid fillers by exogenous hyaluronidases. *Dermatol Surg* 2018;44:1075-83.
17. Buhren BA, Schruppf H, Bolke E, Kammers K, Gerber PA. Standardized in vitro analysis of the degradability of hyaluronic acid fillers by hyaluronidase. *Eur J Med Res* 2018;23:37.
18. Juhasz MLW, Levin MK, Marmur ES. The kinetics of reversible hyaluronic acid filler injection treated with hyaluronidase. *Dermatol Surg* 2017;43:841-7.
19. Dunn AL, Heavner JE, Racz G, Day M. Hyaluronidase: a review of approved formulations, indications and off-label use in chronic pain management. *Expert Opin Biol Ther* 2010;10:127-31.
20. Weber GC, Buhren BA, Schruppf H, Wohlrab J, Gerber PA. Clinical applications of hyaluronidase. In: Labrou N, editor. *Therapeutic enzymes: function and clinical implications*. New York: Springer; 2019. pp. 255-78.
21. Food and Drug Administration. Dermal Fillers Approved by the Center for Devices and Radiological Health. Available from: <https://www.fda.gov/medical-devices/cosmetic-devices/dermal-fillers-approved-center-devices-and-radiological-health> [Last accessed on 6 Jul 2020]
22. Gold MH. What's new in fillers in 2010? *J Clin Aesthet Dermatol* 2010;3:36-45.
23. Agerup B, Berg P, Akermarck C. Non-animal stabilized hyaluronic acid: a new formulation for the treatment of osteoarthritis. *BioDrugs* 2005;19:23-30.
24. Mansouri Y, Goldenberg G. Update on hyaluronic acid fillers for facial rejuvenation. *Cutis* 2015;96:85-8.
25. Philipp-Dormston WG, Wong C, Schuster B, Larsson MK, Podda M. Evaluating perceived naturalness of facial expression after fillers to the nasolabial folds and lower face with standardized video and photography. *Dermatol Surg* 2018;44:826-32.
26. U.S. National Library of Medicine. Study to Evaluate Satisfaction After Treatment with Kysse. Available from: <https://clinicaltrials.gov/ct2/show/NCT03967444> [Last accessed on 24 Apr 2020]
27. Allemann IB, Baumann L. Hyaluronic acid gel (Juvéderm) preparations in the treatment of facial wrinkles and folds. *Clin Interv Aging* 2008;3:629-34.
28. Monheit G, Beer K, Hardas B, Grimes PE, Weichman BM, et al. Safety and effectiveness of the hyaluronic acid dermal filler VYC-17.5L for nasolabial folds: results of a randomized, controlled study. *Dermatol Surg* 2018;44:670-8.
29. Devgan L. Abstract: Juvéderm Volbella for use in periorbital volumization. *Plast Reconstr Surg Glob Open* 2017;5:192-3.
30. Niforos F, Ogilvie P, Cavallini M, Leys C, Chantrey J, et al. VYC-12 injectable gel is safe and effective for improvement of facial skin topography: a prospective study. *Clin Cosmet Investig Dermatol* 2019;12:791-8.
31. Prasetyo AD, Prager W, Rubin MG, Moretti EA, Nikolis A. Hyaluronic acid fillers with cohesive polydensified matrix for soft-tissue augmentation and rejuvenation: a literature review. *Clin Cosmet Investig Dermatol* 2016;9:257-80.
32. Palm MD. Filler frontier: what's new and heading West to the US market. *Semin Cutan Med Surg* 2014;33:157-63.
33. Lee A, Grummer SE, Kriegel D, Marmur E. Hyaluronidase. *Dermatol Surg* 2010;36:1071-7.
34. Landau M. Hyaluronidase caveats in treating filler complications. *Dermatol Surg* 2015;41:S347-53.
35. Delorenzi C. Complications of injectable fillers, part I. *Aesthet Surg J* 2013;33:561-75.
36. Vartanian AJ, Frankel AS, Rubin MG. Injected hyaluronidase reduces Restylane-mediated cutaneous augmentation. *Arch Facial Plast Surg* 2005;7:231-7.

Original Article

Open Access



# Impact of different surgical protocols on dental development in oro-facial cleft children

Rosa Guagnano<sup>1</sup>, Federica Romano<sup>2</sup>, Ernesto Pepe<sup>3</sup>, Patrizia Defabianis<sup>1</sup>

<sup>1</sup>Department of Surgical Sciences C.I.R. Dental School - Section of Paediatric Dentistry, University of Turin, Turin 10126, Italy.

<sup>2</sup>Department of Sciences C.I.R. Dental School - Section of Periodontology, University of Turin, Turin 10126, Italy.

<sup>3</sup>Pediatric Plastic Surgery Division, City of Health and Science, Regina Margherita Children Hospital, Turin 10126, Italy.

**Correspondence to:** Prof. Patrizia Defabianis, Department of Surgical Sciences C.I.R. Dental School - Section of Paediatric Dentistry, University of Turin, Via Nizza 230, Turin 10126, Italy. E-mail: patrizia.defabianis@unito.it

**How to cite this article:** Guagnano R, Romano F, Pepe E, Defabianis P. Impact of different surgical protocols on dental development in oro-facial cleft children. *Plast Aesthet Res* 2020;7:37. <http://dx.doi.org/10.20517/2347-9264.2020.21>

**Received:** 13 Mar 2020 **First Decision:** 11 May 2020 **Revised:** 8 Jun 2020 **Accepted:** 25 Jun 2020 **Published:** 19 Jul 2020

**Academic Editors:** Carroll Ann Trotman, Xu Qian **Copy Editor:** Cai-Hong Wang **Production Editor:** Tian Zhang

## Abstract

**Aim:** To determine the association between dental anomalies and type of facial cleft, gender, ethnicity and timing of hard palate repair surgery.

**Methods:** This observational study comprised a total of 85 non-syndromic cleft children (mean age  $9.7 \pm 3.2$  years) of different ethnicity (68 Caucasians, 7 Asians, 4 Africans, 5 Hispanics and 1 Indian). Sixty-four patients were affected by lip palate cleft, 11 by lip alveolus cleft and 10 by palate cleft. Sixty-one children underwent delayed palate repair at 4.3 years of age, while 21 underwent early palate periosteoplasty at 7.2 months of age. Patients were examined clinically and radiologically to assess dental anomalies. Dental cavities were registered using dmft/DMFT indexes in primary and permanent dentition, while enamel defects were evaluated only in permanent teeth using Aine index.

**Results:** Tooth rotation and agenesis were the most common tooth anomalies affecting 59% and 42.2% of cleft patients, respectively. While a late closure of the cleft palate was associated with a higher number of rotations ( $P = 0.03$ ), an early surgical correction was associated to a higher frequency of tooth agenesis ( $P = 0.02$ ), number of carious lesions in primary dentition ( $P = 0.002$ ) and more severe enamel defects in permanent teeth ( $P < 0.01$ ). A late palate repair increased 3.5 times the likelihood of having at least one rotated tooth ( $P = 0.034$ ), while decreased the odds of having agenesis by 70% ( $P = 0.029$ ) compared to an early surgical repair.

**Conclusion:** Early surgical approaches seem to have more detrimental effects on dental development in both primary and permanent dentition than late surgical protocols. Dental abnormalities in cleft patients have complex etiology combining genetic and external factors and their prevalence can also depend on timing of hard palate surgery.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Keywords:** Caries, cleft lip/palate, dental anomalies, hypoplasia, surgery

## INTRODUCTION

Oro-facial clefts are a heterogeneous group of congenital malformations that recognise similar anomalies in development and fusion of facial processes during embryogenesis, which take place during the tenth week of pregnancy and involve soft and/or hard tissues of the skull.

Typical forms can be categorised into cleft lip with an incidence of 0.29 per 1000 births; cleft palate (CP), with an incidence of 0.31 per 1000 births; and cleft lip and palate (CLP) with an incidence of 0.48 per 1000 births<sup>[1]</sup>. Rare, atypical forms show an occurrence of 1 in over 100,000 births<sup>[1]</sup>. In Italy, about 1 in 800 children are born with a facial cleft.

The aetiology is multifactorial and the causes of facial clefts are unknown but are thought to be caused by a combination of both genetic and environmental factors<sup>[2]</sup>. Lip palate cleft forms because of the maxillary and medial nasal processes fusion failure, disturbance in mesenchyme cells penetration between facial processes or vascular disruption<sup>[3]</sup>. In addition, the size of the facial processes - closely related to the ethnicity - can influence the facial morphology, increasing the susceptibility to develop a cleft: in Asian patients, for example, a smaller and flatter median nasal process, with a smaller third middle of the face and a more frequent trend to develop a skeletal third class, can result in a higher incidence of cleft (2/1000 new-borns) in comparison to Caucasians (1/1000 new-borns) and Afro-Americans (0.4/1000 new-borns)<sup>[4]</sup>.

Previous studies have reported a higher prevalence of dental abnormalities in the permanent dentition of cleft children than in the general population<sup>[5]</sup>. Tooth agenesis, supernumeraries and morphologic irregularities of the crown have been significantly associated with cleft size and severity<sup>[6]</sup>. Embriologically, the formation of tooth germs and the occurrence of cleft defects have a close relationship in terms of timing, thus factors leading to cleft could also affect the dental development<sup>[7,8]</sup>. In this context, recent studies have confirmed that genetic mutations (Interferon Regulatory Factor 6, Msh homeobox 1, Paired Box gene 9 and Transforming Growth Factor-beta) related to oral cleft lead to selective hypodontia and that Amelogenin X isoform gene, involved in the formation of the dental enamel, could also be involved in the development of clefts, suggesting a genetic association between dental anomalies and clefts<sup>[9,10]</sup>.

The literature regarding dental anomalies in cleft patients is heterogeneous, with dental anomalies rates varying more than two-fold. This discrepancy may be partially explained by patient selection criteria but may also suggest a role of external factors in enhancing the risk of tooth anomalies in cleft patients. While the impact of surgical cleft repair protocols on craniofacial growth has been widely investigated<sup>[11-13]</sup>, few studies have focused<sup>[14,15]</sup> on their impact on tooth development. Primary cleft lip repair is usually performed at the age of 3-6 months. Palatal clefts closure is performed using two main techniques depending on surgical timing. The first technique is a two-stage protocol that consists of two separate operations: the first serves to close soft palate and is performed in the first/second year of age and the second serves to close the hard palate and is usually done before the pre-school age. The second technique, called periosteal plastic of the palate, is a one-stage palate repair, closing the soft and the hard palate at the same time before 12 months of age. Bone transplantation can be planned from infancy to adulthood to close the alveolar cleft.

We hypothesise that an early periosteal plastic surgery of the palate could affect dental development by reducing blood supply to developing tooth germs, thus causing more tooth agenesis and enamel defects compared to a delayed plastic surgery of the palate, performed later when the tooth germs are already formed.



The aims of the present study were to assess the association between timing of hard palate surgery and dental anomalies in a sample of Italian cleft children and to investigate the relationship of dental abnormalities with gender, ethnicity and cleft type.

## METHODS

All patients included in the present study were affected by non-syndromic facial cleft and were consecutively selected among outpatients referred for dental examination to the Section of Paediatric Dentistry, C.I.R. Dental School, University of Turin from April to July 2019. Patients were excluded if they were affected by lip or soft palate cleft, suffered from any disease associated with increased risk for dental anomalies or underwent previous extractions or fixed orthodontic treatment so that all dental surfaces could be accessible to the clinical examination and tooth agenesis as well as structural dental anomalies could not be considered iatrogenic.

The protocol of the present study was approved by the local Ethics Committee (No. 0038526), and written, informed consent was obtained from each patient or their parent or guardian. The investigation was performed according to the ethical principles of the Helsinki declaration.

Enrolled patients were classified into two groups depending on the timing the different surgical protocols were carried out. All patients who received one-stage periosteal plastic of the hard palate together with lip and soft palate repair were classified into the early periosteal palate plastic surgery group (EPP). This technique includes the treatment of maxillary defects and the closure of the lip at the age of 2-6 months. Cleft lip was repaired using a modified Tennison-Randall technique or a modified Mulliken technique. Cleft palate was repaired using Bardach technique or Von Langenbeck technique.

The patients included in the delayed palate repair surgery group (DPR) underwent first an infant orthopaedic treatment by the use of a Hotz neonatal plate followed at the age of 3-6 months by a lip repair procedure according to modified Millard or Noordhoff techniques. Soft palate repair was done at 8-10 months of age according to Widmaier-Perko technique, combined with V-Y repositioning of the soft palate without touching either the palatal artery or the palatal periosteum. Hard palate repair was performed at 4 years of age according to Schweckendiek technique with a mucoperiosteal flap. This is the current surgical treatment protocol to which cleft palate children treated at the Plastic Surgery Division of the Regina Margherita Children Hospital of Turin are submitted.

Data on age, gender, ethnicity, concomitant systemic pathologies, type and side of cleft and type and time of surgical corrections were collected from questionnaire and medical records. A specialist in paediatric dentistry evaluated the dental conditions of cleft children. Diagnosis of carious lesions was based on the criteria established by the World Health Organisation<sup>[16]</sup>. Each patient was given a score resulting from the sum of the decayed, missing and filled teeth either in primary (date index) or in permanent dentition (DMFT index). Patients with mixed dentition had two separate scores.

Disturbances of enamel mineralisation were examined on permanent teeth and recorded using the Aine rating scale where Grade I defines qualitative defects (opacities and discolorations), while Grades II, III and IV represent quantitative defects (hypoplasia) of increasing severity<sup>[17]</sup>.

Intraoral examination and panoramic radiographs were used to determine the following dental anomalies: number of impacted, missing, supernumerary or microdontic teeth, abnormalities in crown shape and ectopic eruption of permanent molars. Dental anomalies (fusion of deciduous teeth) were assessed only by intraoral examination for patients younger than 6 years of age.

## Statistical analysis

Data were recorded in a Microsoft Excel file and analysed using the Statistical Package for the Social Sciences (SPSS), version 24.0 (SPSS, Inc., Chicago, IL, USA).

Values of quantitative variables are presented as the mean  $\pm$  standard deviation, while values of categorical variables are presented as frequencies and percentages.

Data were first examined for normality by the Shapiro-Wilk test and if the data did not achieve normality, analyses were performed using non-parametric methods. The  $\chi^2$  test was used to evaluate any potential association between categorical variables and the one-way analysis of variance or the Kruskal-Wallis test were used to assess differences of quantitative variables (Aine, Decayed Missing Filled Teeth index and decayed missing filled teeth index) between gender, ethnicity, cleft types and timings of palate surgery, as appropriate. When there were significant differences, pairwise multiple comparisons were carried out using the Scheffé test or the Dunn test. Logistic regression models were used to analyse the associations between surgical timings and dental anomalies. Estimates are shown as odds ratio (OR) and relative 95% confidence intervals (CIs) adjusted for gender, age, ethnicity and type of cleft.

All tests were two-tailed and *p* values less than 0.05 were considered statistically significant.

## RESULTS

In total, 85 cleft subjects (51 male and 34 female) aged from 3 to 18 years (mean age  $9.7 \pm 3.2$  years) were enrolled in the study, with 68 Caucasians (80%), 7 Asians (8.2%), 5 Hispanics (5.9%), 4 Africans (4.7%) and 1 Indian (1.2%). All but 4 patients were systemically healthy, 1 suffered from heart disease, 1 referred a transient ischemic attack at birth, 1 referred hyposmia due to pituitary gland dysfunction and 1 was affected by a rare Tressier number 7 cleft and presented with bilateral cleft lip, right unilateral alveolar cleft and cleft of the upper maxillary molar region on the right side with macrostomia.

Sixty-four patients were affected by CLP: 29 on the left side (L-UCLP), 20 on the right side (R-UCLP) and 15 bilaterally (BCLP). Ten patients presented with CP and eleven with CLA, with 8 cases on the left side, 1 case on the right side and 1 case bilaterally. The left side was significantly more often affected than the right ( $P < 0.01$ ). There was a statistical association between the type of cleft and gender with CLP more common in males (67.2%) and CP in females (70%) ( $P = 0.047$ ).

Sixty-one cleft patients (mean age  $10.3 \pm 3.3$  years), belonging to the DPR group, had been treated at the Plastic Surgery Division of the Regina Margherita Children Hospital of Turin between January 2002 and December 2017. They underwent lip closure at a mean age of  $6.1 \pm 2.3$  months, in combination with the use of a neonatal palatal plate, soft palate closure at a mean age of  $12.7 \pm 4.3$  months, hard palate closure at  $4.4 \pm 1.5$  years and bone grafting at a mean age of  $12.0 \pm 1.6$  years.

Twenty-one patients (mean age  $7.8 \pm 2.3$  years) in the EPP group were referred from other cleft centres in Italy: they all had been treated with a one-stage periosteal palatoplasty at a mean age of  $7.2 \pm 6.5$  months between January 2006 and December 2015, without using the palatal plate. No information about cleft surgery was available for three patients.

Dental anomalies were assessed clinically and radiologically in 83 patients. The remaining two children were younger than 6 years of age and were submitted only to intraoral examination for assessing the presence of fused teeth. The frequencies of tooth anomalies by gender, ethnicity, cleft type and surgical protocol among subjects aged 6-18 years are summarised in [Table 1](#), while the distribution by tooth type is described in [Table 2](#).

**Table 1. Frequencies of tooth anomalies by gender, ethnicity, types of cleft and surgical protocols in cleft subjects aged 6-18 years**

Variables	Rotations (n, %)	Agenesis (n, %)	Supernumerary (n, %)	Shape anomalies (n, %)	Impaction (n, %)	Ectopic eruption (n, %)
Gender						
Male (n = 50)	30 (60%)	20 (40%)	17 (34%)	8 (16%)	4 (8%)	2 (4%)
Female (n = 33)	19 (57.6%)	15 (45.5%)	8 (24.2%)	9 (27.3%)	3 (9.1%)	4 (12.1%)
Ethnicity						
Caucasian (n = 66)	43 (66.7%)*	26 (39.4%)	18 (27.3%)	14 (21.2%)	5 (7.6%)	5 (7.6%)
Asian (n = 7)	3 (42.9%)	5 (71.4%)**	2 (28.6%)	1 (14.3%)	0	1 (14.3%)
Hispanic (n = 5)	2 (40%)	2 (40%)	2 (40%)	1 (20%)	1 (20%)	0
Indian (n = 1)	1 (100%)	0	0	0	0	0
African (n = 4)	0	2 (50%)	3 (75%)*	1 (25%)	1 (25%)	0
Cleft type						
CLP (n = 62)	41 (67.7%)	28 (45.2%)	20 (32.3%)	13 (12%)	6 (9.7%)	5 (8.1%)
L-UCLP (n = 28)	25 (89.3%)**	11 (39.3%)	6 (21.4%)	3 (10.7%)	4 (14.3%)	3 (10.7%)
R-UCLP (n = 20)	10 (50%)	7 (35%)	5 (25%)	4 (20%)	1 (5%)	2 (10%)
BCLP (n = 14)	6 (42.9%)	10 (71.4%)*	9 (64.3%)*	6 (42.9%)**	1 (7.1%)	0
CP (n = 10)	0	3 (30%)	0	0	0	1 (9.1%)
CLA (n = 11)	8 (72.7%)*	4 (36.4%)	5 (45.5%)*	4 (36.4%)**	1 (9.1%)	
Surgical protocol						
EPP (n = 20)	8 (40%)	13 (65%)*	5 (25%)	3 (15%)	2 (10%)	1 (5%)
DPR (n = 60)	40 (66.7%)*	21 (35%)	20 (33.3%)	14 (23.3%)	4 (6.7%)	5 (8.3%)

Values with superscript asterisks show statistically significant difference between groups: \* $P < 0.05$ ; \*\* $P < 0.01$ . CLP: lip palate cleft; L-UCLP: left unilateral lip palate cleft; R-UCLP: right unilateral lip palate cleft; BCLP: bilateral lip palate cleft; CP: palate cleft; CLA: lip alveolar cleft; EPP: early palate periosteal plastic surgery; DPR: delayed palate repair surgery

**Table 2. Frequencies of tooth anomalies and enamel defects by tooth type in cleft subjects aged 6-18 years**

Tooth type	Rotation (n, %)	Agenesis (n, %)	Supernumerary (n, %)	Shape anomaly (n, %)	Impaction (n, %)	Ectopic eruption (n, %)	Enamel Hypoplasia		
							Aine 1 (n, %)	Aine 2 (n, %)	Aine 3 (n, %)
Upper Central incisors	53 (86.8%)	4 (6%)	2 (6.4%)	2 (9.5%)	4 (44.4%)	0	14 (50%)	17 (68%)	4 (80%)
Upper Lateral Incisors	8 (13.1%)	38 (57.5%)	24 (77.4%)	19 (90.4%)	1 (11.1%)	0	4 (14.2%)	3 (12%)	1 (20%)
Upper Canines	0	0	1 (3.2%)	0	2 (9.5%)	0	2 (7.1%)	0	0
Upper premolars	0	12 (18.1%)	0	0	1 (11.1%)	0	0	2 (8%)	0
Upper molars	0	0	2 (6.4%)	0	0	7 (100%)	1 (3.5%)	0	0
Lower central incisors	0	0	1 (3.2%)	0	0	0	3 (10.7%)	0	0
Lower lateral incisors	0	4 (6%)	1 (3.2%)	0	0	0	1 (3.5%)	0	0
Lower canines	0	0	0	0	0	0	0	0	0
Lower premolars	0	6	0	0	0	0	0	1 (4%)	0
Lower molars	0	2 (3%)	1 (3.2%)	0	1 (11.1%)	0	3 (10.7%)	2 (8%)	0
Total	61	66	31	21	9	7	28	25	5

No significant gender difference in the prevalence of tooth anomalies was observed, while ethnicity, cleft type and surgical timing were statistically significantly related to their frequency. Rotation was the most common development anomaly of dentition (59%), affecting one tooth in 37 patients (44.6%) and two teeth in 12 patients (14.5%). Caucasians and DPR patients exhibited tooth rotations more often than other racial groups as well as more often than EPP patients (both  $P = 0.03$ ). Rotations were also more frequent in L-UCLP and CLA, while they were absent in CP patients ( $P < 0.01$ ). The upper central left permanent incisor was the most frequently affected tooth (50.8%).

Agenesis affected one tooth in 19 subjects (22.9%), two/three teeth in 14 (16.9%) subjects and four/five teeth in 2 subjects (2.4%). It was more frequent in Asians ( $P < 0.01$ ) and BCLP subjects ( $P = 0.014$ ) and those submitted to EPP ( $P = 0.02$ ). Upper lateral incisors were the teeth more commonly involved in this anomaly.

One or two supernumerary teeth were found in 25 patients (30.1%), involving more often the upper lateral incisors. Their frequency was higher in Africans ( $P = 0.04$ ), CLA (45.5%) and CLP (32.3%) subjects, in particular those with R-UCLP ( $P = 0.01$ ).

**Table 3. Enamel defects on permanent teeth (Aine index) by gender, ethnicity types of cleft, and surgical protocols (mean  $\pm$  SD)**

Variables	Aine			
	0	1	2	3
Gender				
Male ( $n = 42$ )	14.0 $\pm$ 7.0	0.4 $\pm$ 1.1	0.1 $\pm$ 0.4	0.1 $\pm$ 0.2
Female ( $n = 23$ )	13.3 $\pm$ 8.5	1.1 $\pm$ 2.8	0.5 $\pm$ 0.9	0.1 $\pm$ 0.4
Ethnicity				
Caucasian ( $n = 53$ )	14.7 $\pm$ 7.8*	0.7 $\pm$ 1.7	0.3 $\pm$ 0.6	0.1 $\pm$ 0.2
Asian ( $n = 5$ )	4.2 $\pm$ 1.8	0.4 $\pm$ 0.9	0.2 $\pm$ 0.5	0.0 $\pm$ 0.0
Hispanic ( $n = 3$ )	13.0 $\pm$ 7.8	0.0 $\pm$ 0.0	0.3 $\pm$ 0.6	1.0 $\pm$ 0.9
Indian ( $n = 1$ )	10.0	0.0	0.0	0.0
African ( $n = 3$ )	10.3 $\pm$ 10.1	5.0 $\pm$ 8.7	2.3 $\pm$ 2.1	0.0 $\pm$ 0.0
Cleft type				
CLP ( $n = 50$ )	14.0 $\pm$ 8.3	1.0 $\pm$ 0.7*	0.4 $\pm$ 0.8	0.1 $\pm$ 0.4
CP ( $n = 5$ )	9.6 $\pm$ 1.8	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0
CLA ( $n = 10$ )	13.3 $\pm$ 8.2	0.2 $\pm$ 0.4	0.4 $\pm$ 0.7	0.1 $\pm$ 0.3
Surgical protocol				
EPP ( $n = 16$ )	15.3 $\pm$ 7.9**	1.0 $\pm$ 2.7	0.5 $\pm$ 0.9**	0.0 $\pm$ 0.2
DPR ( $n = 49$ )	8.1 $\pm$ 5.8	0.2 $\pm$ 0.6	0.1 $\pm$ 0.4	0.2 $\pm$ 0.6

Values with superscript asterisks show statistically significant difference between groups: \* $P < 0.05$ ; CLP: lip palate cleft; CP: palate cleft; CLA: lip alveolar cleft; EPP: early palate periosteal plastic; DPR: delayed palate plastic

Tooth impaction was observed in seven subjects (8.4%), involving one tooth in five subjects (6%) and two teeth in two subjects (2.4%). The upper central incisors (3.7%) and canines (1.8%) were the most frequently impacted teeth. A minority of subjects (7.2%) presented with ectopic eruption of permanent molars, with two teeth erupted ectopically in only one case.

Morphological abnormalities of dental crowns were detected in 17 subjects (20.5%) affecting one (15.6%) or two teeth (4.8%) and were observed more frequently in B-CLP subjects (42.9%) ( $P = 0.001$ ). Twelve patients (14.5%) exhibited microdontic upper lateral incisors and five patients (6.0%) the fusion of two deciduous teeth (lower lateral incisor with canine 4.7% and upper central incisors together 1.2%). No primary fused teeth were found in children younger than 6 years of age.

Logistic analysis adjusted for gender, age, ethnicity and cleft type showed a significant association between timing of palate surgery, tooth rotation and agenesis. DPR children exhibited a 3.5-fold higher likelihood of having at least one rotated tooth than EPP children (OR = 3.50, 95%CI: 1.10-11.13,  $P = 0.034$ ) but lower likelihood of having at least one agenesis tooth (OR = 0.26, 95%CI: 0.08-0.87,  $P = 0.029$ ).

As summarised in Table 3, in total 984 permanent teeth were also examined in 65 patients for enamel defects: 13 patients (20%) showed opacities and discolorations (Aine 1), 15 (23%) mild (Aine 2) and 4 (6%) evident structural defects (Aine 3). No Aine 4 defects were found. As described in Table 3, CLP subjects showed higher mean number of enamel defects (Aine 1) compared to CLA and CP subjects ( $P = 0.012$ ). More severe enamel defects (Aine 3) were also observed in patients submitted to EPP compared to those who underwent DPR ( $P < 0.01$ ). The upper central and lateral incisors were the most frequently involved teeth [Table 1].

Table 4 summarises data on caries experience in both primary and permanent dentition. The mean dmft index was higher in EPP subjects compared to DPR subjects ( $P = 0.002$ ), while no statistically significant differences were observed among males and females, ethnic groups and cleft types. In addition, no statistically significant differences were observed for DMTF index scores.

## DISCUSSION

A recent systematic review on frequency of dental anomalies in cleft patients emphasised that data in the literature are difficult to compare because of the heterogeneity in terms of surgical cleft closure techniques,

**Table 4. Dental caries in primary (dmft) and permanent teeth (DMFT) by gender, ethnicity types of cleft, and surgical protocols in the study sample (mean  $\pm$  SD)**

Variables	dmft	DMFT
Gender		
Male ( $n = 51$ )	2.4 $\pm$ 2.9	0.9 $\pm$ 1.5
Female ( $n = 34$ )	1.8 $\pm$ 2.8	1.1 $\pm$ 2.1
Ethnicity		
Caucasian ( $n = 68$ )	1.9 $\pm$ 2.9	1.8 $\pm$ 0.4
Asian ( $n = 7$ )	3.5 $\pm$ 2.6	0.4 $\pm$ 0.5
Hispanic ( $n = 5$ )	4.0 $\pm$ 2.9	1.0 $\pm$ 1.1
Indian ( $n = 1$ )	4.0	0.0
African ( $n = 4$ )	1 $\pm$ 1.1	1 $\pm$ 1.1
Cleft type		
CLP ( $n = 64$ )	2.2 $\pm$ 1.0	1.1 $\pm$ 2.4
CP ( $n = 10$ )	2.5 $\pm$ 1.0	0.3 $\pm$ 0.9
CLA ( $n = 11$ )	1.5 $\pm$ 0.9	0.7 $\pm$ 1.0
Surgical protocol		
EPP ( $n = 21$ )	3.9 $\pm$ 3.0*	0.8 $\pm$ 1.2
DPP ( $n = 61$ )	1.5 $\pm$ 2.6	1.1 $\pm$ 1.9

Values with superscript asterisks show statistically significant difference between groups: \* $P < 0.05$ . CLP: lip palate cleft; CP: palate cleft; CLA: lip alveolar cleft; EPP: early palate periosteal plastic; DPR: delayed palate plastic

interval time between surgeries, study design and length of follow-up<sup>[6]</sup>. Moreover, little information is available on the potential effects that different surgical timings of hard palate repair, early or late, could have on dental development<sup>[14,15]</sup>. It is indeed well known that all types of surgical repair of oro-facial clefts are detrimental to maxillary growth and development of permanent teeth.

The present results suggest that surgical timing could impact only on the frequency of rotations and agenesis, while other tooth anomalies were not significantly affected. Interestingly, a late surgical repair increased 3.5 times the likelihood of having at least one rotated tooth, while decreased the odds of having agenesis by 70%.

A delayed surgical repair of the cleft palate could favour tooth rotation because of the persistence of lack of bone and space for incisor eruption<sup>[18]</sup>. This situation could even be worsened by the concomitant presence of supernumeraries in the cleft area. It should be considered that tooth rotation is the most frequently observed anomaly at the cleft side, mainly in subjects with L-UCLP, especially affecting the maxillary central incisors.

By contrast, tooth agenesis was found more commonly in children who underwent early cleft palate surgical closure. Surgical trauma during early palatal periosteal plastic and reduction of the blood supply due to tissue tension and excessive scarring associated with palatal defects, as well as absence of early orthopaedic treatment to optimise the position of maxillary fragments, have been suggested as external causes of agenesis and enamel defects in upper permanent incisors<sup>[14]</sup>.

Notably, we observed a prevalence of tooth agenesis of 42.2%, that was lower than that reported in the literature, ranging from 45% to 67.6%<sup>[19-23]</sup>. This lower frequency could be explained by the fact that most of the patients were Caucasian, an ethnicity with a low incidence of agenesis, and underwent a late surgical closure of the hard palate, at 4 years of age or older. Tooth agenesis affected more frequently BCLP subjects (71.4%), in which the alveolar defect is more severe than in other cleft types. According to the literature, upper lateral incisors were the more common missing teeth (40%), followed by upper and lower second premolars (18%), lower left incisors and lower second molars (2%)<sup>[6]</sup>.

Morphological abnormalities of dental crowns were observed in 20.5% of the patients, being the lateral incisors the most frequently affected teeth. In particular, microdontia was found in 14.5% of cleft children,



a lower percentage compared to that reported in the literature, ranging from 18% to 37%<sup>[19-24]</sup>. Conversely, only a minority of patients (6%) showed primary fused teeth, as previously observed by Suzuki *et al.*<sup>[21]</sup>. Notably, fusion of deciduous teeth in the present study was always followed by the agenesis of the permanent teeth.

Tooth impaction was observed in 8.4% of the patients and ectopic eruption of molars in 7.2% of the cases. These percentages were lower compared to data reported in the literature, which varied from 10% to 50%<sup>[19-25]</sup> and from 15% to 28%<sup>[26]</sup>, respectively.

Worth *et al.*<sup>[27]</sup> reported that the dental caries prevalence in cleft lip palate patients is higher than that observed in healthy children, in both deciduous and permanent dentition, with a pooled mean difference in dmft of 0.63 (95%CI: 0.47-0.79) and in DMFT of 0.28 (95%CI: 0.22-0.34). In the present cleft population, caries experience was two-fold higher in primary than in permanent teeth. Dental anomalies in the deciduous dentition may predispose the affected teeth to greater accumulation of bacterial plaque and consequently to dental caries<sup>[28]</sup>. Thus, counselling and follow-up are important to maintain the integrity of teeth in order to maintain the supportive bone structures that may be defective at the cleft area.

Finally, in line with previous studies<sup>[29]</sup>, enamel defects, varying from opacity and discoloration to mild and evident structural changes, were more frequently observed in upper left lateral and central permanent incisors. In agreement with Korolenkova *et al.*<sup>[14]</sup>, we observed a statistically significant association between early periosteal plastic surgery of the palate and higher number of carious lesions in deciduous teeth or severe degree of enamel defects in permanent dentition and a higher prevalence of permanent tooth agenesis. A possible explanation could be that an early surgery on hard tissues, when primary teeth are erupting and permanent teeth (particularly the incisors) are developing, can interfere with blood supply of dental buds, resulting in more enamel defects up to agenesis. Indeed, the lower percentage of damage to dental enamel reported in our study (49% vs. 87.9%<sup>[29]</sup>) could be related to the fact that most of the patients underwent late surgical repair of the hard palate, with less impact on dental development, while the most severe consequences were observed in patients treated early in life.

The limitation of the present study is the heterogeneity of enrolled patients in terms of type of cleft, ethnicity and surgical procedures for cleft repair. In addition, the study sample was a convenience sample, but it provided significant results about the association between surgical timings and dental anomalies in cleft lip palate patients, suggesting that prevalence of dental abnormalities may also depend on treatment protocol.

Further multi-centre studies with larger numbers of cleft children should be performed to investigate timing, type of surgical cleft repairs, clinical and patient-related outcomes in order to identify the most appropriate surgical approach to optimise both speech outcomes and maxillary bone development while at the same time limiting the detrimental impact on both primary and permanent dentition. While early hard palate repair improves speech production, delayed repair allows for better maxillary growth<sup>[30]</sup>. Intensive speech therapy directed at the correction of articulation errors should be implemented as soon as possible to improve overall communication.

Interdisciplinary management and proper follow-up of these patients are crucial and paediatric dentists must be conscious about the dental needs of these subjects in order to improve their quality of life.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study: Guagnano R, Defabianis P

Performed data acquisition and provided administrative, technical, and material support: Guagnano R

Performed data analysis and interpretation: Romano F

Performed cleft surgeries: Pepe E

Made substantial contributions in writing the paper: Guagnano R, Defabianis P

### **Availability of data and materials**

Not applicable.

### **Financial support and sponsorship**

None.

### **Conflicts of interest**

All authors declared that there are no conflicts of interest.

### **Ethical approval and consent to participate**

The Institutional Ethics Committee of the “AOU Città della Salute e della Scienza”, of Turin (Italy) approved the research protocol (No. 0038526). The study was performed according to the ethical principles of the Helsinki declaration. Written and informed consent was obtained from participants or their parents or legal guardians.

### **Consent for publication**

Not applicable.

### **Copyright**

© The Author(s) 2020.

## **REFERENCES**

1. Tolarova MM, Cervenka J. Classification and birth prevalence of orofacial cleft. *Am J Med Genet* 1998;75:126-37.
2. Raut JR, Simeone RM, Tinker SC, Canfield MA, Day RS, et al. Proportion of orofacial clefts attributable to recognized risk factors. *Cleft Palate Craniofac J* 2019;56:151-8.
3. Rice DP. Craniofacial anomalies: from development to molecular pathogenesis. *Curr Mol Med* 2005;5:699-722.
4. Gundlach KK, Maus C. Epidemiological studies on the frequency of clefts in Europe and world-wide. *J Craniomaxillofac Surg* 2006;34:1-2.
5. da Silva AP, Costa B, de Carvalho Carrara CF. Dental anomalies of number in the permanent dentition of patients with bilateral cleft lip: radiographic study. *Cleft Palate Craniofac J* 2008;45:473-6.
6. Tannure PN, Oliveira CA, Maia LC, Vieira AR, Granjeiro JM, et al. Prevalence of dental anomalies in nonsyndromic individuals with cleft lip and palate: a systematic review and meta-analysis. *Cleft Palate Craniofac J* 2012;49:194-200.
7. Stahl F, Grabowski R, Wigger K. Epidemiology of Hoffmeister's “genetically determined predisposition to disturbed development of the dentition” in patients with cleft lip and palate. *Cleft Palate Craniofac J* 2006;43:457-65.
8. Howe BJ, Cooper ME, Vieira AR, Weinberg SM, Resick JM, et al. Spectrum of dental phenotypes in nonsyndromic orofacial clefting. *J Dent Res* 2015;94:905-12.
9. Simioni M, Araujo TK, Monlleo IL, Maurer-Morelli CV, Gil-da-Silva-Lopes VL. Investigation of genetic factors underlying typical orofacial clefts: mutational screening and copy number variation. *J Hum Genet* 2015;60:17-25.
10. Oliveira FV, Dionísio TJ, Neves LT, Machado MA, Santos CF, et al. Amelogenin gene influence on enamel defects of cleft lip and palate patients. *Braz Oral Res* 2014;28:1-6.
11. Stoltz JF, Nicolas A. Study of amino groups of the human platelet membrane. *Acta Haematol* 1978;60:304-9.
12. Salgado KR, Wendt AR, Fernandes Fagundes NC, Maia LC, Normando D, et al. Early or delayed palatoplasty in complete unilateral cleft lip and palate patients? A systematic review of the effects on maxillary growth. *J Craniomaxillofac Surg* 2019;47:1690-8.
13. Farronato G, Kairyte L, Giannini L, Galbiati G, Maspero C. How various surgical protocols of the unilateral cleft lip and palate influence the facial growth and possible orthodontic problems? Which is the best timing of lip, palate and alveolus repair? Literature review. *Stomatologija* 2014;16:53-60.
14. Korolenkova MV, Starikova NV, Udalova NV. The role of external aetiological factors in dental anomalies in non-syndromic cleft lip and palate patients. *Eur Arch Paediatr Dent* 2019;20:105-11.
15. Korolenkova MV, Starikova NV, Ageeva LV. Risk factors for teeth aplasia and hypoplasia in cleft lip and palate children. *Stomatologija (Mosk)* 2016;95:59-62.

16. World Health Organization. (1997). Oral health surveys : basic methods, 4th ed. World Health Organization. Available from: <https://apps.who.int/iris/handle/10665/41905> [Last accessed on 16 Jul 2020]
17. Aine L, Mäki M, Collin P, Keyriläinen O. Dental enamel defects in celiac disease. *J Oral Pathol Med* 1990;19:241-5.
18. Smahel Z, Tomanová M, Müllerová Z. Position of upper permanent central incisors prior to eruption in unilateral cleft lip and palate. *Cleft Palate Craniofac J* 1996;33:219-24.
19. Tortora C, Meazzini MC, Garattini G, Brusati R. Prevalence of abnormalities in dental structure, position, and eruption pattern in a population of unilateral and bilateral cleft lip and palate patients. *Cleft Palate Craniofac J* 2008;45:154-62.
20. Germec Cakan D, Nur Yilmaz RB, Bulut FN, Aksoy A. Dental anomalies in different types of cleft lip and palate: is there any relation? *J Craniofac Surg* 2018;29:1316-21.
21. Suzuki A, Takahama Y. The maxillary lateral incisor of subject with cleftlip and/or palate (part 1). *Cleft Palate Craniofac J* 1992;29:376-9.
22. Aizenbud D, Camasuvi S, Peled M, Brin I. Congenitally missing teeth in the Israeli cleft population. *Cleft Palate Craniofac J* 2005;42:314-7.
23. Al Jamal GA, Hazza'a AM, Rawashdeh MA. Prevalence of dental anomalies in a population of cleft lip and palate patients. *Cleft Palate Craniofac J* 2010;47:413-20.
24. Suzuki A, Nakano M, Yoshizaki K, Yasunaga A, Haruyama N, et al. A longitudinal study of the presence of dental anomalies in the primary and permanent dentitions of cleft lip and/or palate patients. *Cleft Palate Craniofac J* 2017;54:309-20.
25. Hinrichs JE, El-deeb ME, Waite DE, Bevis RR, Bandt CL. Periodontal evaluation of canines erupted through grafted alveolar cleft defects. *J Oral Maxillofac Surg* 1984;42:717-21.
26. da Silva Filho OG, De Albuquerque MV, Kurol J. Ectopic eruption of maxillary first permanent molars in children with cleft lip. *Angle Orthod* 1996;66:373-80.
27. Worth V, Perry R, Ireland T, Wills AK, Sandy J, et al. Are people with an orofacial cleft at a higher risk of dental caries? A systematic review and meta-analysis. *Br Dent J* 2017;223:37-47.
28. Cheng LL, Moor SL, Ho CT. Predisposing factors to dental caries in children with cleft lip and palate: a review and strategies for early prevention. *Cleft Palate Craniofac J* 2007;44:67-72.
29. Shen CA, Guo R, Li W. Enamel defects in permanent teeth of patients with cleft lip and palate: a cross-sectional study. *J Int Med Res* 2019;47:2084-96.
30. Shaffer AD, Ford MD, Losee JE, Goldstein J, Costello BJ, et al. The association between age at palatoplasty and speech and language outcomes in children with cleft palate: an observational chart review study. *Cleft Palate Craniofac J* 2020;57:148-60.

Original Article

Open Access



# Olecranon bone grafting for the treatment of nonunion after distal finger replantation

Burak Sercan Ercin<sup>1,2</sup>, Fatih Kabakas<sup>1,2</sup>, Musa Kemal Keles<sup>1,2</sup>, Ismail Bulent Ozcelik<sup>1,3,4</sup>, Berkan Mersa<sup>1,3,4</sup>

<sup>1</sup>IST-EL Microsurgery Group, Istanbul 34245, Turkey.

<sup>2</sup>Department of Plastic Surgery and Hand Surgery, Medicalpark Gebze Hospital, Kocaeli 41400, Turkey.

<sup>3</sup>Department of Hand Surgery, Yeni Yuzyil University, Gaziosmanpasa Hospital, Istanbul 34245, Turkey.

<sup>4</sup>Nisantasi University, Istanbul 34398, Turkey.

**Correspondence to:** Dr. Burak Sercan Ercin, Department of Plastic Surgery and Hand Surgery, Medicalpark Gebze Hospital, Kocaeli 41400, Turkey. E-mail: bsercin@gmail.com

**How to cite this article:** Ercin BS, Kabakas F, Keles MK, Ozcelik IB, Mersa B. Olecranon bone grafting for the treatment of nonunion after distal finger replantation. *Plast Aesthet Res* 2020;7:38. <http://dx.doi.org/10.20517/2347-9264.2020.56>

**Received:** 29 Mar 2020 **First Decision:** 4 Jun 2020 **Revised:** 12 Jun 2020 **Accepted:** 7 Jul 2020 **Published:** 19 Jul 2020

**Academic Editor:** A Thione **Copy Editor:** Cai-Hong Wang **Production Editor:** Tian Zhang

## Abstract

**Aim:** Although not very popular, the olecranon bone graft is a useful option for this type of operation due to the minimal donor morbidity and its ease of use in small bone defect reconstruction and non-union therapy. To our best knowledge, few studies have evaluated the use of the olecranon bone graft as a treatment for non-union after distal finger replantation. Our aim in this report was to present our experience of using olecranon grafts in our nonunion patients undergoing distal replantations.

**Methods:** Between 2013 and 2019, a total of 14 patients who developed nonunion or had segmental bone defects due to the injury were included in the study. Retrospectively the results were analyzed in terms of complication and union rates.

**Results:** The mean follow-up period was 37 months (range 8-72 months). No major complications were seen in the donor region or recipient regions. One patient developed necrosis in the nail bed and one patient had a hematoma in the donor site. The minor complications were solved without any problem.

**Conclusion:** In conclusion, we found the olecranon bone grafting for the treatment of nonunion after distal finger replantation is a safe and convenient method. It can be preferred as the first choice for nonunions of distal finger replantations.

**Keywords:** Bone, olecranon, graft, replantation, nonunion



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



## INTRODUCTION

The first successful finger replantation was performed by Tamai, and major advances in replantation surgery have been made since then<sup>[1]</sup>. Today, the survival of the replanted finger is no longer a sufficient criterion for success; rather, functional recovery is considered crucial.

Distal replantations, and especially those distal to the distal interphalangeal joint, are technically difficult, but many are likely to be functionally successful<sup>[2]</sup>. Adequate vascular repair affects replant viability in the short term, but functional success is largely affected by bone fixation. The rate of the union in distal phalanx bone fractures is quite high; however, nonunion sometimes occurs due to inadequate fixation, segmental bone loss, soft tissue problems, and infection<sup>[3]</sup>. Patients with nonunion often experience instability, pain, and radiological abnormalities. In those cases, a bone graft may be required.

Although not very popular, the olecranon bone graft is a useful option for this type of an operation due to the minimal donor morbidity and its ease of use in small bone defect reconstruction and nonunion therapy. To our best knowledge, few studies have evaluated the use of the olecranon bone graft as a treatment for nonunion after distal finger replantation. Our aim in this report was to present our experience of using olecranon grafts in our nonunion patients undergoing distal replantations.

## METHODS

This study included a total of 14 (13 male and 1 female) patients who developed nonunion or segmental bone loss after replantation surgery between 2013 and 2019. Finger amputations of these patients were distal to the distal interphalangeal joint. Four amputations were at the Tamai zone 2 level and ten amputations were at the Tamai zone 1 level. The mean age was 34.5 years (range 19-54). Two patients had multiple finger amputations. Seven patients had crush amputations and seven patients had avulsion amputations. Two patients had segmental bone loss secondary to injury during the first surgery, and these defects were not reconstructed in the first operation to avoid vascular problems. In the other 12 patients, nonunion was diagnosed at the end of the fourth month based on radiological and clinical findings.

During the initial treatment, K-wires were used for all amputated fingers. Infection occurred in 3 patients, partial necrosis of the nail plate in 6 patients, segmental bone loss in 2 patients before initial surgery, and possibly inappropriate fixation in 3 patients that caused nonunions. The initial injuries were located at the arthrodesis site in 1 patient, at the tuft in three patients, and at the shaft of the distal phalanx in 10 patients.

All patients showed radiological and clinical findings (deformity, pain, and discomfort) before cancellous bone grafting. The overall mean time delay before the secondary treatment was 3.9 months (range 1-5 months).

### Surgical technique

All surgeries were performed by two senior surgeons under axillary regional anesthesia. A 3 cm incision for bone graft harvesting was made starting 1-1.5 cm distal to the tip of the olecranon [Figure 1]. The periosteum was incised and elevated off the donor site of the olecranon. A cortical fenestra was elevated and preserved to expose the medullary space. The cancellous bone graft was harvested with an osteotome. The cortical piece was replaced and closed after harvesting the graft to prevent irregularity at the donor site. Cancellous bone grafts were used in all patients. The periosteum was repaired with absorbable sutures, with the skin sutured individually. Postoperatively, no splint was used for elbow immobilization.

The recipient site was reached through a unilateral incision made either lateral to the nail plate or through the nail plate, depending on the nail plate situation. The fibrous tissue was removed at the nonunion line





**Figure 1.** A 3 cm incision for bone graft harvesting was made starting 1-1.5 cm distal to the tip of the olecranon

**Table 1. Location of nonunion and complications with the management of them**

Patient No	Non-union area	Complication	Note
1	Thumb distal phalanx	Hematoma	Resolved after drainage
2	Index distal phalanx	None	-
3	Ring Finger distal phalanx	None	-
4	Index distal phalanx	Nail bed necrosis	Local flap
5	Long Finger distal phalanx	None	-
6	Little finger distal phalanx	None	-
7	Ring Finger distal phalanx	None	-
8	Little finger distal phalanx	None	-
9	Thumb distal phalanx	None	-
10	Long Finger distal phalanx	None	-
11	Ring Finger distal phalanx	None	-
12	Long Finger distal phalanx	None	-
13	Thumb distal phalanx	None	-
14	Little finger distal phalanx	None	-

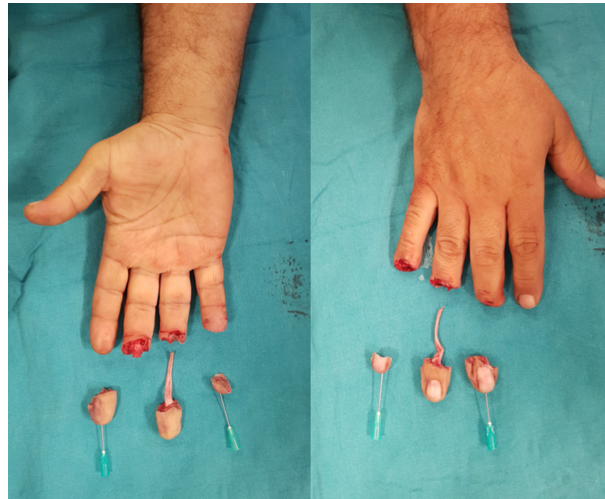
until the healthy bone was excised. The bone graft was placed and fixed with one or two K-wires. The size of the bone grafts ranged from 20 mm × 6 mm × 6 mm to 3 mm × 3 mm × 4 mm.

Patients were discharged from the hospital on the same day as the operation. They were all given parenteral antibiotics during the hospital stay and continued to take oral antibiotics for 5 days after the operation. The operated finger was splinted for 6 weeks. The K-wires were removed at the end of the sixth week. The patients were assessed for pain, deformity, and instability after removal of the K-wires and during the post-operative visits. All patients had a final X-ray taken at least 6 months after bone grafting.

## RESULTS

The mean follow-up period was 37 months (range 8-72 months). No major complications were seen in the donor region of the olecranon. One patient developed a hematoma in the donor area, but this issue was resolved after drainage. No fracture, palpable irregularity, discomfort, or pain was detected in the donor area in any of the patients.

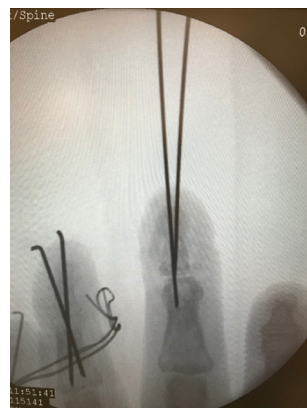
One patient developed necrosis of the nail bed and the graft was exposed. The defect was covered with a local flap and no complications were seen after this revision. No viability problems were experienced in any replant after bone reconstruction. The radiological union was detected in all 14 patients at 6 months postoperatively [Table 1]. No pain, deformity, or instability was detected in the fingertip in any patient. We haven't seen any hypertrophic scars in the donor area.



**Figure 2.** The patient had three fingers amputation due to an industrial accident



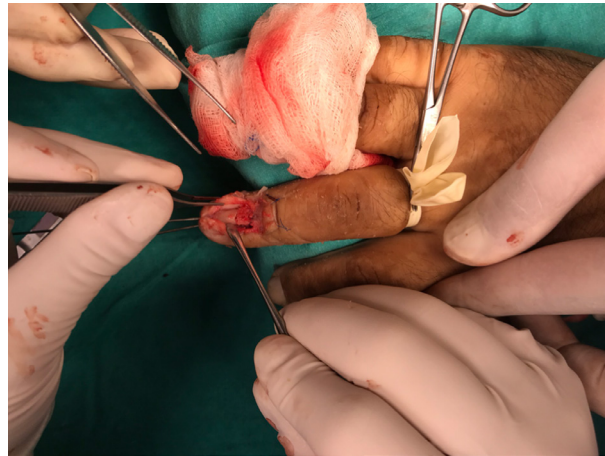
**Figure 3.** Segmental bone defect can be seen in the distal phalanx of the third finger



**Figure 4.** At 7 weeks after the first operation, an arthrodesis with an olecranon graft was performed on the 3rd distal interphalangeal joint

### Case 1

A 45-year-old male patient underwent operations for 3 finger amputations [Figure 2]. The initial surgery, performed as a 3 finger replantation, was successful. Segmental bone loss was evident in the amputated 3rd finger, and the union line was probably distracted during fixation with the K-wire. These features resulted



**Figure 5.** For this case, the thickness and stability of the nail bed was suitable, so grafting was performed through the incision in the middle of the nail bed



**Figure 6.** Six months after the olecranon bone grafting, the bony healing was complete

in nonunion in the 3rd finger, as confirmed by x-rays taken in the 6th week [Figure 3]. At 7 weeks after the first operation, an arthrodesis with an olecranon graft was performed on the 3rd distal interphalangeal joint [Figure 4]. The thickness and stability of the nail bed were suitable, so grafting was performed through the incision in the middle of the nail bed [Figure 5]. Six months after the olecranon bone grafting, the bony healing was complete [Figure 6].

## DISCUSSION

Prehensile ability is essential for hand functions and requires a sensible and stable fingertip grip<sup>[4]</sup>. Whenever it is technically possible, distal fingertip replantation is the gold standard method for replacing a missing fingertip<sup>[2]</sup>. Replantation gives the best result, both cosmetically and functionally, in fingertip amputations, but the functional outcome depends on sensory recovery and stability. A nonunion impairs the stability of the fingertip<sup>[5]</sup>.

Bone fixation methods used for replantation should be simple, rapid, and consistent, so they should cause minimal injury to bone and soft tissue<sup>[5]</sup>. The reported nonunion rate after replantation is between 3% and 19% in different studies<sup>[1,6-8]</sup>. Autologous bone grafts harvested from the iliac crest are commonly used in reconstructive surgery. The bone promotes bone healing in fractures and provides structural support for reconstructive surgery<sup>[9]</sup>. Iliac bone grafting can be performed under general anesthesia, but major complications include abdominal hernia, vascular injuries, deep infections at the donor site, neurologic injuries, hematoma, and iliac bone fractures. Harvesting of an iliac crest bone graft can, therefore, be associated with significant morbidity<sup>[10]</sup>.

Another option for cancellous bone grafting is the distal radius cancellous graft<sup>[9]</sup>. This graft type has the important advantage that it can be performed under an axillary block and in the same surgical field. The complication rates for harvesting bone grafts over the distal radius are as low as 1.7%<sup>[9]</sup>, but they can include fracture at the donor site, local infection, DeQuervain's tenosynovitis, and neuroma of the superficial radial nerve<sup>[11]</sup>. Patel *et al.*<sup>[11]</sup> reported an overall complication rate, including bone graft failure, of 4%, as 38 patients (2.3%) required re-grafting with iliac bone, 21 patients (1.3%) developed DeQuervain's tenosynovitis, and 2 patients (0.1%) developed superficial radial nerve neuromas.

Although not very popular in previous studies, the olecranon graft is used as the first choice by the authors for the treatment of a distal phalanx nonunion. This graft has the important advantage that it can be performed under an axillary block and in the same surgical field. The graft can be used as a bone chip in amorphous, irregular, and small defects or it can be used as a structural cancellous graft in longitudinal defects. Harvesting takes about 10 min by the author. In addition to the short duration of the surgery, its other advantage is that the graft can be easily shaped with a scalpel. The donor site is also distant from important neurovascular structures, so the dissection can be done quite easily. The site can also be harvested with a trephine, which provides a further advantage of minimal scarring<sup>[12]</sup>.

A major disadvantage of the olecranon graft is that a sufficient graft may not be obtained for large defects. This graft is also not recommended for use in elderly or osteoporotic patients<sup>[9]</sup>. Ozçelik *et al.*<sup>[3]</sup> reported the successful use of cancellous olecranon grafts for treating nonunion of distal phalangeal fractures in 11 cases.

The timing of the bone grafting is also important. Jupiter *et al.*<sup>[13]</sup> recommended waiting 4 months after the initial surgery, based on clinical and radiological findings. We haven't experienced any spontaneous union after 4 months too. Therefore, we had 12 of our patients wait 4 months for the surgical intervention for their nonunions. In two patients, the defect present at the replantation stage was evaluated as a segmental defect. Therefore, after vascular stabilization, grafting was performed without waiting for 4 months.

When using this technique, we preferred a mid-lateral approach to the distal phalanx as it does not disturb the vascular circulation. Ozçelik *et al.*<sup>[3]</sup> preferred a mid-lateral incision for the 11 patients in their study. In cases where the nail bed is stable and thick, grafting can also be done through the nail bed. However, although this approach is more advantageous in terms of exposure, the graft may become exposed after dehiscence of the nail bed. Itoh *et al.*<sup>[4]</sup> preferred grafting with a mid-palmar incision, which is advantageous in terms of exposure but has a theoretically higher risk of vascular injury and the possibility of painful scarring. With the mid-palmar technique, which Itoh *et al.*<sup>[4]</sup> applied in 6 patients, no patient had any vascular or sensory problems.

In conclusion, we found that olecranon bone grafting is a safe and convenient method for the treatment of nonunion after distal finger replantation. It can be a preferred first choice for the treatment of nonunions of distal finger replantations.

## DECLARATIONS

### Authors' contributions

Corresponding, writing: Ercin BS

Data search: Ercin BS, Keles MK

Editing discussion part: Kabakas F

Editing: Ozcelik IB

Review: Mersa B

### Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author, (initials). The data are not publicly available due to (restrictions e.g. their containing information that could compromise the privacy of research participants).

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

All ethical approvals and informed consents were obtained according to Helsinki Declaration.

### Consent for publication

Authors provide consent to publish before publication of the work.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Tamai S. Twenty years' experience of limb replantation--review of 293 upper extremity replants. *J Hand Surg Am* 1982;7:549-56.
2. Venkatramani H, Sabapathy SR. Fingertip replantation: technical considerations and outcome analysis of 24 consecutive fingertip replantations. *Indian J Plast Surg* 2011;44:237-45.
3. Özçelik IB, Kabakas F, Mersa B, Purisa H, Sezer I, et al. Treatment of nonunions of the distal phalanx with olecranon bone graft. *J Hand Surg Eur Vol* 2009;34:638-42.
4. Itoh Y, Uchinishi K, Oka Y. Treatment of pseudoarthrosis of the distal phalanx with the palmar midline approach. *J Hand Surg Am* 1983;8:80-4.
5. Lee SW, Lee DC, Kim JS, Roh SY, Lee KJ. Analysis of bone fixation methods in digital replantation. *Arch Plast Surg* 2017;44:53-8.
6. Nunley JA, Goldner RD, Urbaniak JR. Skeletal fixation in digital replantation. Use of the "H" plate. *Clin Orthop Relat Res* 1987;66-71.
7. Hoffmann R, Buck-Gramcko D. Osteosynthesis in digital replantation surgery. *Ann Chir Gynaecol* 1982;71:14-8.
8. Whitney TM, Lineaweaver WC, Buncke HJ, Nugent K. Clinical results of bony fixation methods in digital replantation. *J Hand Surg Am* 1990;15:328-34.
9. Mersa B, Özçelik IB, Kabakas F, Sacak B, Aydin A. Olecranon bone graft: revisited. *Tech Hand Up Extrem Surg* 2010;14:196-9.
10. Arrington ED, Smith WJ, Chambers HG, Bucknell AL, Davino NA. Complications of iliac crest bone graft harvesting. *Clin Orthop Relat Res* 1996;300-9.
11. Patel JC, Watson K, Joseph E, Garcia J, Wollstein R. Long-term complications of distal radius bone grafts. *J Hand Surg Am* 2003;28:784-8.
12. Özçelik B, Mersa B, Yesiloglu N. An easy way of harvesting olecranon bone graft in adults by using bone biopsy trephine. *J Hand Surg Am* 2013;38:622-3.
13. Jupiter JB, Koniuch MP, Smith RJ. The management of delayed union and nonunion of the metacarpals and phalanges. *J Hand Surg Am* 1985;10:457-66.



Review

Open Access



# Strategies for operative management of abdominal wall hernia after solid organ transplant

Devinder Singh<sup>1</sup>, Luther Holton<sup>2</sup>, Lauren Antognoli<sup>2</sup>, Salman Choudhry<sup>2</sup>

<sup>1</sup>Division of Plastic Surgery, University of Miami, Miller School of Medicine, Miami, FL 33136, USA.

<sup>2</sup>Division of Plastic Surgery, Anne Arundel Medical Center, Annapolis, MD 21401, USA.

**Correspondence to:** Dr. Devinder Singh, Division of Plastic Surgery, University of Miami, Miller School of Medicine, 1150 NW 14th St #701, Miami, FL 33136, USA. E-mail: dsingh.md@gmail.com

**How to cite this article:** Singh D, Holton L, Antognoli L, Choudhry S. Strategies for operative management of abdominal wall hernia after solid organ transplant. *Plast Aesthet Res* 2020;7:39. <http://dx.doi.org/10.20517/2347-9264.2019.76>

**Received:** 13 Feb 2020 **First Decision:** 27 May 2020 **Revised:** 23 Apr 2020 **Accepted:** 22 Jun 2020 **Published:** 19 Jul 2020

**Academic Editor:** Sahil Kuldip. Kapur **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

About 5%-11% of all abdominal surgery results in incisional hernia. This rate can be even higher among high-risk populations such as transplant patients. Lifetime incidence of incisional hernia following liver transplant is as high as 43% in recent studies. The transplant population is at higher risk for incisional hernia precisely because of their immunosuppressive therapy. Thus, it is imperative to understand the risk factors for incisional hernia in this unique patient population. This article focuses on understanding preoperative, intraoperative, and postoperative risk factors for failure of hernia repair in the transplant population in addition to discussing risk stratification for incisional hernia in this population. Furthermore, we discuss the utility of panniculectomy in abdominal organ transplantation. Additionally, we discuss the value of mesh placement in abdominal wall closure. Finally, we review the concept of vascularized composite allograft as a method for achieving abdominal wall closure for patients who have failed more traditional repairs and who are left with inadequate tissue for successful repair.

**Keywords:** Abdominal organ transplant, incisional hernia, vascularized composite allograft, panniculectomy, human acellular dermal matrix, porcine acellular dermal matrix

## INTRODUCTION

Approximately 5%-11% of all abdominal surgery incisions result in incisional hernia, but this rate can exceed 30% in complex wounds among high-risk patients such as those undergoing solid organ



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



transplants<sup>[1]</sup>. The transplant population is uniquely susceptible to wound healing complications, such as wound infection and incisional hernia for multiple reasons. Lifetime incidence of incisional hernia following liver transplant has been reported to be as high as 43% in recent studies<sup>[2-4]</sup>. Wound complications can plague the postoperative patient experience as they cause discomfort, repeat hospitalizations, increased health care costs, and diminished quality of life. For transplant patients, the stakes are particularly high because wound infection, dehiscence, or incisional hernia have the potential to compromise graft function and viability and because efforts to heal are obtunded by the presence of often powerful immunosuppressive medications. Notably, efforts to modulate immunosuppression levels during events such as wound infections or malignancies have been thought to correlate with graft dysfunction and failure. Accordingly, preventing surgical site infection (SSI) is critical in these patients. Operative and perioperative strategies to prevent SSI and wound breakdown in the transplant population are similar to those of other surgical disciplines. In addition to maintaining a clean field with minimal spillage, use of drains, and a complex multi-layered closure of the fascia and subcutaneous tissue, for this particularly susceptible population, we have routinely used negative-pressure wound therapy in order to sequester the incision from outside contamination as well as aid perfusion across the incision. In fact, in May 2019, the Federal Drug Administration approved the PREVENA<sup>TM</sup> negative-pressure incision management system to help reduce superficial SSIs in patients at high risk of postoperative infections in Class I and II wounds. It is the first and only negative-pressure medical device indicated to aid in the reduction of SSIs. If a wound complication does occur, it should be addressed as early as possible to prevent progression. Active wound management with early debridement and washout can prevent worsening infection.

## ETIOLOGY AND RISK FACTORS

### Immunosuppression

Transplant patients are at especially high risk for hernia due to their immunosuppressed state and comorbid conditions which can hinder adequate healing and even hasten wound breakdown<sup>[1]</sup>. Induction immunosuppression is short-term, intense immunosuppression therapy administered to recipients at the time of transplantation in an attempt to prevent rejection in the first few weeks after transplant, when risk for rejection is highest. Modern induction immunosuppression in solid organ transplant recipients is not only focused on preventing rejection but also minimizing steroid use and mitigating the negative side effects of long-term immunosuppression. Current induction therapy commonly utilizes agents such as basiliximab, antithymocyte globulin, and alemtuzumab to reduce the use of steroids immediately after transplantation. The goal of maintenance therapy is to provide adequate long-term rejection prophylaxis through multiple immunosuppressive drugs with different mechanisms of action. Maintenance therapy often consists of tacrolimus, mycophenolate mofetil, and, at times, steroids. Vitamin A is also used as an adjunct to assist with wound healing in these patients on chronic steroids. Obviously, variation in induction and maintenance protocols exists between transplant centers, and all have potentially deleterious effects on wound healing. Aside from steroids used in induction and maintenance immunosuppression, steroid boluses are often used to treat patients experiencing rejection episodes. Thus, a lifetime of immunosuppression in transplant patients may allow their graft to survive, but at the cost of poor healing, wound breakdown, and hernias, which can potentially threaten the transplanted organ(s).

When planning elective surgery such as incisional hernia repair, careful consideration must be given to each patient's immunosuppressive regimen. A multidisciplinary approach is highly recommended for care for the transplant patient, including transplant pharmacologists to assist with drug modulation in the perioperative period. Decisions regarding adjustments to immunosuppressive therapy should be made on an individual and real-time basis and with the expertise of the involved team. Unfortunately, there are currently no data available from randomized, double-blind controlled clinical trials on how to guide immunosuppressive therapy in the perioperative setting for this patient population, making evidence-based

recommendations difficult. Furthermore, chronic immunosuppression remains a cofactor for recurrence and subsequent hernia repairs, thus adding to the complexity of hernia repair in this population.

### Patient comorbidities

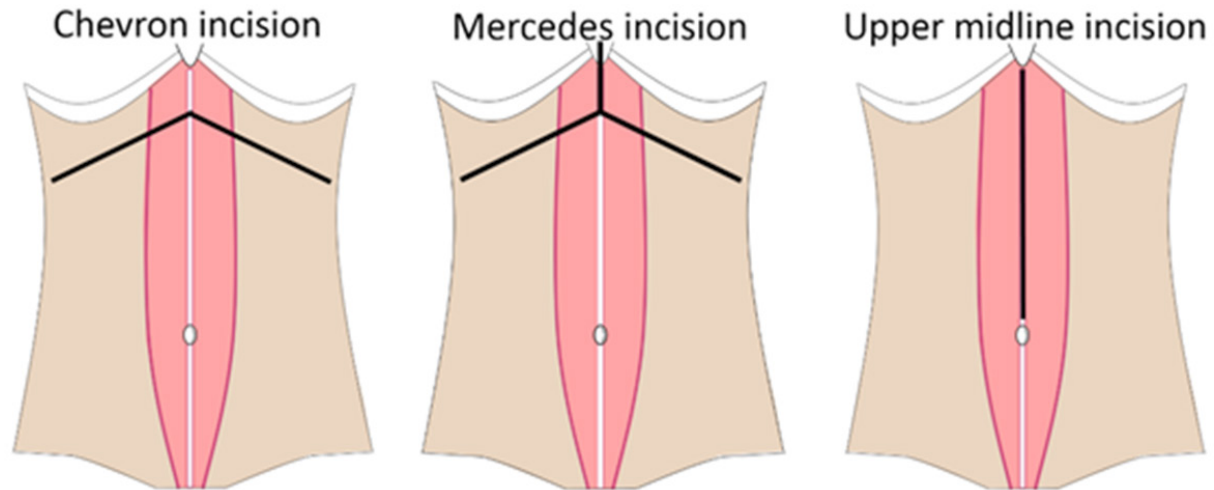
Preoperative patient factors that predispose to incisional hernia in transplant patients include male gender, advanced age (studies cite ages greater than 45-60 as risk factors), elevated BMI (BMI > 25), smoking, malnutrition (serum albumin levels less than 3.5 g/L), connective tissue disorders (e.g., osteogenesis imperfecta, certain subtypes of Ehlers-Danlos syndrome, and Marfan syndrome), immediately preoperative chemotherapy and/or radiation to the operative site or region, presence of large volume ascites, COPD, previous surgery in the operative area, anemia, steroid use, diabetes mellitus, and immunosuppression<sup>[2-6]</sup>. Furthermore, patients with end-stage renal or liver disease live in catabolic states in which muscle atrophies and the abdominal wall weakens.

Postoperative factors associated with incisional hernia include wound infection, pulmonary complications, prolonged ICU stay, severe ascites, anemia, thrombocytopenia, acute rejection with steroid treatment, and same-site repeat surgery with fascial reopening and reclosure<sup>[2-6]</sup>.

Common to these risk factors - obesity, ascites, and COPD - is increased intra-abdominal pressure, which puts mechanical stress on the fascial closure and weakens the abdominal wall, making patients more prone to wound necrosis, breakdown, and hernia<sup>[2,4]</sup>. Furthermore, medical issues such as benign prostatic hypertrophy chronic constipation, and chronic cough can also significantly increase intra-abdominal pressure and thus jeopardize the integrity of hernia repairs. Lower abdominal incisions are also more prone to hernia, as increased abdominal pressure is greater in the dependent abdomen and a pannus may put tension on healing fascia. A patient undergoing abdominal organ transplant should be optimized to the greatest extent possible in the preoperative period. This should include careful medical management of weight, pulmonary function, and ascites to decrease postoperative intra-abdominal pressure, thus decreasing the incidence of developing incisional hernia. Unfortunately, patients often spend months and years on a transplant list and are often not adequately optimized for surgery when an organ actually becomes available.

### Technique-related risk factors

Technique-related factors that increase risk for an incisional hernia after transplant include excess tension upon fascial closure, emergency surgery, and type of incision<sup>[2-6]</sup>. A surgeon's choice of incision for solid organ transplant surgery depends on the type of organ transplanted, surgeon preference, and patient-specific factors such as previous surgeries and organ size. The larger incisions with liver transplants are, in part, why liver transplant patients have a much higher incidence of hernia compared to renal transplant patients. Incisions commonly used in liver transplants include bilateral subcostal, Mercedes, and upper midline incisions, among multiple other variants [Figure 1]. Bilateral subcostal and Mercedes incisions are the most commonly used, as they provide excellent exposure. However, these large incisions with hours of stout retraction can lead to greater areas of fascial weakening, thus increased risk of future hernia. The Mercedes incision carries an increased risk of incisional hernia when compared to bilateral subcostal incisions<sup>[2]</sup>. The triple point of the Mercedes incision is a common area of decreased perfusion and increased tension, which can lead to wound ischemia, necrosis, and dehiscence at the levels of the fascia and/or the skin. Few studies exist regarding optimal closures for liver transplant incisions to prevent hernia. In a retrospective study from 2010, Aydin *et al.*<sup>[7]</sup> described a unique approach for closure of abdominal fascia in Mercedes incisions, designed to reinforce the triple point. In this technique, the transverse components of the incision are closed in two overlapping layers, and the vertical component is closed in one layer using a single suture that overlaps the medial corners of the transverse incisions before running cephalad to the xiphoid. Patients whose fascia was closed in this method had significantly lower rates of



**Figure 1.** Examples of incisions used in liver transplant recipients

hernia than those closed in a single layer without this locking suture at the triple point. However, there is still no consensus on ideal closures for these large incisions, and the method of closure varies greatly between surgeons.

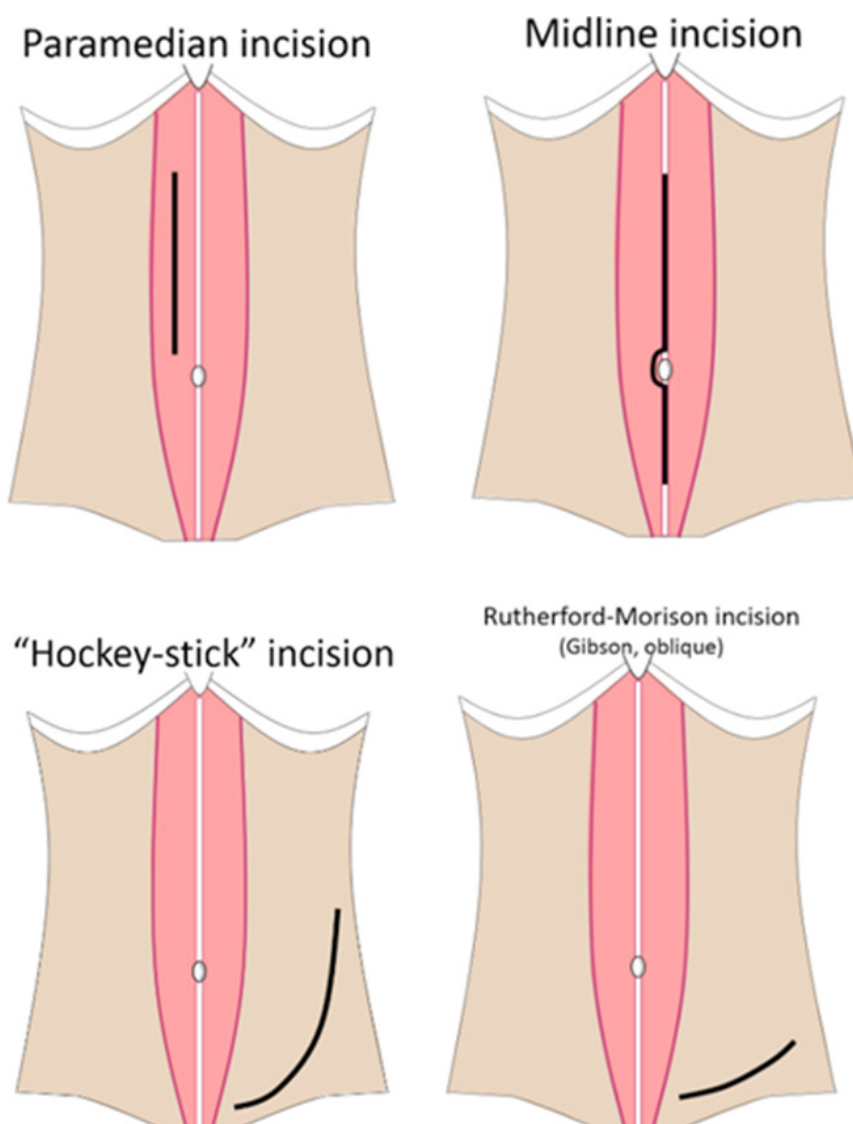
Common incisions described for kidney transplant recipients include paramedian, midline, and low oblique muscle-cutting incisions, such as the Rutherford - Morison incision [Figure 2]. Midline abdominal incisions are more prone to incisional hernia formation and carry a significantly higher risk of herniation compared to transverse and paramedian incisions<sup>[2]</sup>. Low oblique incisions result in fewer hernias than the “Hockey-stick” incision.

Finally, focused intraoperative assessment of fascia is essential. Although there may be concern for excessive fascial tension or even difficulty/inability to close the abdomen if there is not enough fascia, poor quality fascia must be debrided prior to closure. Including ischemic, nonviable fascia in a closure will greatly increase risk for wound breakdown, dehiscence, and exposure of the graft to infection.

## RISK STRATIFICATION

Understanding how to avoid hernia complications in transplant patients is perhaps more valuable than understanding how to repair these hernias. Important measures to avoid hernia include active incision management (e.g., careful tissue handling, closing the operative area in multiple layers, placement of drains, and use of negative pressure incision management), as well as early identification of wound complications. Should wound complications occur, early management with operative debridement and washout as well as the use of adjunct modalities such as negative-pressure therapy with antibiotic solution irrigation may prove useful. However, hernias still occur in this complex patient population despite meticulous technique and early management of complications. Accordingly, risk stratification is helpful.

There is an established grading system created by the Ventral Hernia Working Group (VHWG) to predict surgical site occurrences in patients undergoing ventral hernia repair based on patient risk factors and comorbidities. This was initially described in 2002 and consisted of four grades (low risk, comorbid, potentially contaminated, and infected). The grading system was then redefined in 2012, which resulted in Grades 3 and 4 being combined [Table 1]<sup>[8]</sup>. Transplant patients by virtue of immunosuppression are classified as at least Grade 2.



**Figure 2.** Examples of incisions used in kidney transplant recipients. Please note that some incisions may be right- or left-sided, depending on patient anatomy and involved organ(s)

**Table 1. Hernia Grading System as described by the Ventral Hernia Working Group<sup>[8]</sup>**

Hernia Grading System		
Grade 1 (low risk)	Grade 2 (comorbid)	Grade 3 (contaminated)
<ul style="list-style-type: none"> <li>·No history of wound complications</li> <li>·No evidence of contamination</li> <li>·No comorbidities</li> </ul>	<ul style="list-style-type: none"> <li>·Comorbid conditions (DM, smoking, immunocompromised, COPD, obesity)</li> <li>·No evidence of contamination</li> </ul>	<ul style="list-style-type: none"> <li>·Presence of nearby stoma</li> <li>·History of wound infection</li> <li>·Violation of GI tract</li> <li>·Septic dehiscence</li> <li>·Infected mesh</li> <li>·Active infection</li> </ul>

In addition to the grading system developed by VHWG, multiple other tools are used for risk stratification of complications developed after ventral hernia repair. Such models include the American College of Surgeons National Surgical Quality Improvement Program (ACS-NSQIP), European Hernia Society (EHS), The HERNIAScore, and Ventral Hernia Risk Score (VHRS). The HERNIAScore developed by Goodenough *et al.*<sup>[9]</sup> helps to identify risk factors for ventral incisional hernia formation. Laparotomy, hand-assisted laparoscopy, COPD, and BMI greater than 25 are all independent risk factors for hernia



development. Depending on the score from zero to six, the risk for hernia ranges from 5.5% to 55%<sup>[9]</sup>. The HERNIAScore has also been improved to include length of incision, COPD, and prior abdominal operations<sup>[10]</sup>.

VHRS is an additional tool available for risk assessment of surgical site infection (SSI). Based on VHRS, risk factors for SSI include concomitant hernia repair, raising of skin flaps, wound Class 4 (i.e., dirty), BMI greater than 40, and ASA Class 3 or greater<sup>[11]</sup>. Both the HERNIAScore and VHRS have been externally validated and shown to be more effective in identifying SSI/SSO risk when compared to other risk models. Overall, while some models are helpful, not all have been internally and externally validated. Validated grading scales can help counsel patients and set realistic expectations for possible complications following ventral hernia repair.

## CURRENT TECHNIQUES IN HERNIA MANAGEMENT

### Abdominal wall closure in transplant patients

Surgical closure of an abdomen following solid organ transplant is a critical aspect of the transplant, as a failure of closure greatly increases the risk for graft failure and infection. Primary closure of the abdominal wall is, of course, ideal, however cannot be achieved in approximately 20% of patients<sup>[12]</sup>. A common reason for failure of primary closure is lack of intra-abdominal domain. Some initial loss of domain is transient from edema and resolves with diuresis, *etc.* A rush to primary closure only increases the risk of dehiscence and wound breakdown. Patients may also present with presence of fistulae, ostomies, extensive scar tissue, significant skin lesions from healed fistulae/wounds, or even a frozen abdomen secondary to dense adhesions from multiple prior procedures, thus making primary closure challenging or even impossible to perform. In these patients with such anatomic complexities, an open approach may be favorable.

Significant tension on the abdominal wall from closure in patients with loss of domain can also lead to abdominal compartment syndrome, which in a newly transplanted graft can lead to graft failure. Foley catheters are commonly left in place postoperatively, often to monitor urine output, but also to monitor bladder pressures in the setting of possible abdominal compartment syndrome. It is imperative that the patient is intubated, paralyzed, and fully supine in order to obtain accurate bladder pressures. Increased abdominal pressure that does not cause compartment syndrome can still impede flow within the inferior vena cava and decrease portal venous flow to the liver. This is a known risk factor for graft failure following liver transplantation. Low flow states can also lead to poor venous return, resulting in decreased cardiac output and perfusion. Increased abdominal pressure after fascial closure can also lead to respiratory compromise, especially in patients with loss of domain. Thus, it is important to communicate with the anesthesia team intraoperatively to monitor peak pressures after closure, as some patients may require continued intubation in the immediate postoperative period. Elevated ventilatory pressure or changes in peak pressures may also serve as a real time indication of impending compartment syndrome so our group routinely solicits feedback from anesthesia throughout closure.

### Panniculectomy and transplantation

Obesity remains a significant risk factor for morbidity after abdominal surgery. Transplantation in general carries a higher degree of morbidity among obese patients. Large overhanging skin folds create a moist environment that increases the risk of postoperative infection. Furthermore, the large amount of subcutaneous tissue that must be traversed and potentially undermined in patients undergoing transplant has a higher risk of necrosis and infection. A large pannus may also create additional tension and stress on the incision, which may lead to separation of wound edges<sup>[13]</sup>. Individual centers have specific protocols and regulations regarding optimal BMI prior to transplantation. While there is no consensus, some centers suggest having a BMI less than 30 to decrease the risk of perioperative complications<sup>[14]</sup>. A prophylactic panniculectomy can be performed prior to transplant to enable obese patients to meet criteria

for transplantation. Performing panniculectomy in advance will allow patients to be better optimized for transplantation and allow them to heal without the influence of immunosuppression. However, this staged approach may add cost relative to simultaneous panniculectomy and transplant. It should be acknowledged that, while the pre-transplant panniculectomy patients are often not pharmacologically immunosuppressed, they are still relatively immunosuppressed due to their end-stage organ dysfunction.

Ngaage *et al.*<sup>[15]</sup> discussed panniculectomy at the time of living donor renal transplantation. In their study, panniculectomy was performed prior to standard oblique muscle cutting incisions such as a Gibson or Rutherford-Morison incision for transplantation. Among 58 patients with an average BMI of 35.2 who underwent simultaneous panniculectomy and renal transplant, the authors noted comparable rates of wound complications when compared to panniculectomy without transplant. Furthermore, there was no significant difference in allograft complications compared to the standard two-staged procedure, nor was there a significant difference in length of hospital stay<sup>[15]</sup>.

In the single-stage operation by Ngaage *et al.*<sup>[15]</sup>, the reconstructive surgeon mobilizes, undermines, and reflects the redundant soft tissue, exposing entirely the underlying fascia. This exposure provides access to the abdominal fascia and then facilitates closure of the defect. Simultaneous panniculectomy appears to be performed without a significant increase in wound healing morbidity even though these single-stage patients must face the challenge of healing after induction and maintenance of immunosuppression<sup>[15]</sup>. It is noteworthy that the study by Ngaage *et al.*<sup>[15]</sup> did not have homogeneous immunosuppressive regimens, which may have skewed the interpretation of wound complication rates.

### Use of mesh in post-transplant incisional hernia repair

The optimal type of mesh to use in hernia repair - and hernia prevention - is widely debated. However, there has recently been a greater shift towards the use of biologic mesh over synthetic mesh. Biological materials such as human acellular dermal matrix (HADM) and porcine acellular dermal matrix (PADM) have multiple advantages over their synthetic counterparts. Biologic mesh provides a scaffold that allows cellular and vascular ingrowth, thus benefitting from possible incorporation into native abdominal wall tissue<sup>[9]</sup>. The vascularization of acellular dermal matrix (ADM) assists the immune system in combating bacterial contamination. Furthermore, ADM has low antigenic potential and elicits a milder host inflammatory response compared to synthetic mesh. This allows for a less adhesiogenic environment in comparison to prosthetic mesh, making ADM desirable for intraperitoneal use<sup>[16]</sup>. ADM also contains collagen cross-linkages, which provide strength and resistance to mechanical stress<sup>[16]</sup>. Among popular acellular dermal matrices (AlloDerm, FlexHD, Strattice, Neoform, and DermACELL), AlloDerm (a HADM) has been shown to be more effective against bacterial growth. This was supported by Fahrenbach *et al.*<sup>[17]</sup> who showed that AlloDerm was resistant to penetration with *S. aureus* and *S. pyogenes*. However, AlloDerm contains more elastin than its porcine equivalent; thus, it is more prone to stretching and can lead to recurrence, bulging and weakness when used in the abdominal wall.<sup>17</sup> Generally speaking, non-crosslinked porcine ADM has been found to be beneficial in higher risk patients, including the transplant population.

Multiple studies have shown that mesh implantation during incisional hernia repair can decrease the risk of hernia recurrence without increasing risk for infection in the transplant population<sup>[2,5]</sup>. A study published by our group compared clinical outcomes of incisional hernia repair in 87 patients receiving solid organ transplants in which PADM, HADM, or synthetic mesh was utilized. We found that patients whose hernias were repaired using synthetic mesh had a significantly higher incidence of wound infection, hernia recurrence, and mesh removal<sup>[1]</sup>. Another study observing 104 patients undergoing renal, liver, or pancreas transplants had a statistically significant odds ratio of 11 and 8.7, respectively, for wound infection and mesh removal when comparing HADM with synthetic mesh<sup>[18]</sup>. It is important to note that neither of these studies was blinded, therefore inherent selection bias is possible in both.

As mentioned, HADM has a greater tendency to stretch over time, given the higher relative content of elastin within the matrix when compared to Porcine versions. Some studies have documented increased rates of bulging and hernia recurrence in patients with large complex hernias repaired with HADM. Our group found that, unlike its human analog, PADM resists early stretching after implantation while still providing comparable tensile strength, thus demonstrating a clinically relevant but statistically non-significant benefit to using PADM in abdominal wall reconstruction in the transplant population<sup>[1]</sup>. Despite advantages and disadvantages to using either HADM or PADM, at this time, there is little evidence showing a significant benefit of one type of ADM over another.

### Operative approaches

Multiple operative techniques are currently used by general, plastic, and transplant surgeons to repair incisional hernias in transplant patients. Primary suture repair is often limited to primary ventral hernias with small defects less than 3 cm. However, some surgeons still opt for mesh reinforcement in small repairs due to higher rates of recurrence in this patient population. Repair with mesh is recommended for repair of incisional hernias larger than 2 cm in a non-infected field<sup>[19]</sup>. Laparoscopic intraperitoneal onlay mesh (IPOM) is a commonly-used repair technique for small- to medium-sized hernias, although it has been described as useful in facial defects up to 10 cm<sup>[19]</sup>. IPOM may also be useful in transplant patients who have undergone multiple abdominal surgeries and either lack a viable peritoneum or are missing a portion of posterior rectus sheath, thus making them ineligible for retro-rectus repair. In any mesh repair, it is important to ensure adequate overlap of the defect by at least 3-5 cm to decrease the risk of recurrence and to offload tension<sup>[19]</sup>. Furthermore, recognizing there is a growing adoption of posterior approaches, there still may be benefit for a modified onlay approach in properly selected patients, as described by the authors<sup>[20]</sup>. In this component separation onlay approach, PADM is anchored to the donor site cut edge of external oblique on either side of the defect, thus providing a spanning and load-sharing structure that reinforces the midline closure with low rate of hernia recurrence and surgical site occurrence<sup>[20]</sup>.

Open sublay (e.g., retro-rectus) mesh repair has been shown in some studies to have similar recurrence rates as compared to IPOM, but these sublayed repairs are notable for increased perioperative morbidity and hospital length of stay<sup>[21]</sup>. However, the retro-rectus approach has several advantages when compared to IPOM and is being used more frequently in incisional hernia repairs in both post-transplant and general populations. Retro-rectus hernia repair, such as the Rives-Stoppa technique, maintains extraperitoneal access, which allows the surgeon to avoid contact with intraperitoneal adhesions and other organs, including the graft. By existing outside of the peritoneum, this technique also prevents the formation of adhesions that may hinder future surgical interventions, including graft repair and re-transplantation.

Sublay repair is often used in conjunction with component separation techniques in order to restore the linea alba and medialize the rectus muscles. Component separation in post-transplant patients is often challenging, as the native planes are often distorted and scarred down. Additionally, as mentioned above, incisions such as Mercedes incisions have both horizontal and vertical components, which can further complicate plane dissection and hernia repair. Black *et al.*<sup>[22]</sup> described a modified component separation for abdominal wall reconstruction in 19 liver and kidney transplant patients, where open perforator-sparing component separation techniques (e.g., posterior external oblique dissection) were used in conjunction with biologic mesh underlay. Their data show comparable rates of healing and long-term hernia recurrence compared to other techniques<sup>[22]</sup>. More research is needed to examine outcomes and hernia recurrence after sublay/retro-rectus repair in transplant patients.

While component separation is often a necessary maneuver to achieve fascial re-approximation, it is not without potential complications. Anterior component separation (ACS) requires creation of large subcutaneous flaps, which can disrupt the blood supply via transection of trans-rectus epigastric perforator

to the overlying fat and skin, leading to skin necrosis and its downstream sequelae. As mentioned above, this dissection also creates large flaps, which often harbor enormous areas of dead space and associated fluid collections. It should be noted that perforator-preserving ACS techniques have been described with improved outcomes. A posterior component separation (PCS) allows for less subcutaneous dissection and also may be preferred in patients who have undergone prior ACS repairs. In cases where patients lack adequate retro-rectus space, a unilateral or bilateral transverse abdominis release (TAR) may be required to minimize tension while avoiding the neurovascular bundles at the lateral border of the rectus muscle. As a general rule, we recommend that the retro-rectus space be at least twice the size of the defect transversely in order to undergo repair without the need for TAR. An advantage of TAR is the ability to create a wide retro-muscular space that can extend to the psoas muscles, Cooper's ligaments, and the central tendon of the diaphragm.

A meta-analysis from 2018 comparing open ACS to PCS with TAR showed similar hernia recurrence rates and wound complication rates; however, both larger and comparative studies still need to be performed<sup>[23]</sup>. It is difficult to discern which approach is superior. Ultimately, the choice of repair is multifactorial and will vary based on patient comorbidities, hernia morphology, and surgeon experience.

Finally, robotic-assisted hernia repair can aid in abdominal wall reconstruction for complex and large-sized hernias. A robotic approach may facilitate dissection and component separation in the setting of dense adhesions and complicated post-transplant anatomy. It is our recommendation that such cases should be performed at hernia centers that specialize in both complex hernia repair as well as transplant surgery such that, if there are complications, the transplant team is readily available.

Significant variability exists between size criteria and the appropriateness of individual techniques among surgeons; therefore, surgeon experience, patient factors, and hernia morphology must be considered when choosing how to repair incisional hernia in solid organ transplant patients. Currently, there are no definitive algorithms for hernia repair in this population for multiple reasons. First, a reparative approach can be determined by size of fascial defect, patient risk factors, or even hernia stages. There is no consensus as to which category is preferred in terms of stratifying patients to follow a certain algorithmic path. It is this lack of a universal classification system that has prevented development of algorithms in the past and this remains true today. Secondly, there is a great deal of variation in hernia repair technique that differs among institutions and surgeons, based on individual operator preference, experience, and available resources. What may be an acceptable approach at one institution may be deemed outdated, less desirable, or even impossible at another. Finally, there is so much nuance regarding incisional hernias in transplant patients - hernia size and location, degree of immunosuppression, comorbidities, loss of domain, and history of previous surgeries and hernia repairs to name a few - that there are infinite possible approaches to repair. Algorithms fail to be useful when they are too convoluted. This sentiment is best stated by Malangoni and Rosen in the 20th Edition of the Sabiston Textbook of Surgery: "The absence of a universal classification system has hindered comparisons within the literature and at meetings, indirectly delaying meaningful conversations about repair techniques and prosthetic choice. The TNM model for cancer staging is an enviable model to strive for in hernia repair"<sup>[24]</sup>. As of now, a comparable model does not exist.

### **Autologous tissue flaps**

While not common, some transplant patients' hernias are so complicated, or have failed one or more biological mesh repairs, that surgeons must turn to autologous tissue flaps such as tensor fascia lata or thigh flaps. These autologous flaps were performed more frequently in the past and are now mostly of historical interest because complicated hernias are so effectively managed by biological mesh and novel methods of tissue expansion that complicated flap procedures are rarely necessary.

### Abdominal wall vascularized composite allograft

Multiple techniques have been described to help achieve abdominal wall closure including component separation, Gortex patch, use of biologic mesh (as described above), autologous flaps, and more recently abdominal wall transplantation. Abdominal wall transplantation, more commonly known as abdominal wall vascularized composite allograft (AW-VCA), is a modern alternative to abdominal wall closure which is typically reserved for truly complex defects. Utilization of AW-VCA can be separated into three categories: (1) patients receiving AW-VCA in conjunction with intestinal transplant; (2) patients receiving AW-VCA who already have a visceral organ transplant such as liver, kidney, or pancreas; and (3) AW-VCA performed as an isolated soft tissue transplant, which has shown promise in cadaver models. It is important to note that in non-transplant patients performing AW-VCA will subject patients to lifelong immunosuppression. On the contrary, for transplant patients, they are already immune suppressed.

AW-VCA can be either partial or full-thickness. In full-thickness transplants, the abdominal wall - including the peritoneum, rectus abdominis muscle(s), and variable amounts of oblique muscle, as well as skin and soft tissue - are harvested en bloc. Partial-thickness reconstruction involves vascularized or non-vascularized fascia in patients with adequate skin cover, but insufficient or inadequate fascia<sup>[25]</sup>.

Two techniques have been described for abdominal wall transplants. In the conventional method, blood supply from the donor is taken from the inferior epigastric vessels, which are left in continuity with the femoral and iliac vessels and then anastomosed to the recipient's common iliac artery and vein<sup>[12]</sup>. The second method uses a microvascular technique to anastomose the donor's inferior epigastric vessels to those of the recipient<sup>[26]</sup>. The fascia of the donor is then sutured to the abdominal wall fascia of the recipient. A layered closure involving the subcutaneous tissues and skin of the donor and recipient is then performed, thereby completing graft integration. In both techniques, the abdominal donor graft is taken from a beating-heart donor. Procurement ideally is taken from the same donor of the abdominal organs. Interestingly, there have been reports of procurement from abdominal wall-specific donors.

Levi's group in 2003 was the first group to report their experience in a nine-case series of abdominal wall transplants<sup>[12]</sup>. This series included adults and children and was later pooled with additional patients in 2009<sup>[27]</sup>. Noted indications for intestinal transplant in their series included Gardner's syndrome, trauma, Churg-Strauss vasculitis, and intestinal motility disorders such as Hirschsprung and pseudo-obstruction<sup>[12]</sup>. Since the initial report in 2003, there have been 35 cases of AW-VCA transplantation with flap survival rates as high as 88% with immunosuppression<sup>[25,28]</sup>.

Postoperative immunosuppression protocols are center-specific. These regimens commonly include antibody induction with maintenance therapy using tacrolimus and/or mycophenolate mofetil. Steroids may also be used during maintenance therapy. One study noted a rejection rate of 17.7% among a cohort of 17 patients<sup>[29]</sup>. Rejection of the graft is often accompanied by a maculopapular rash or skin breakdown. This is in part due to the strong antigen surveillance role played by Langerhan cells within the skin, which may demonstrate signs of rejection when other composite tissues may not. Early skin changes on the abdominal wall are an indication of rejection and thus treatments such as steroid boluses and medication adjustments can be initiated sooner. Rejection may respond to topical immunosuppressive application as well or in conjunction with oral steroids. The added benefit of a skin rash is being a sentinel marker of infection that provides a visual which patients may share with their transplant team, prompting intervention sooner than would be otherwise. Furthermore, because transplanted skin can act as a marker of visceral rejection via a rash, patients may have immunosuppressive regimens titrated down as long as such symptoms do not occur<sup>[30]</sup>. It is worth noting that multiple studies have reported that deaths after transplantation have not been directly related to abdominal wall transplants.



Since the results published by Levi *et al.*<sup>[12]</sup>, numerous research studies have focused on AW-CVA as an isolated soft tissue transplant without viscera. Quigley *et al.*<sup>[31]</sup> (2013) conducted complete isolated AW-VCA transplantation with femoral micro-anastomosis in rats. They used an immunosuppressive regimen consisting of Tacrolimus and showed 100% graft survival at 100 days, however with limited chimerism<sup>[31]</sup>. Chimerism involves a patient having hematopoietic stem cells of both donor and recipient origin. A lower degree of chimerism in patients can lead to graft failure; however, it can be halted with immunosuppression. Over time, tolerance between the two cell lines can occur, known as mixed chimerism, leading to patients requiring less immunosuppression, making rejection less likely.

To date, there is no literature showing AW-VCA being performed outside of transplant patients as an isolated procedure. The application of isolated AW-VCA (without viscera) has significant potential in patients with large abdominal wall defects including multiple prior surgeries or trauma patients with profound loss of domain. These patients are often plagued by poor functional status and have undergone multiple attempts at repair. There is, however, some thought that the risks of lifelong immunosuppression outweigh the benefits of AW-VCA transplantation, which is in part why AW-VCA as an isolated soft tissue transplant has not yet been performed.

While AW-VCA is a solution for patients with profound domain loss, the transplanted abdominal walls are essentially defunctionalized mechanical retainers of abdominal contents. These denervated transplants lack all motor function which leads quickly to atrophy, fibrosis, and loss of strength. Thus, while the patient may have a vascularized abdominal wall, he or she may have significant physical dysfunction and deformity.

In cases where innervated AW-VCA have been attempted, innervation is often unsuccessful or incomplete. From promising results in rat models, there have been multiple cadaver studies describing innervated AW-VCA to preserve both motor and sensory functions. Using a component separation technique involving the external oblique, the thoracolumbar nerves can be isolated from the donor to allow for the AW-VCA to retain both sensation and motor function<sup>[32,33]</sup>. We designed and executed a cadaver study which combined the concepts of functional hernia repair with the goal of innervated abdominal wall allotransplantation through preparation of the graft using a “multi-layered” component separation technique that carefully identifies individual segmental intercostal nerves beneath the internal oblique muscle. By preserving the nerve supply, the rectus muscle can theoretically remain innervated after transplant, which would allow for faster functional rehabilitation, increased strength, and decreased complications from a denervated abdominal wall such as bulge or hernia. In this study, they harvested the lowermost portion of the ribcage with the innervated soft tissue abdominal wall specimens in two cadavers. This required plates and screws for osteosynthesis but came with the advantage of presence of bone and bone marrow in the graft, which is thought to potentially promote immunogenic chimerism and thus decrease immunosuppressive requirements<sup>[32]</sup>.

## CONCLUSION

Management of incisional hernia remains very complex, even more so in the post-transplant population. When planning any hernia repair in this patient population, one must consider patient comorbidities and risk factors, hernia morphology, and surgeon experience. Furthermore, a multidisciplinary approach should be used regarding each patient's immunosuppression regimen, ideally including a transplant pharmacologist. While a variety of repair options exist, it has not been possible to create an algorithmic approach to such a heterogeneous population. Therefore, each patient must be approached systematically to determine the most appropriate repair. Lastly, AW-VCA is an option for very complex defects or in patients with significant loss of domain, and new techniques may allow innervation in the transplanted abdominal wall.

## DECLARATIONS

### Author's contributions

Made substantial contributions to the literature review, writing, and editing of this manuscript: Singh D, Holton L, Antognoli L, Choudhry S

Performed image creation and formatting: Antognoli L

Performed table creation and formatting: Choudhry S

### Availability of data and materials

Not Applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

Singh D is the consultant to 3M-KCI, Allergan, and Gore; Holton L is the consultant to 3M-KCI, Allergan, and Stryker; Antognoli L and Choudhry S have no conflicts of interest.

### Ethical approval and consent to participate

Not Applicable.

### Consent for publication

Not Applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Gowda AU, McNichols CH, Asokan I, Matthews JA, Buckingham EB, et al. Porcine acellular dermal matrix for hernia repair in transplant patients. *Ann Plast Surg* 2016;77:674-7.
2. Garmpis N, Spartalis E, Schizas D, Patsouras D, Damaskos C, et al. Incisional hernias post liver transplantation: current evidence of epidemiology, risk factors and laparoscopic versus open repair. A review of the literature. *In Vivo* 2019;33:1059-66.
3. Ayvazoglu Soy EH, Kirnap M, Yildirim S, Moray G, Haberal M. Incisional hernia after liver transplant. *Exp Clin Transplant* 2017;15:185-9.
4. Fikatas P, Schoening W, Lee JE, Chopra SS, Seehofer D, et al. Incidence, risk factors and management of incisional hernia in a high volume liver transplant center. *Ann Transplant* 2013;18:223-30.
5. Hegab B, Abdelfattah MR, Azzam A, Al Sebayel M. The usefulness of laparoscopic hernia repair in the management of incisional hernia following liver transplantation. *J Minim Access Surg* 2016;12:58-62.
6. Piazzese E, Montalti R, Beltempo P, Bertelli R, Puviani L, et al. Incidence, predisposing factors, and results of surgical treatment of incisional hernia after orthotopic liver transplantation. *Transplant Proc* 2004;36:3097-8.
7. Aydin U, Pinar Y, Murat K. Optimal technique for abdominal fascial closure in liver transplant patients. *Asian J Surg* 2010;33:1-7.
8. Kanters AE, Krpata DM, Blatnik JA, Novitsky YM, Rosen MJ. Modified hernia grading scale to stratify surgical site occurrence after open ventral hernia repairs. *J Am Coll Surg* 2012;215:787-93.
9. Goodenough CJ, Ko TC, Kao LS, Nguyen MT, Holihan JL, et al. Development and validation of a risk stratification score for ventral incisional hernia after abdominal surgery: hernia expectation rates in intra-abdominal surgery (the HERNIA Project). *J Am Coll Surg* 2015;220:405-13.
10. Cherla DV, Moses ML, Mueck KM, Hannon C, Ko TC, et al. External validation of the HERNIA score: an observational study. *J Am Coll Surg* 2017;225:428-34.
11. Liang MK, Goodenough CJ, Martindale RG, Roth JS, Kao LS. External validation of the ventral hernia risk score for prediction of surgical site infections. *Surg Infect (Larchmt)* 2015;16:36-40.
12. Levi DM, Tzakis AG, Kato T, Madariaga J, Mittal NK, et al. Transplantation of the abdominal wall. *Lancet* 2003;361:2173-6.
13. Troppmann C, Santhanakrishnan C, Kuo JH, Bailey CM, Perez RV, et al. Impact of panniculectomy on transplant candidacy of obese patients with chronic kidney disease declined for kidney transplantation because of a high-risk abdominal panniculus: a pilot study. *Surgery* 2016;159:1612-22.

14. Dudley C, Harden P. Assessment of the potential kidney transplant recipient. Available from: <https://renal.org/wp-content/uploads/2017/06/assessment-of-the-potential-kidney-transplant-recipient-5th-edition-1.pdf>. [Last accessed on 9 Sep 2019]
15. Ngaage LM, Elegbede A, Tadisina KK, Gebran SG, Masters BM, et al. Panniculectomy at the time of living donor renal transplantation: an 8-year experience. *Am J Transplant* 2019;19:2284-93.
16. Coccolini F, Catena F, Bertuzzo VR, Ercolani G, Pinna A, et al. Abdominal wall defect repair with biological prosthesis in transplanted patients: single center retrospective analysis and review of the literature. *Updates Surg* 2013;65:191-6.
17. Fahrenbach EN, Qi C, Ibrahim O, Kim JY, Alam M. Resistance of acellular dermal matrix materials to microbial penetration. *JAMA Dermatol* 2013;149:571-5.
18. Brewer MB, Rada EM, Milburn ML, Goldberg NH, Singh DP, et al. Human acellular dermal matrix for ventral hernia repair reduces morbidity in transplant patients. *Hernia* 2011;15:141-5.
19. Earle D, Roth JS, Saber A, Haggerty S, Bradley JF 3rd, et al; SAGES Guidelines Committee. SAGES guidelines for laparoscopic ventral hernia repair. *Surg Endosc* 2016;30:3163-83.
20. Singh DP, Zahiri HR, Gastman B, Holton LH 3rd, Stromberg JA, et al. A modified approach to component separation using biologic graft as a load-sharing onlay reinforcement for the repair of complex ventral hernia. *Surg Innov* 2014;21:137-46.
21. Alizai PH, Lelaona E, Andert A, Neumann UP, Klink CD, et al. Incisional hernia repair of medium- and large-sized defects: laparoscopic IPOM versus open SUBLAY technique. *Acta Chir Belg* 2019;119:231-5.
22. Black CK, Zolper EG, Walters ET, Wang J, Martinez J, et al. Utility of a modified components separation for abdominal wall reconstruction in the liver and kidney transplant population. *Arch Plast Surg* 2019;46:462-9.
23. Hodgkinson JD, Leo CA, Maeda Y, Bassett P, Oke SM, et al. A meta-analysis comparing open anterior component separation with posterior component separation and transversus abdominis release in the repair of midline ventral hernias. *Hernia* 2018;22:617-26.
24. Malangoni MA, Rosen MJ. Hernias. In: Townsend Jr CM, Beauchamp RD, Evers BM, Mattox KL, editors. *Sabiston Textbook of Surgery: The Biological Basis of Modern Surgical Practice*. 20th ed. Philadelphia, PA: Saunders Elsevier; 2017. pp.1109-10.
25. Giele H, Vaidya A, Reddy S, Vrakas G, Friend P. Current state of abdominal wall transplantation. *Curr Opin Organ Transplant* 2016;21:159-64.
26. Cipriani R, Contedini F, Santoli M, Gelati C, Sgarzani R, et al. Abdominal wall transplantation with microsurgical technique. *Am J Transplant* 2007;7:1304-7.
27. Selvaggi G, Levi DM, Cipriani R, Sgarzani R, Pinna AD, et al. Abdominal wall transplantation: surgical and immunologic aspects. *Transplant Proc* 2009;41:521-2.
28. Park SH, Eun SC. Abdominal wall transplant surgery. *Exp Clin Transplant* 2018;16:745-50.
29. Berli JU, Broyles JM, Lough D, Shridharani SM, Rochlin D, et al. Current concepts and systematic review of vascularized composite allotransplantation of the abdominal wall. *Clin Transplant* 2013;27:781-9.
30. Barnes J, Issa F, Vrakas G, Friend P, Giele H. The abdominal wall transplant as a sentinel skin graft. *Curr Opin Organ Transplant* 2016;21:536-40.
31. Quigley MA, Fletcher DR, Zhang W, Nguyen VT. Development of a reliable model of total abdominal wall transplantation. *Plast Reconstr Surg* 2013;132:988-94.
32. Broyles JM, Berli J, Tuffaha SH, Sarhane KA, Cooney DS, et al. Functional abdominal wall reconstruction using an innervated abdominal wall vascularized composite tissue allograft: a cadaveric study and review of the literature. *J Reconstr Microsurg* 2015;31:39-44.
33. Hunter DJ, Hankinson SE, Laden F, Colditz GA, Manson JE, et al. Plasma organochlorine levels and the risk of breast cancer. *N Engl J Med* 1997;337:1253-8.

Review

Open Access



# Prevention of hyper- and hypertrophic scars through surgical incisions in the direction of the “main folding lines” of the skin

Gottfried Lemperle

Division of Plastic Surgery, University of California, San Diego, CA 92103-8890, USA.

**Correspondence to:** Prof. Gottfried Lemperle, Plastic Surgeon, Wolfsgangstr. 64, Frankfurt am Main D-60322, Germany.  
E-mail: lempelerle8@aol.com

**How to cite this article:** Lemperle G. Prevention of hyper - and hypertrophic scars through surgical incisions in the direction of the “main folding lines” of the skin. *Plast Aesthet Res* 2020;7:40. <http://dx.doi.org/10.20517/2347-9264.2020.14>

**Received:** 30 Apr 2019 **First Decision:** 1 Jun 2020 **Revised:** 17 Jun 2020 **Accepted:** 20 Jun 2020 **Published:** 26 Jul 2020

**Academic Editor:** Alexis Desmoulière, Jérôme Laloze **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Langer's lines are still the recommendation and matrix for surgical incisions in most surgical textbooks, even if they were never meant to be by their first describer in 1861. To achieve minimal scarring, surgeons should attempt to make incisions parallel to skin tension lines, i.e., in skin folds or skin creases. On the basis of visible stretch marks (striae distensae) in the skin, which always appear in the same direction against skin tension in men and women, the direction of skin tension lines can be manifested also in the skin of children and young patients. These invisible or virtual tension lines are the same as the main folding lines (MFL) in adults and run perpendicular to the stretch marks. While well-established on the face and abdomen, these folding lines may not be obvious on other parts of the body. On chest, back and extremities, optimal direction of surgical skin incisions should take into account the patterns of striae distensae, which develop perpendicular to skin tension lines. MFL should be used in elective incisions in children, adolescents, and young women as a guide for the prevention of later visible hyper- or hypertrophic scars.

**Keywords:** Surgical incisions, folding lines, skin tension lines, Langer's lines, striae distensae, stretch marks, hypertrophic scar

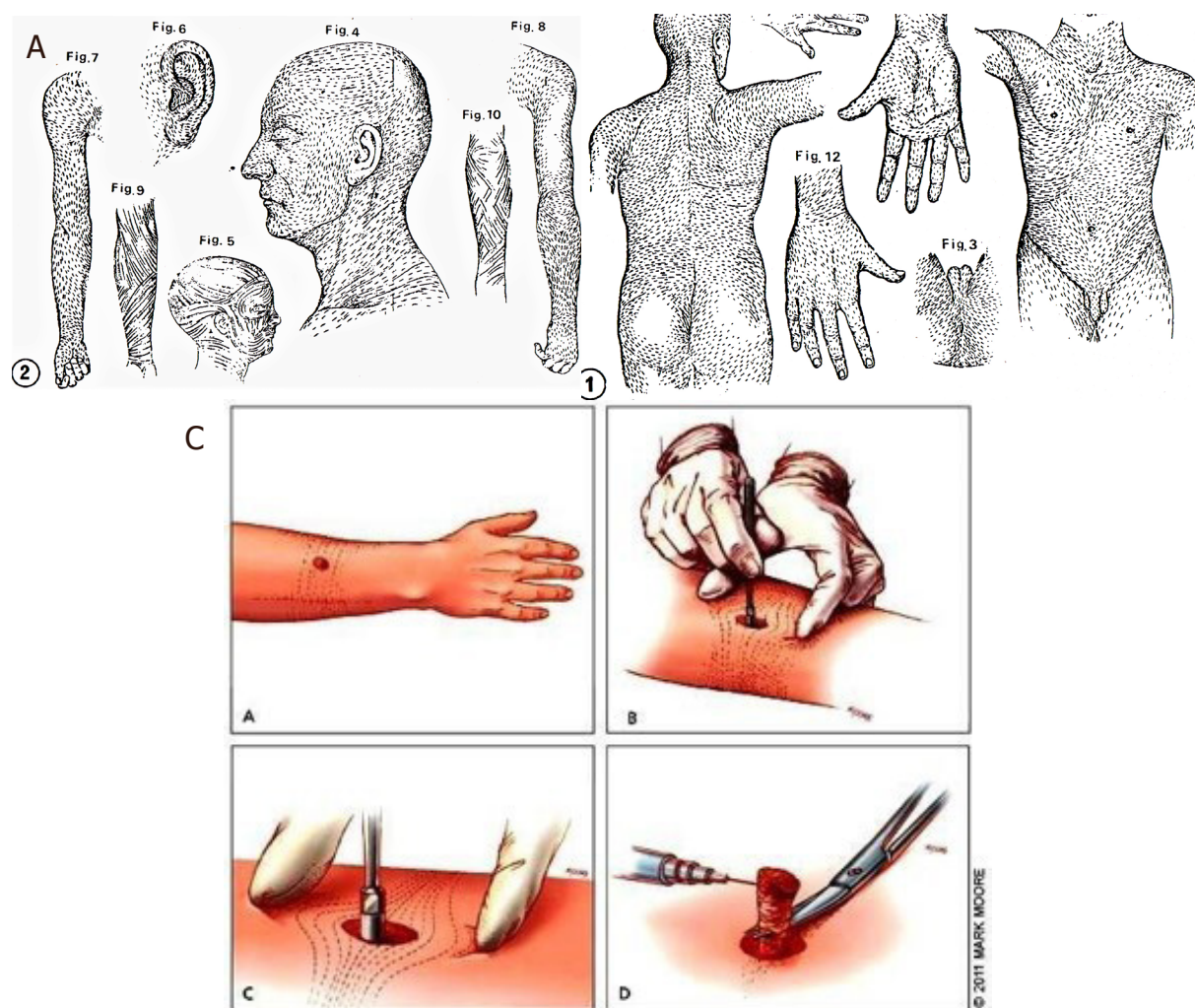
## INTRODUCTION

Minimally invasive and endoscopic surgeries have revolutionized thoracic, abdominal, and orthopedic surgery, and have become today's gold standard, resulting in much smaller scars. However, children and



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** Some Langer's "cleavage lines" run oblique or perpendicular to the recommended "main folding lines" on forehead, lower abdomen, buttocks and extremities (A); on the other hand, some Langer's cleavage lines run parallel to the "main folding lines" over neck, shoulders, upper abdomen and back and therefore correctly in the direction of the proposed "tension lines" (B) (both figures are reproduced with permission from Lemperle *et al.*<sup>[11]</sup>); Langer punched round holes into cadaver skin at various sites to determine invisible "cleavage lines" (C) (Drawings by Mark Moore 2011)

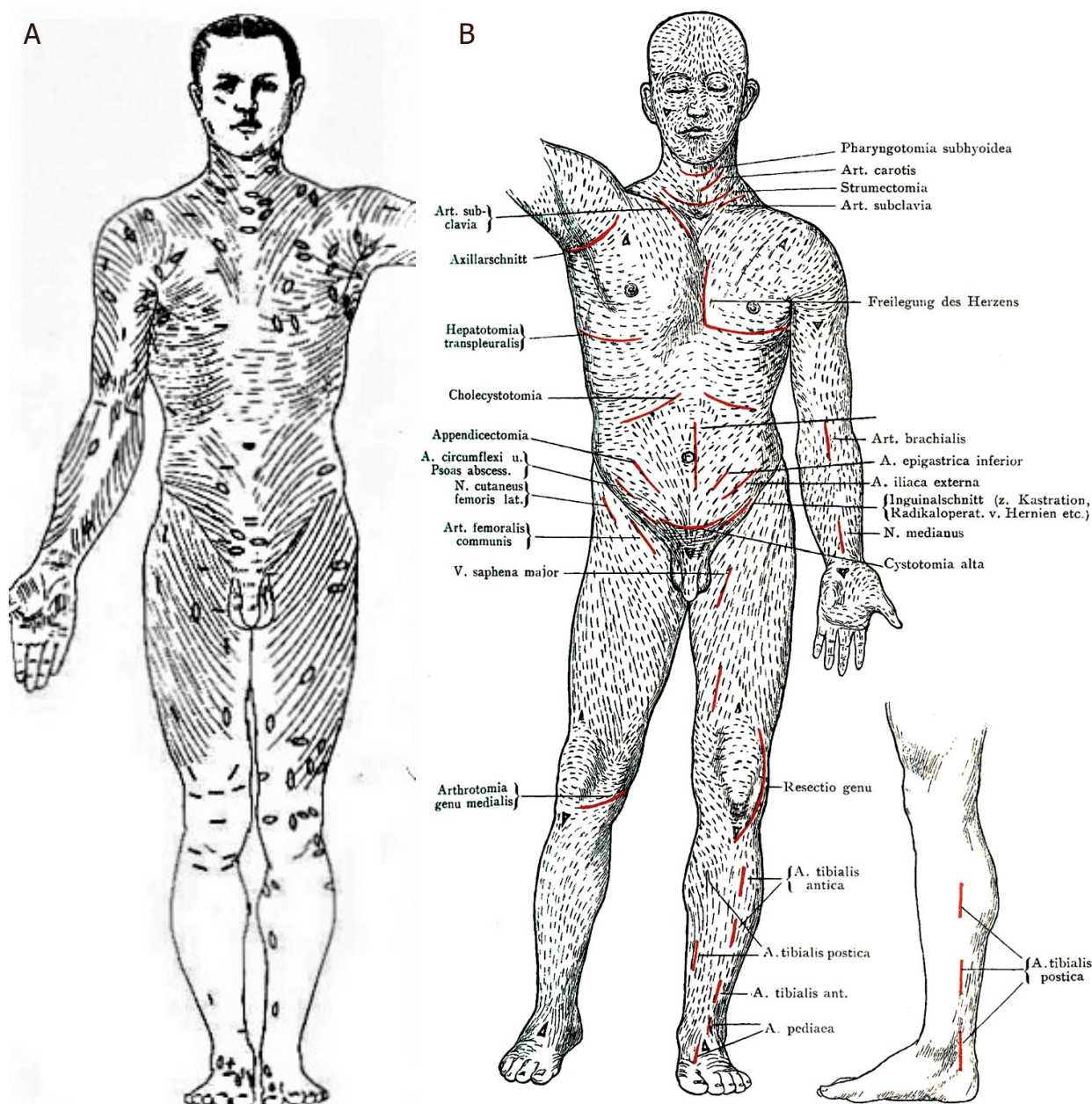
adolescents are still prone to develop hypertrophic scars, which could be prevented by surgical incisions in the direction of the main folding lines (MFL) of the skin. The list of ineffective treatments for scars is long, including heparin, panthenol and silicone creams, needling rollers, and laser therapy. However, pressure dressings and corticosteroid injections are the only effective treatments in fresh and surgical excision of mature hypertrophic scars, while injectable dermal fillers can be used in broad indented hypertrophic scars.

Surgical and orthopedic textbooks do not mention natural folding lines for optimal surgical incisions. The purpose of this review is to facilitate the determination of optimal incision lines perpendicular to the direction of striae distensae.

### History of surgical incision lines

When consulted to correct conspicuous scars from prior surgeries, some surgeons still rely on Langer's invisible "cleavage lines" described in 1861<sup>[1]</sup>, which run perpendicular to skin folds in several regions of the body [Figure 1A and B]. However, folds and folding lines are easily determined in adults by bending a joint or pinching the skin in different directions.

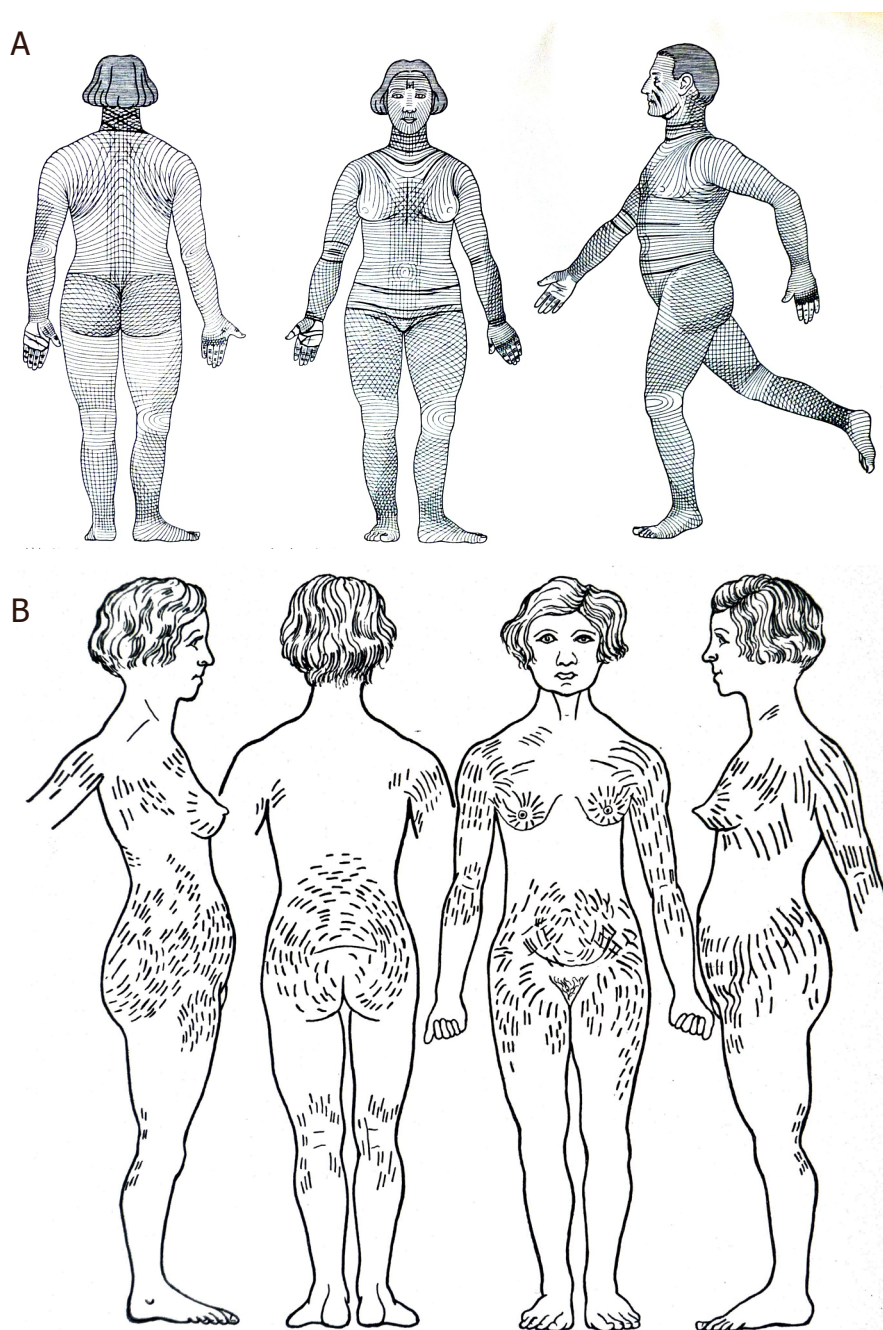




**Figure 2.** As early as 1892, Kocher recommended “Langer’s cleavage lines” for all surgical excisions and incisions (A); especially on extremities of children and adolescents, the lengthwise running scars often become hypertrophic (B) (both figures are reproduced with permission from Lemperle *et al.*<sup>[1]</sup>)

Langer, an anatomist in Vienna, Austria, had no surgical incisions in mind during his well-known experiments on cadaver skin. After punching round holes into cadaver skin, he suggested the best direction of the wound margins by invisible “cleavage lines”, which eventually became oval-shaped<sup>[1]</sup> [Figure 1C]. In 1892 Kocher, a famous surgeon and Nobel laureate in Bern, Switzerland, promoted Langer’s lines worldwide as direction for skin excisions and incisions<sup>[2]</sup> [Figure 2A and B].

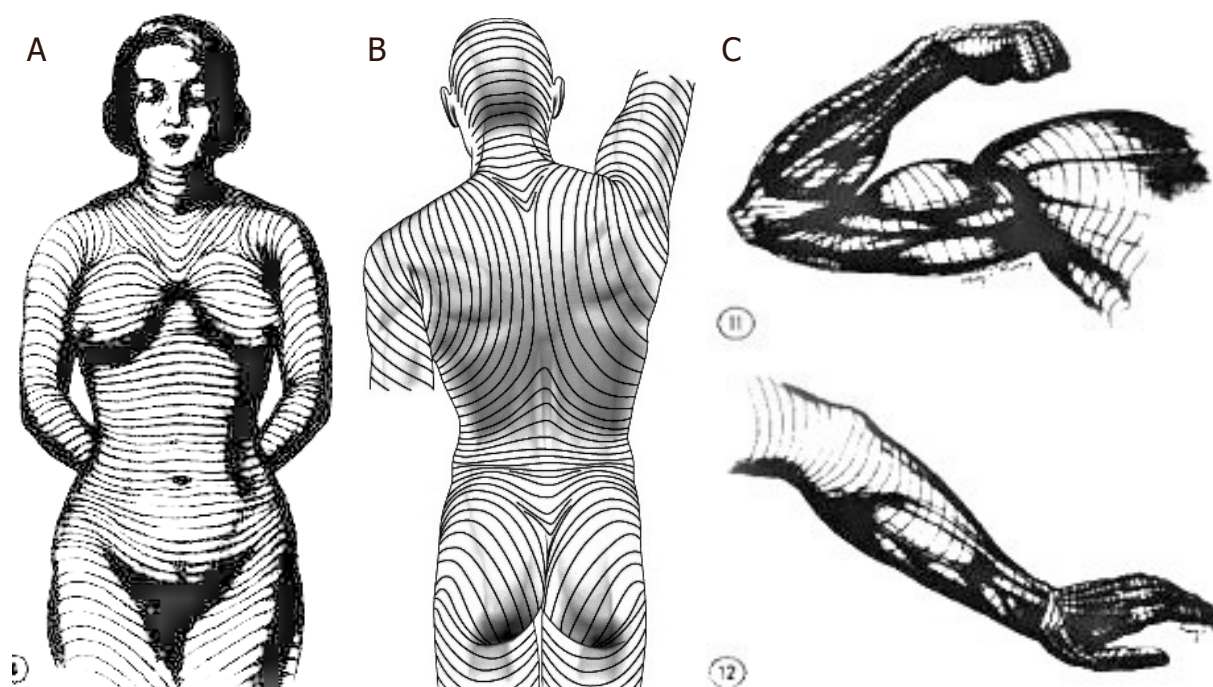
To achieve minimal scar formation, surgical incisions must be placed in “main folding lines”<sup>[3-5]</sup> [Figure 3A and B] or in the also invisible “relaxed skin tension lines” of Borges<sup>[6-8]</sup>. Although facial and abdominal folds can be easily identified, folding lines may be difficult to determine on the back, arms and legs, and may



**Figure 3.** Pinkus' "main folding lines" (MFL) of the skin facilitate optimal incision lines. These drawings, however, are irritating to a surgeon because they are not clearly defined at the extremities (A); a drawing of collected striae gravidarum by Pinkus in 1927 (B) (both figures are reproduced with permission from Lemperle *et al.*<sup>[11]</sup>)

be absent in younger patients. Interestingly, the stripes of tigers and zebras, and the folds in Sharpei dogs follow similar lines, transverse at the torso and extremities, and concentric towards the inner side of large joints.

Aesthetic surgeons learn to follow specific directions for incisions, and reconstructive surgeons may be uncertain of the optimal direction. General surgeons focus on the fastest and most direct way to reach the abdominal cavity, while orthopedic surgeons focus on the most convenient way to access a bone or joint. Surgical and orthopedic textbooks do not mention natural folding lines for optimal incisions<sup>[9,10]</sup>. The



**Figure 4.** Kraissl's lines (1954) run perpendicular to the direction of underlying muscles but are not correct over breasts, lower back and buttocks (A-C) (these figures are reproduced with permission from Lemperle *et al.*<sup>[11]</sup>)

concept of using natural striae distensae for optimal incision lines is new<sup>[11]</sup> and an extension of the earlier presentations of Kraissl<sup>[4,5]</sup> and Borges<sup>[6-8]</sup>.

During the past century, thirty-eight various guidelines have been developed regarding elective skin incisions<sup>[6,12]</sup>. Most surgical textbooks include Kraissl's "incisions perpendicular to muscle action" of 1951<sup>[5]</sup> [Figure 4A-C] or Langer's "cleavage lines" of 1861<sup>[1,12,13]</sup> [Figure 1A and B], despite the fact that these incisions run oblique or even perpendicular to skin folds on the forehead, cheeks, breasts, and abdomen. They run vertically across the antecubital region, the wrist, thigh, and distal regions of the extremities, although concordant on the neck, shoulders, back and buttocks. Therefore, new oblique, horizontal or partly circumferential directions for incisions on extremities have to be proposed.

In 1927, dermatologist Felix Pinkus was the first to question Langer's lines as a guide for skin incisions, and he described the "main folding lines" [Figure 3A] as the ideal direction for elective incisions<sup>[3]</sup>. He also described and illustrated the location of striae [Figure 3B], but did not relate them to skin incisions. The publication in a German dermatological textbook<sup>[3]</sup> did not reach the surgical community.

In the 1950s, Cornelius Kraissl<sup>[4,5]</sup> proposed lines oriented perpendicular to the action of the underlying muscles [Figure 4A-C] by demonstrating histologically that the adherent connective tissue bands run from the skin to the underlying fascia, perpendicular to the long axis of the muscles, especially at joints such as wrist and knee. His drawing of the facial folds shows long-accepted incision lines on the face.

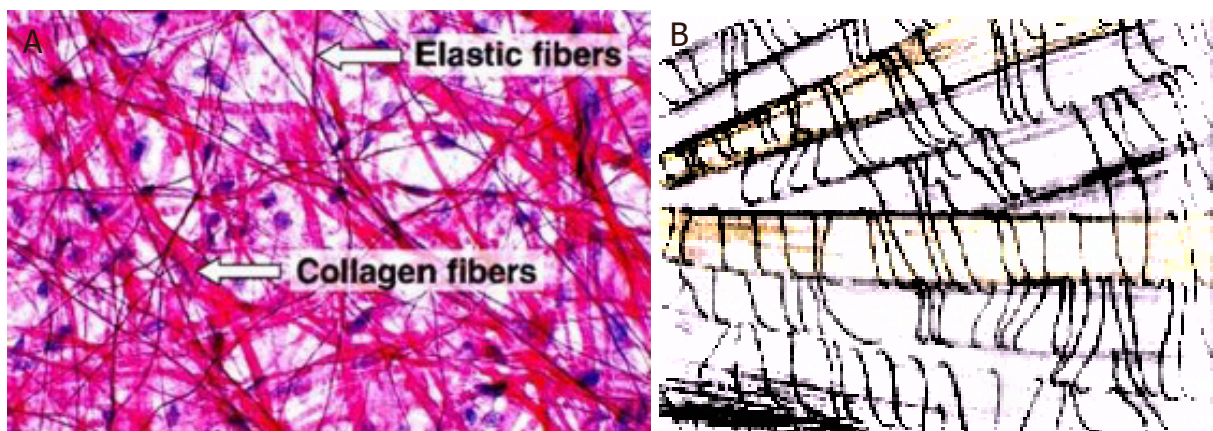
In 1962, Alberto Borges<sup>[6,7]</sup> described the "relaxed skin tension lines" (RSTL) on the face and in 1984 on the body<sup>[8]</sup>. RSTL follow furrows produced by pinching the skin in different directions - which had been recommended earlier by Pinkus<sup>[2]</sup> and Kraissl<sup>[4]</sup>.

This "pinching", however, is difficult to practice on the back and extremities of younger patients, especially on the forearm in both, the supine and pronate position<sup>[14]</sup>. Courtiss in 1963<sup>[15]</sup> and Barile in 1976<sup>[16]</sup>





**Figure 5.** If all patients presented with these clear folding lines, the choice of skin incisions would be easy (A and B)



**Figure 6.** The network of fibers in the dermis will be compressed beneath a skin fold. The transverse elastic fibers will remain together with the parallel collagen fibers; those diagonal to the fold will be displaced or absorbed as the fold deepens (A and B) (EM of R. Roth, Tübingen)

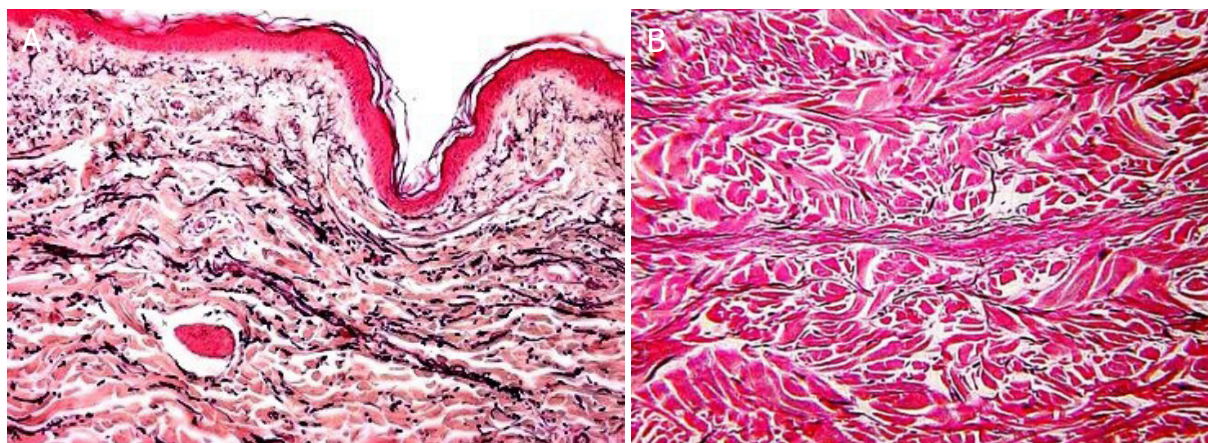
essentially copied the article and illustrations of Kraissl<sup>[4]</sup> and recommended, just like Borges<sup>[8]</sup>, to follow Kraissl's lines for the rest of the body.

Uncertainty about the best direction of surgical incisions led to more proposals. Carmichael<sup>[13]</sup> used a “Reviscometer” to measure electrical waves travelling radially on the skin, and Paul<sup>[17,18]</sup> recommended a bi-directional “Tensiometer”, a type of broad plastic forceps connected to an electrical indicator to measure skin tension. There appears to be no practical value, as nature already presents skin tension lines as folds in elderly patients [Figure 5A and B] or as striae distensae in many younger ones.

### Histology of tension lines

The presence of tension lines and normal wrinkle lines in the skin depends on the interrelation between elastic fibers and collagen fibers, as well as on the anchorage of collagen bundles one upon another<sup>[19]</sup> [Figure 6A and B]. While collagen fibers underlying Langer's cleavage lines are arranged irregularly and interweaving, they are lined up parallel beneath folds in their direction.

The fibroblasts in striae possess a contractile phenotype more akin to myofibroblasts<sup>[19,20]</sup>, and the elastic fiber network proximal to the epidermal- dermal junction appears to be more prone to destruction in



**Figure 7.** A skin fold with reduced collagen fibers underneath but compressed elastic fibers (black) running diagonal to the fold. The collagen fibers are mainly running parallel to the fold but cut across the fold (A and B)

active striae. The collagen lattice can be ruptured under the influence of steroids and especially estrogens. The newly synthesized collagen becomes reorganized by tension and is aligned in the direction of the presumed stress. The same happens in wound healing, and therefore, striae are considered dermal scars<sup>[21,22]</sup> [Figure 7A and B].

### Striae distensae

Striae distensae or striae gravidarum are seen in many patients and can often act as a guide in planning elective incisions. Regardless of their etiology and slight variation, they have the same direction and clinical appearance [Figure 3A and B].

The fibroblasts in striae possess a contractile phenotype more akin to myofibroblasts<sup>[19,20]</sup> and the elastic fiber network proximal to the epidermal- dermal junction appears to be more prone to destruction in active striae. The collagen lattice can be ruptured under the influence of steroids and especially estrogens. The newly synthesized collagen becomes reorganized by tension and is aligned in the direction of the presumed stress. The same happens in wound healing, and therefore, striae are considered dermal scars<sup>[21,22]</sup>.

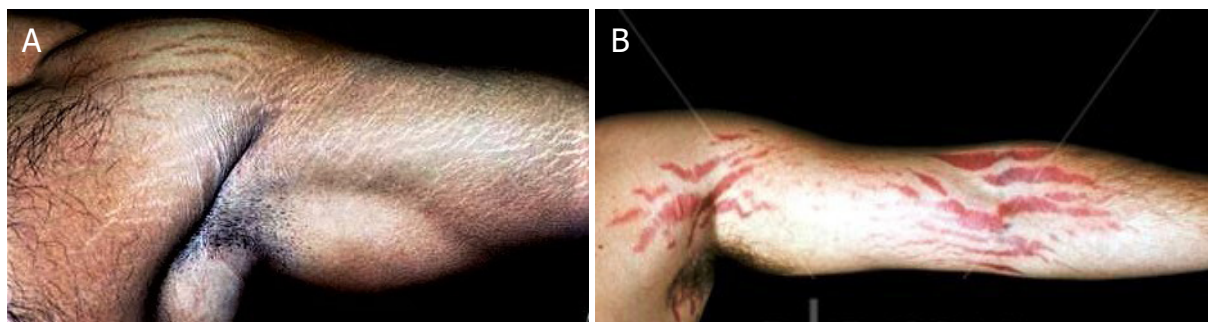
Striae distensae are characterized by linear, smooth bands of atrophic skin that are reddish at first and ultimately turn pale. In young adolescents, they can appear during growth spurts on hips, inner thighs, and female breasts, as well as on the shoulders, lower back, and outer thighs of boys without measurable changes in their hormone levels<sup>[22]</sup> [Figures 8-11]. Eighty percent of adolescents in Korea show striae: in girls more pronounced on the buttocks, thighs and calves, and in boys on the buttocks, knees and lower back<sup>[23]</sup>.

During pregnancy, striae distensae appear in the abdominal skin often during the first months of pregnancy and before tension is caused by the growing fetus and uterus. Obesity, oral contraceptives, and breast augmentation can also cause striae in a small percentage of women<sup>[24]</sup>. The widest and deep red striae are seen in Cushing patients and in those under chronic systemic or local cortisone therapy<sup>[25,26]</sup> [Figure 12].

Women with pelvic relaxation and prolapse demonstrate striae twice as often as healthy women. There appears to be a strong association between the presence of striae, varicosis, and the development of pelvic relaxation<sup>[27]</sup>; genetically weak connective tissue appears to be the reason for all three deformities<sup>[28]</sup>.

The claim of mechanical stretching being the main cause of striae is disputable: striae often occur in early pregnancy when there is no obvious skin stretching by the growing uterus. They also appear over the hips

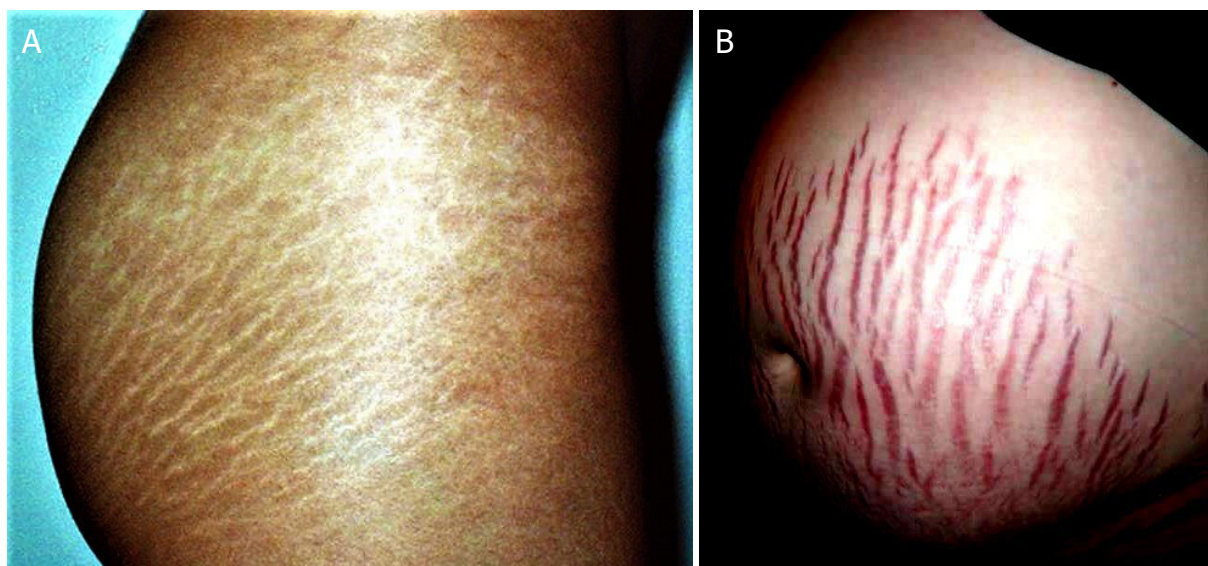




**Figure 8.** Striae in a body builder and a Cushing patient run parallel to the direction of the muscle bundles: therefore, all incisions on extremities should be made oblique but should not interfere with major nerves and arteries (A and B)



**Figure 9.** Radial fresh striae on the breasts during pregnancy and mature striae postpartum (A and B)

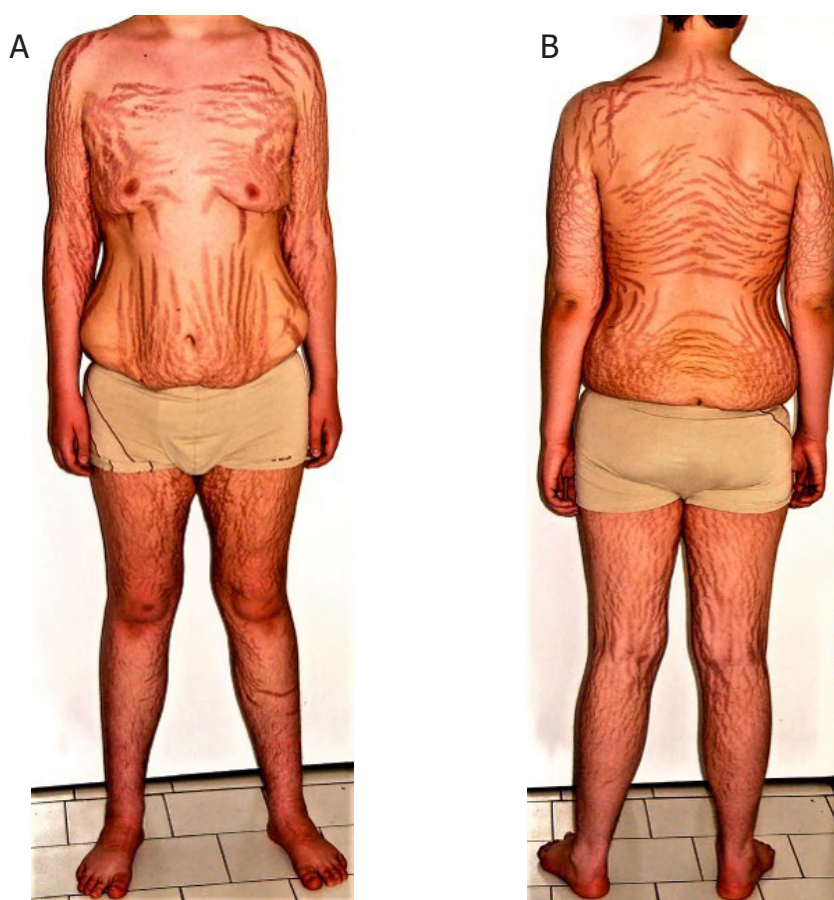


**Figure 10.** As an exception, striae distensae over the buttocks run perpendicular to the muscle fibers of the underlying gluteus major muscle (A and B) (Figure 10A is reproduced with permission from Lemperle *et al.*<sup>[1]</sup>)

of adolescents, where there is little stretching during the day or sleep. If skin expansion is the only reason, the use of inflatable silicone expanders should be accompanied by a certain incidence of striae formation, but it is not.



**Figure 11.** Striae on the inner thigh run oblique and vertical over the knee joint; therefore, incisions should be made perpendicular to them, i.e., horizontally in the popliteal groove (A and B) (Figure 11A is reproduced with permission from Lemperle *et al.*<sup>[11]</sup>)



**Figure 12.** Extreme striae distensae in a 14-year-old boy with encephalitis, treated with common doses of dexamethasone over 9 months (A and B) (both figures are reproduced with permission from Lemperle *et al.*<sup>[11]</sup>)



Also, if excessive skin stretching were a factor, athletes, gymnasts, and heavy-lifting workers would be expected to develop more stretch marks than the average population. Yet, the only athletes known to develop them are weightlifters and probably due to the use of anabolic steroids. In general, potential causes for striae development are family history, difficult hormonal equilibrium during adolescence, obesity, and the presence of varicosis<sup>[29]</sup>.

Recent research on estrogen receptors in the extracellular matrix suggests the importance of estrogen in the development of striae<sup>[30]</sup>. When estrogen, androgen and glucocorticoid receptors in the skin of patients with striae were compared to those in healthy skin, their number was actually double<sup>[31]</sup>. These findings indicate that under certain conditions, there is an increase in hormone receptor expression in the skin. Hormone receptor activity may influence the metabolism of the extracellular matrix, pointing to the important involvement of estrogens in striae formation.

In general, people with a lack of elastic components in the extracellular matrix of their dermis are more prone to hypotrophic than hypertrophic scar formation. To date, there still remains a lack of logical and effective, evidence-based treatments for stretch marks, including non-surgical skin tightening procedures, such as laser treatments or topical preparations that claim “collagen remodeling”<sup>[32]</sup>.

## MATERIALS, METHODS, RESULTS

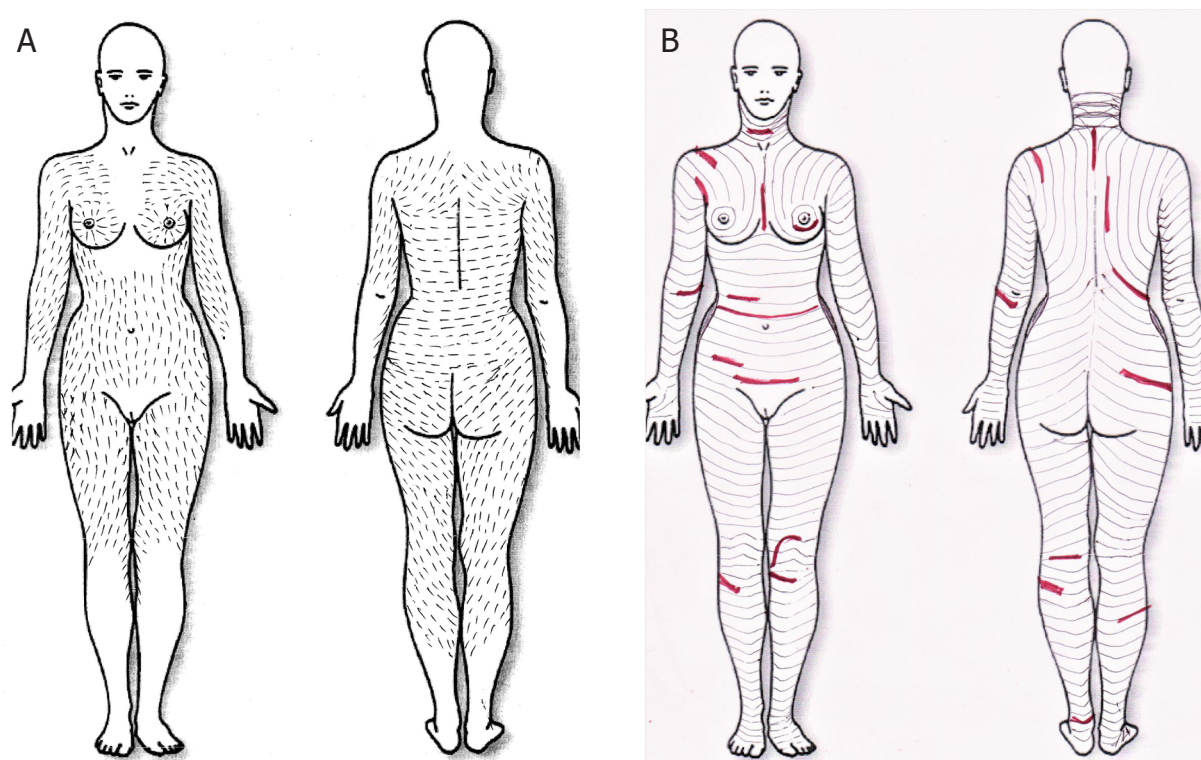
A total of 213 photographs of patients with striae as adolescents were examined, during and after pregnancy, diagnosed with “linear focal elastosis”<sup>[33,34]</sup> and after Cushing’s disease or steroid use and abuse. Sources included our own image files (78 photographs) as well as an extensive Google search on “stretch marks” and “striae” (135 photographs). All relevant striae were copied onto blank templates and 3 overall direction charts were created [Figure 13A]. Regardless of their etiology, all striae demonstrated a similar clinical appearance and same direction in both male and female skin. Furthermore, the direction of the lamellae in patients with linear ichthyosis was the same as the skin’s MFL across the entire body<sup>[35]</sup>.

Optimal incision lines were established from a slide collection of the Department of Plastic Surgery at Markus Hospital in Frankfurt/Main, Germany, including hundreds of surgical scar corrections from the past 50 years. Each direction of a “right or wrong” incision was compared with the direction of the folding lines and judged either “optimal” within the MFL, or “suboptimal”, if the scar was hypertrophic or wider than expected. In addition, 276 images of unknown surgical incisions and scars were retrieved from the Internet, and their direction and width were compared to the MFL.

Photographs of striae did not include all regions of the body. The logical elongation of the direction of striae over the front of the lower leg was retrieved from the folding lines of elderly patients and patients with linear ichthyosis. In children and adolescents, the main skin folding lines on the face, neck, hand and foot were found in skin folds by moving the head or limbs.

The striae-derived tension or MFL [Figure 13B] were consistent with the direction of Kraissl’s lines on the shoulder, chest, abdomen, arms and legs, but not on the breasts, lower back, and buttocks, where they run parallel to the muscle fibers of the gluteus maximus muscle. However, they disproved Langer’s lines on the lower abdomen, back, buttocks, posterior thigh, and foot, but coincided with Langer’s lines on the upper back, chest, upper abdomen, anterior thigh, knee, and lower leg.

Alternatively, Pinkus<sup>[3]</sup> described several directions of skin folding lines over body and limbs [Figure 4], rather than an ideal direction for surgeons to follow. Striae distensae follow the natural anti-tension lines of the skin of all ages and races and are therefore an objective indicator for the direction of the real tension lines, e.g., always perpendicular to them.



**Figure 13.** A compound of striae lines collected from 213 photographs of patients with different underlying etiology. The resulting “main folding lines” (MFL) run perpendicular to the striae lines (A and B)

### “Pinch test”

The “pinch test”<sup>[3]</sup> is an easy and practical tool to find the “main folding lines” in old and middle-aged people, but less valuable in children and adolescents. The skin has to be reasonably loose and movable, and must be able to slide over the underlying muscle fascia to create folds in the concerned area [Figure 14A and B]. In addition, one can measure the thickness of the subcutaneous fat layer.

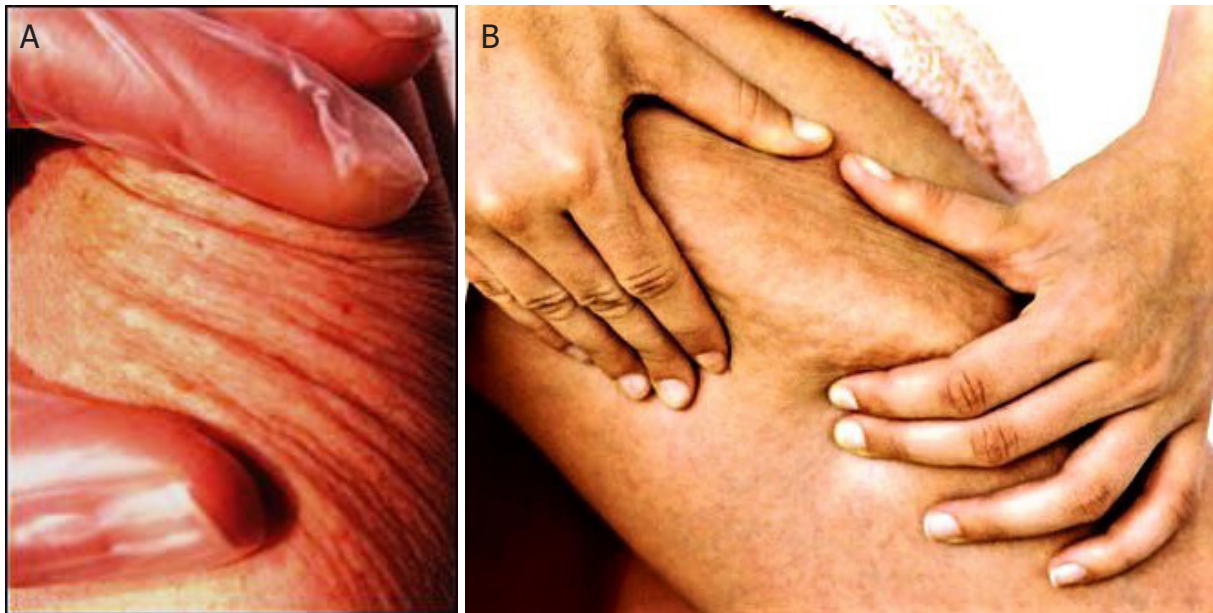
## DIRECTION OF OPTIMAL INCISIONS

### Face and neck

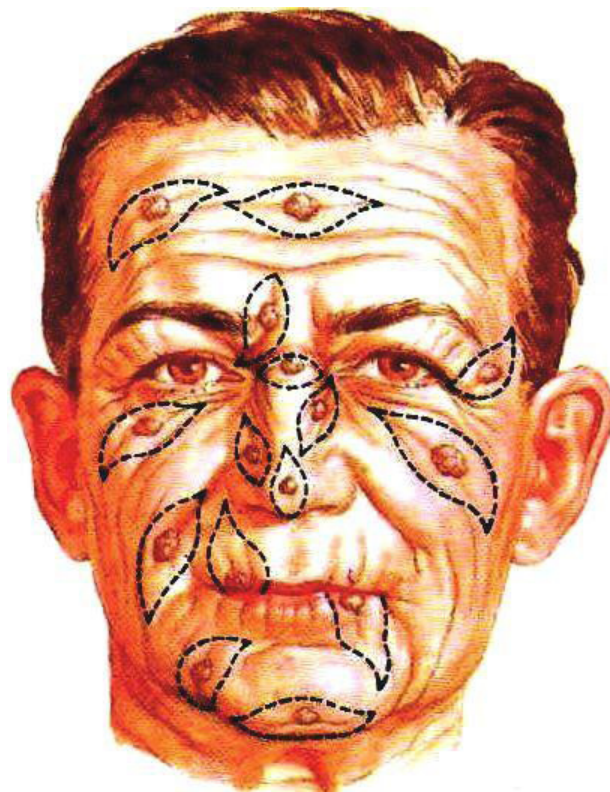
Surgical mistakes must be kept to an absolute minimum on the face, where existing folds and wrinkles<sup>[36]</sup> determine the logical direction of an incision or a fusiform skin excision [Figure 15]. For young patients, mimic movement of the face [Figure 16A and B] or a textbook on facial incisions<sup>[2,4,6-8]</sup> may serve as a guide for optimal directions.

Incision lines on the neck should run horizontally and preferably inside existing horizontal neck folds. Interestingly, Chinese adolescents frequently have already developed two or three pronounced horizontal neck folds, which should be used. On the other hand, Asian women in general develop nasolabial folds and glabellar frown lines much later than Caucasian women.

Incisions for tracheotomies<sup>[36]</sup>, thyroidectomies, or access to cervical discs should always be done higher up in the lower horizontal neck fold and clear of the jugulum to avoid hypertrophic scarring<sup>[5]</sup> [Figure 17A]. The “Kocher collar incision” dates back to the late 19th century<sup>[2]</sup>, when women were wearing high-necked dresses or heavy jewelry. Vertical tracheotomy incisions against the folding lines of the neck are often followed by ugly contracted scars<sup>[36]</sup> [Figure 17B].



**Figure 14.** The “pinch test” is an easy way to find the best direction for a surgical incision or excision on the extremities of adults and older patients, but less valuable in children, younger women, and obese patients (A and B)

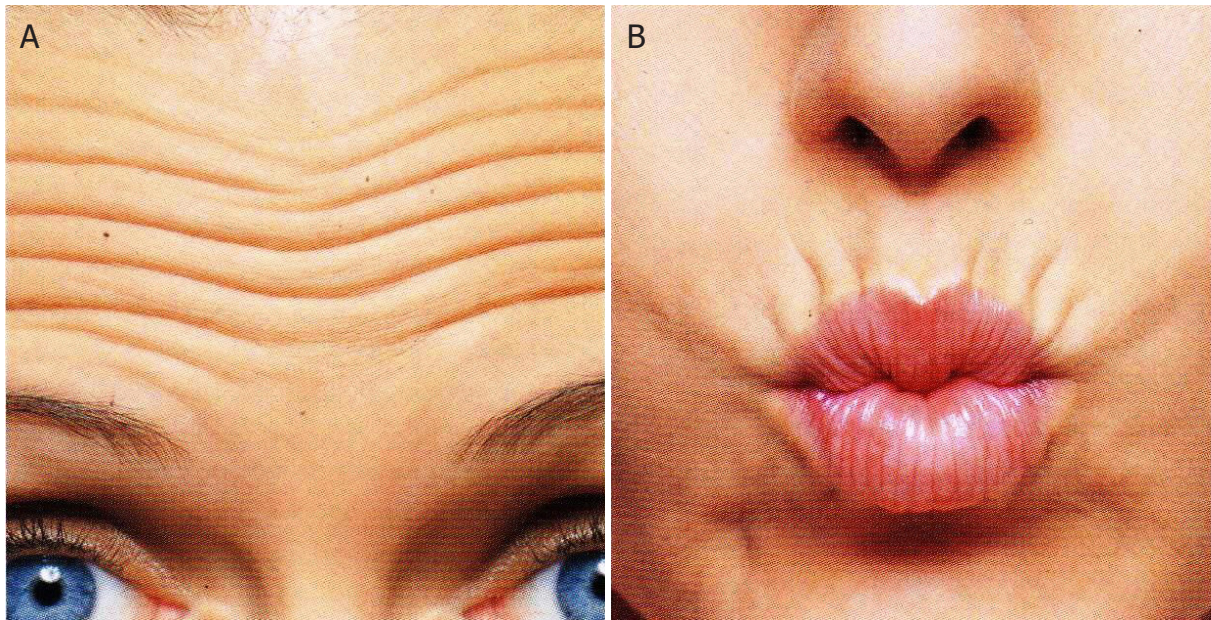


**Figure 15.** “Main folding lines” on the face and the logical directions of excisions (drawings by Frank H. Netter 1994)

### Shoulder and arm

Striae observed in body builders and in patients with linear focal elastosis or Cushing syndrome all point in one direction [Figure 8A]: horizontal over pectoral and deltoid muscles. Viewed from the front, the



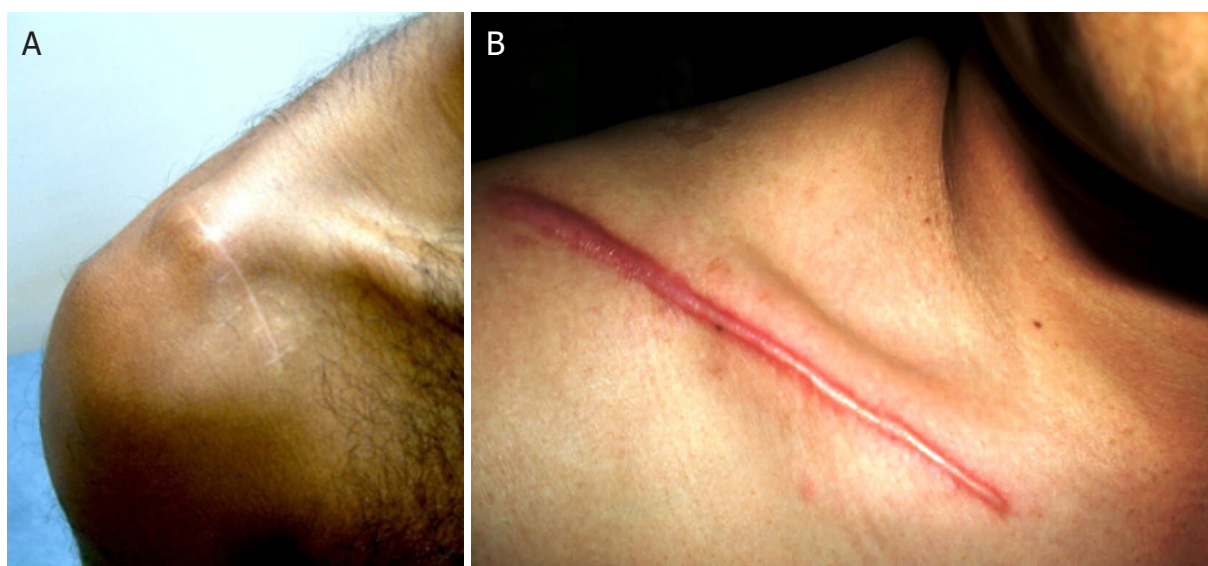


**Figure 16.** Folds can be formed during facial expressions already in young patients (A and B)



**Figure 17.** "Kocher's collar incision" over the sternal notch may leave a hypertrophic scar. The thyroid is more easily approached through an incision in the lower cervical fold. Very obvious vertical tracheotomy scar of Elisabeth Taylor (photograph: Douglas Kirkland, 1961) (A and B)

MFL therefore appear vertical between neck and shoulders, but in reality, if viewed from the side, they are virtually horizontal.



**Figure 18.** Correct incision in the main folding lines over the shoulder. The typical hypertrophic scar after fixation of a clavicle fracture could have been prevented with a shorter vertical incision in the main folding lines (A and B) (Figure 18B is reproduced with permission from Lemperle *et al.*<sup>[11]</sup>)

While fine scars are seen in the direction of folding lines [Figure 18A], wide and hypertrophic scars often develop over the shoulder, over the AC-joint and after open reposition of a clavicle fracture [Figure 18B]. Therefore, in young patients, anterior incisions across the joint and deltoid muscle should be avoided in favor of posterior vertical incisions between axilla and upper arm head.

On the arms, striae do not develop straight vertically, but somewhat obliquely from the anterior axilla to the inner elbow. The tension lines on the upper arm and forearm are not perpendicular to the muscle pull<sup>[4]</sup> or circumferential, but somewhat oblique and proceed over the joints into the horizontal skin folds. Longitudinal and vertical incisions to expose a bone fracture on the upper arm must be avoided in favor of oblique and semi-circumferential incisions, which will heal inconspicuously.

In planning an incision, the direction of the underlying cutaneous nerves and larger blood vessels must be considered. Larger cutaneous nerves of the extremities may run perpendicular to a recommended incision and must be preserved.

Longitudinal scars on the radial quadrant of the distal forearm skin envelope are typically observed to be wider than those on the ulnar quadrant and have an increased incidence of hypertrophy. The MFL on the forearm appear different in pronation and supination [Figure 14A]. Forearm rotation movements may produce differential skin tensions within the forearm skin envelope, and this may lead to differential scarring patterns<sup>[14]</sup>. Since the forearm is seen either from the inside or outside, incisions should be planned obliquely on the outer side in pronation and on the inner side in supination.

Wound healing in the hand is good in general, especially in the palm. Attention must be paid to the vascularity of the raised flaps to prevent tip necrosis, and palmar incisions should be made inside the natural creases [Figure 19A]. On the dorsum of the hand, horizontal incisions will fall into the “main folding lines”, and vertical incisions [Figure 19B] should be avoided or hidden in the ulnar thenar or on the sides of the fingers.





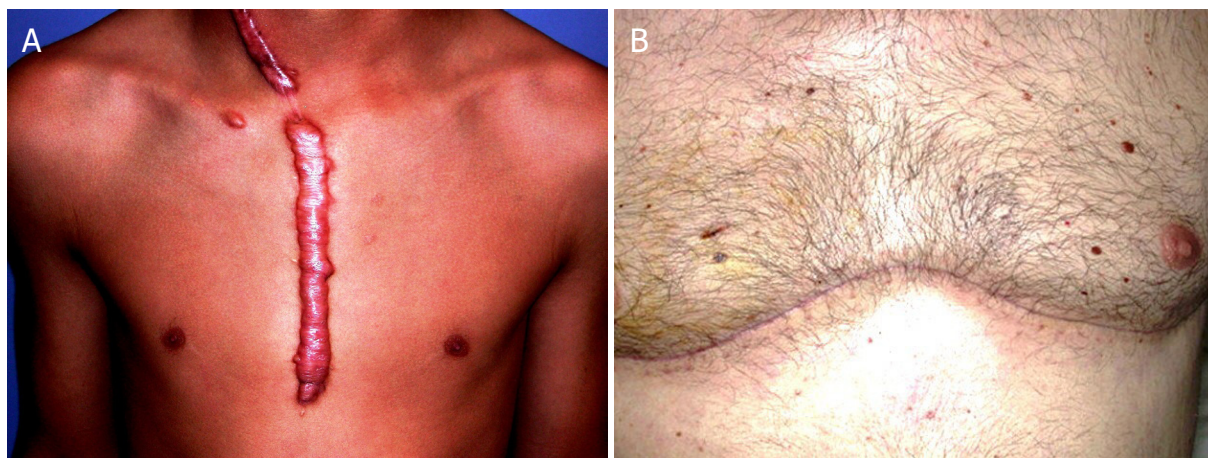
**Figure 19.** In the volar hand, visible folding lines are used to prevent hypertrophic scars. In the dorsum of the hand, horizontal incisions with preserving nerves and vessels would have produced fine scars (B), and produces fine scars (A and B)

### Chest and breast

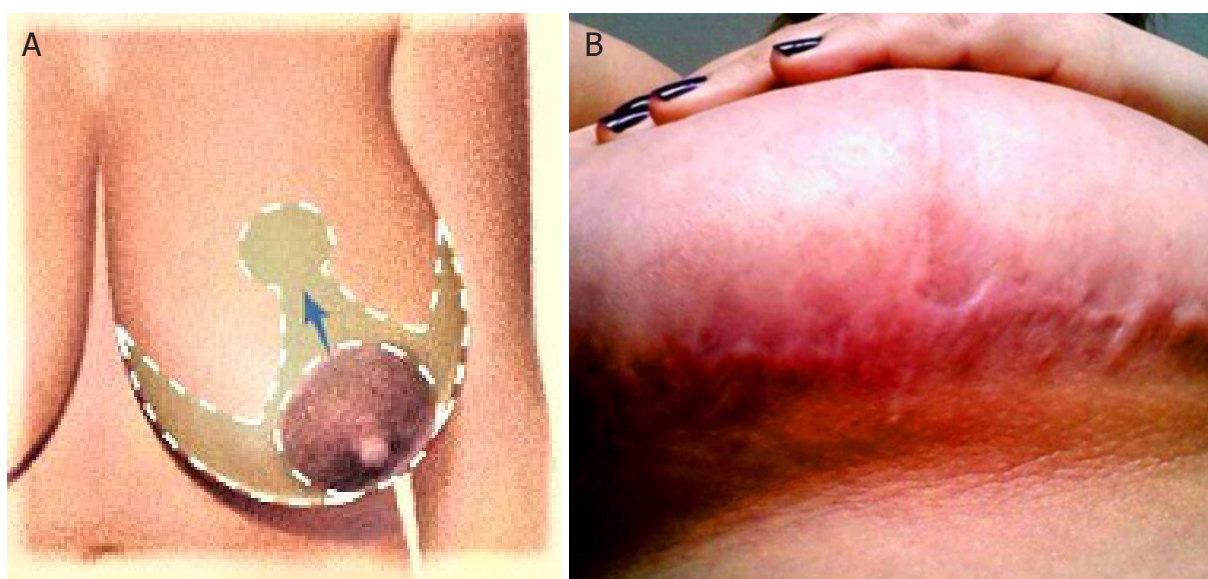
Striae over the upper chest develop mainly laterally in a horizontal direction over the pectoral muscle and run more horizontally over the deltoid muscle to the front of the horizontally stretched arm [Figure 8A]. Therefore, the lines on the chest are oblique and become more circular towards the arm, while gravitational forces and movement of the mammary gland may alter this pattern. Women, who have been sleeping on the side for many years, develop “main folding lines” in their décolleté, which originate parallel to the clavicles and run in the form of a “V” towards the mid-sternum. Therefore, incision lines should be chosen either parallel to the sternum or further caudally, and in the case of skin or breast tumors in women, circumferential at a distance around the areola, i.e., obliquely towards the sternum. Spontaneous keloids, such as the well-known “butterfly keloids”, are believed to originate from simple skin pimples and spread horizontally over the sternum often in the direction of the striae.

In performing a sternotomy in adolescents, the median skin incision should be as caudal as possible by sparing the manubrium and undermining the skin up to the sternal notch, since hypertrophic scarring is more pronounced in the upper part [Figure 20A]. Hypertrophic scarring after heart surgery may be prevented in children and young women by performing a wide, half-circumferential, horizontal incision in both inframammary folds (“clamshell incision”); the 4th intercostal space and jugulum can then be reached with the saw by bluntly raising both breasts<sup>[37]</sup> [Figure 20B].

A lateral incision through the rib cage is always performed horizontally or slightly obliquely parallel to the ribs. In young female patients, the incision should be hidden anteriorly in the respective sub-mammary fold.



**Figure 20.** In children and adolescents, open heart surgery may cause severe hypertrophic scarring, which could be avoided by a “clamshell-incision” (A and B) (Figure 20B is reproduced with permission from Lemperle *et al.*<sup>[11]</sup>)



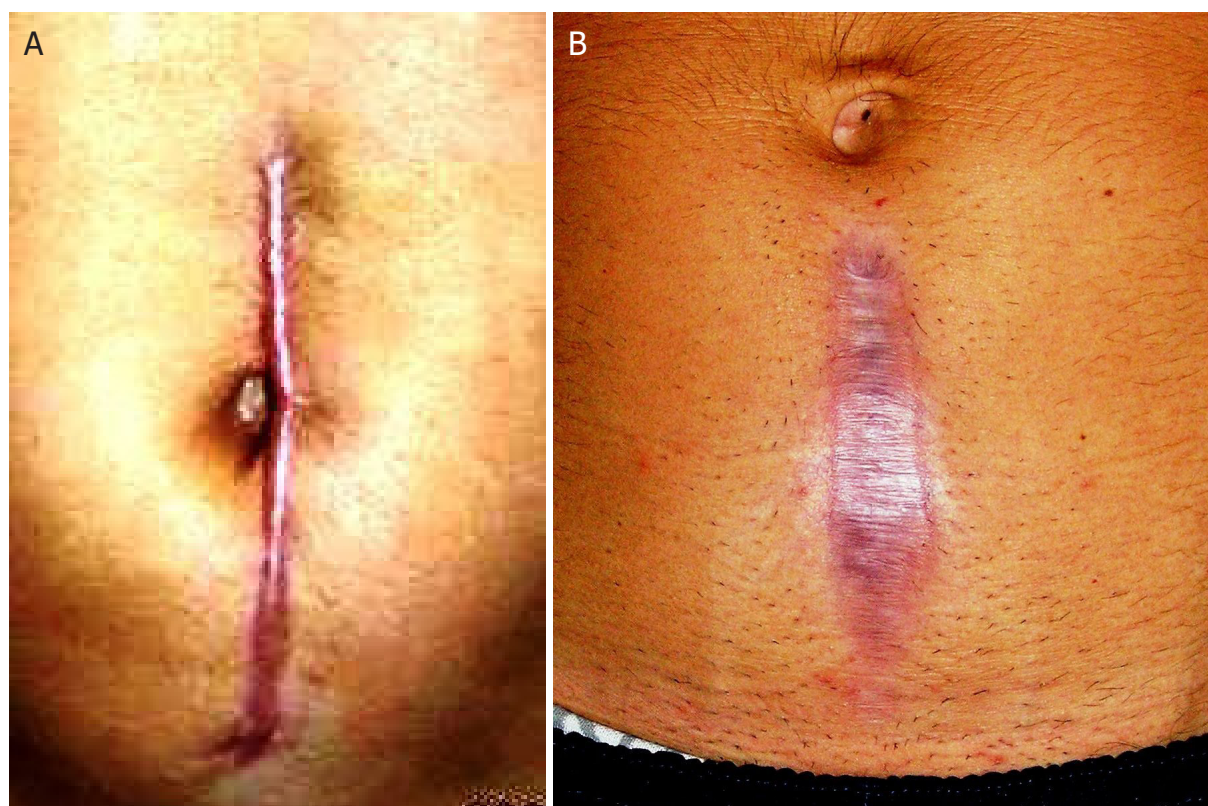
**Figure 21.** For reduction mammoplasty, the “Wise Pattern” is commonly used: The wound edges of the vertical scar run parallel to the tension lines, but the horizontal edges run perpendicular to the main folding lines. Therefore, the horizontal scar often becomes hypertrophic, whereas the vertical scar appears flat and narrow (A and B) (Figure 21A is a drawing from ADAM Health solutions)

In the breast, striae radiate from the areola outwards [Figure 9B], and therefore, optimal incisions run circumferentially. In augmentation mammoplasty, periareolar horizontal axillary<sup>[38]</sup>, or submammary incisions are performed routinely. After reduction mammoplasty, the often inconspicuous vertical scar may be due to the right angle of the incision line on the Wise pattern [Figure 21A] relative to the real or virtual direction of the radial striae on the breast. The often seen hypertrophy of the horizontal scar in the inframammary fold occurs because 2 wound edges with cross-cut collagen fibers are adapted [Figure 21B].

### Abdomen

There are two ways to open the abdominal cavity in elective general surgery: vertically or transversely. Striae distensae always develop perpendicular to the abdominal skin folding lines [Figure 10B], therefore, skin incisions should be made horizontally wherever possible [Figure 13B]. It has long been shown<sup>[39]</sup> that wide transverse incisions along the natural folds of the upper abdomen not only yield optimal access to





**Figure 22.** Incisions in abdominal surgery are still performed vertically, i.e., perpendicular to the main folding lines. In adolescents, vertical midline incisions often result in hypertrophic scars, which then widen under triamcinolone injections (A and B)

all organs, but also result in improved healing with significantly less complications than vertical incisions through the linea alba.

A meta-analysis of various clinical studies<sup>[39]</sup> has postulated that a transverse approach is superior in regard to postoperative complications. This discrepancy between existing recommendations from clinical trials and clinical practice may be explained by a general mistrust of clinical studies or an unwillingness to accept a change for familiar procedures [Figure 22A and B].

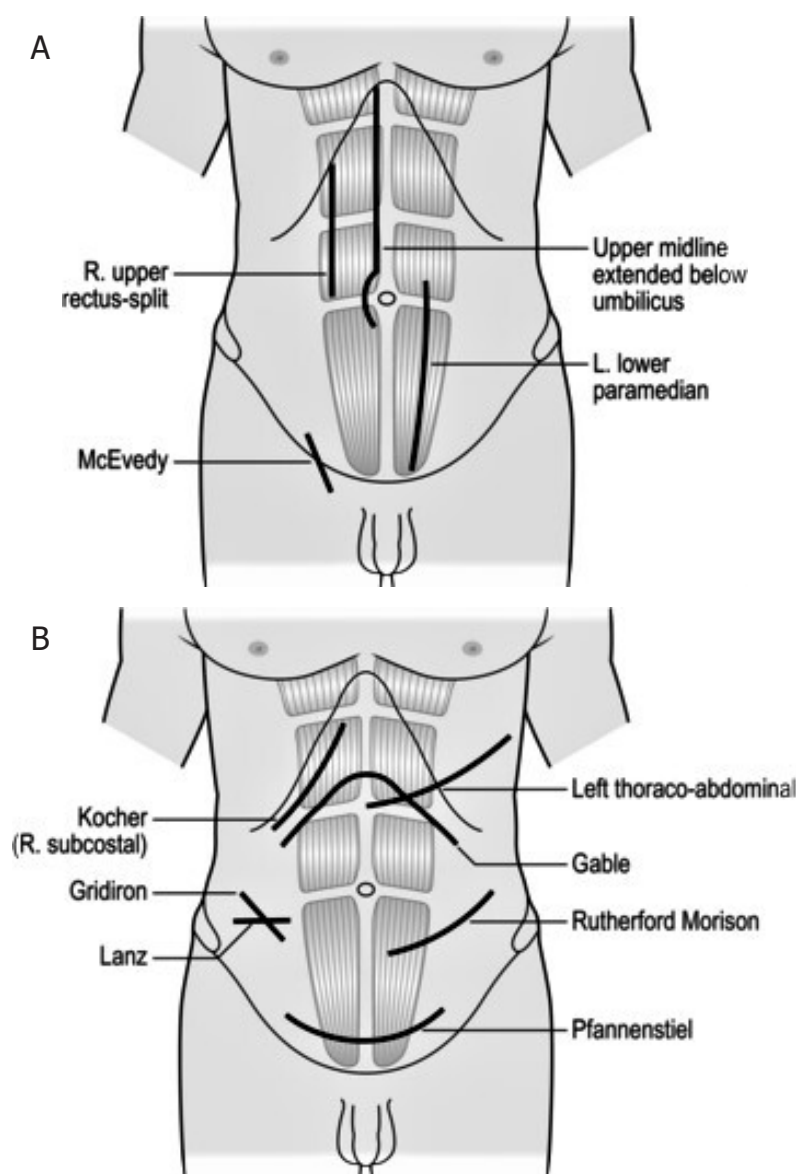
After healing, the mean width of scars was found to be  $8.3 \pm 1.4$  mm for midline incisions, while the mean width of scars after transverse incision was  $3.3 \pm 1.2$  mm<sup>[39]</sup>. However, general surgeons continue to use the traditional vertical midline incision in 90% of patients [Figure 23A and B], despite the additional risk of midline hernias in up to 17% in elderly obese patients with multi-morbidities<sup>[40,41]</sup>. Rectus abdominis atrophy is a rare complication following transverse incisions.

Unsightly vertical scars after open gallbladder and appendix removal have become obsolete due to modern endoscopic surgery. Old scars are best revised by a longer, but ultimately less obvious, fusiform horizontal excision [Figure 24A and B].

### Back and buttocks

The simple experiment of approximating the scapulae and extending the arms will reveal many lines in elderly people. This generally vertical pattern in the upper back is altered by the flexion of the head into transverse lines in the neck [Figure 25A and B]. On the contrary, incisions in the back should be performed vertically in the midline or paramedially [Figure 13B], except in women, where they can be hidden



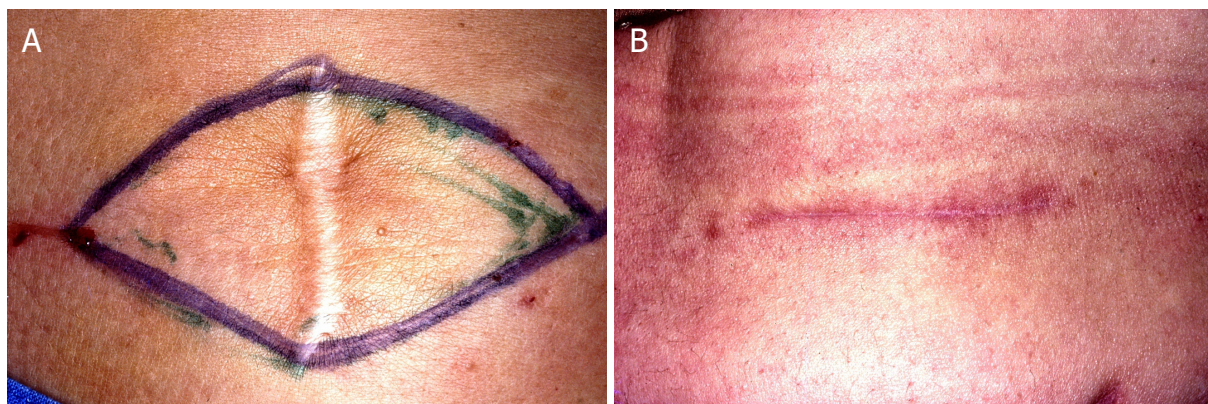


**Figure 23.** Most abdominal incisions are still perpendicular to the main folding lines, even if some surgeons are using wide horizontal incisions in patients with acute abdominal pain (from iknowledge: R.E. D'Souza and R. Novell: Pancreatectomy and Whipple 2015) (A and B)

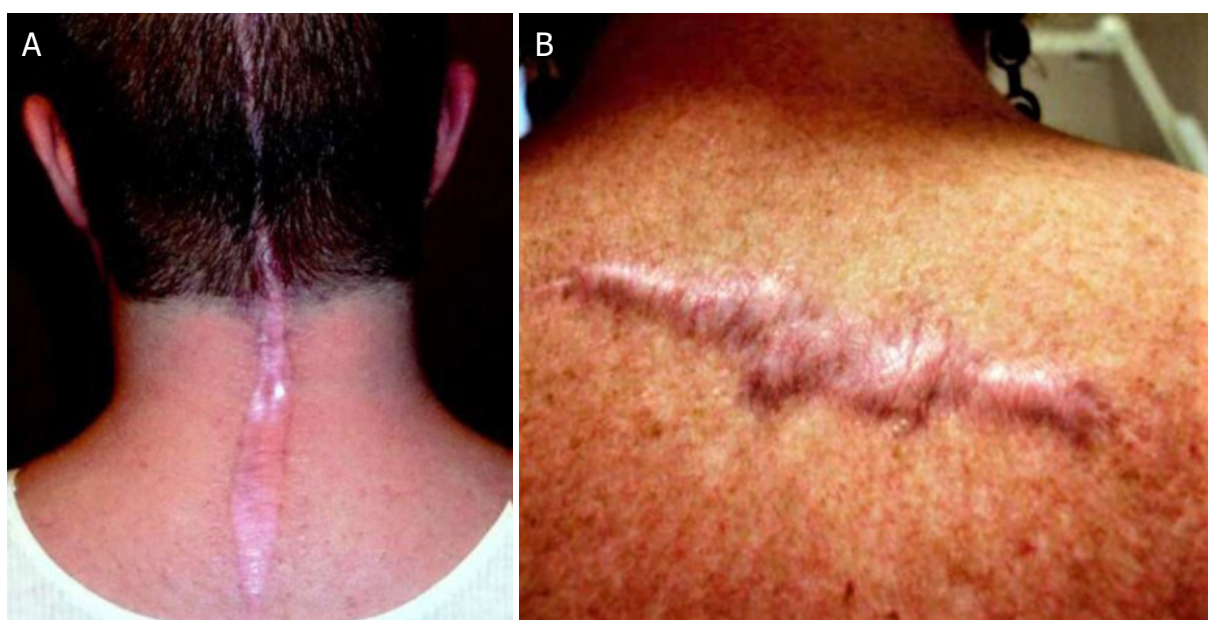
beneath a horizontal bra or bikini strap. Elevating a myocutaneous latissimus dorsi flap must be performed according to the defect. The skin island can often be designed horizontally to hide the scar behind a bra. Towards the lateral rib cage, incisions are made obliquely, following the direction of the ribs.

On the back and over the buttocks, the anti-striae lines differ from the Langer and Kraissl lines in a right-angle fashion. Adolescents develop inconspicuous striae in an oblique direction over the hip and gluteus muscle [Figure 10A], it is therefore recommended to consider skin incisions in hip joint surgery in children and adolescents in an oblique direction, but parallel to the fibers of the gluteus maximus between the posterior iliac crest and trochanter major [Figure 26A and B].

Mini-invasive incisions in total hip arthroplasty are a marketing tool for patients refusing a large lateral or anterior incision as a hip prosthesis flag<sup>[42]</sup>. An incision in the direction along the folding lines would produce the best scars.



**Figure 24.** Vertical pararectal hypertrophic scar after cholecystectomy, corrected by horizontal conversion in the main folding lines (A and B) (both figures are reproduced with permission from Lemperle *et al.*<sup>[m]</sup>)

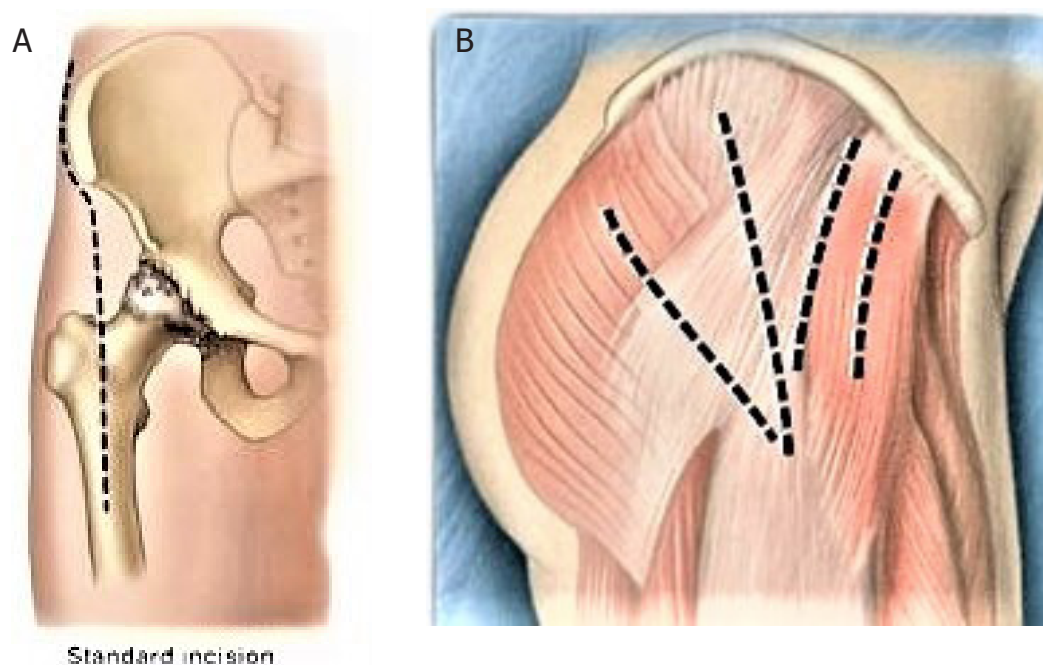


**Figure 25.** On the dorsal neck, the folding lines run horizontally, but vertically above the scapula. In young patients, horizontal incisions may develop hypertrophy (A and B)

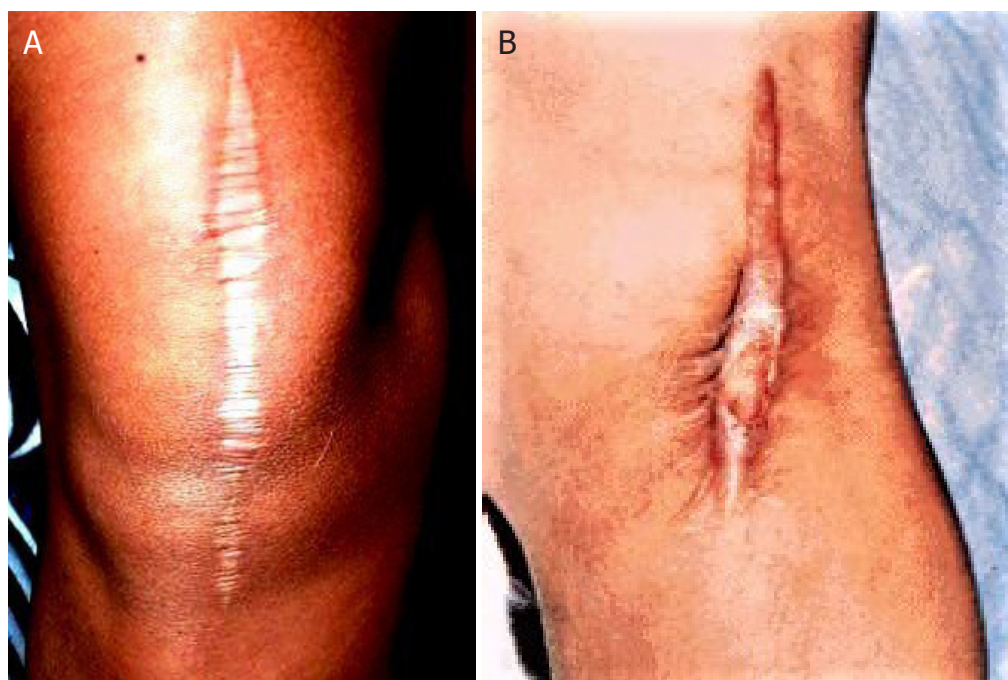
### Leg and foot

Inconspicuous striae often develop on the inner thigh and posterior knee in a vertical direction [Figure 11A] and only rarely on the anterior surface of the thigh, where they develop vertically. Above the knee, horizontal folding lines surround the patella and should be used for the excision of suprapatellar fat pads and half-circular incisions either medial or lateral to the patella [Figure 13B], rather than the commonly used vertical incision straight across the knee [Figure 27A and B].

On the lower leg across the calf, striae generally run vertically and in an oblique direction from lateral proximal to medial distal. The skin folds on the lower extremities are analogous to those of the upper limbs. Therefore, oblique incisions from medial proximal to lateral distal over the calf and oblique incisions from proximal posterior to distal anterior over the fibula, are recommended in young patients with selected indications.



**Figure 26.** Common incisions for hip surgery run vertically. A more oblique incision parallel to the fibers of the gluteus maximus muscle will result in less conspicuous scars (A and B), (reproduced from Michael Kang, MD, New York, NY)



**Figure 27.** Common vertical scar after knee surgery. The wide opening to the joint after a horizontal incision prevents this conspicuous vertical scar. A vertical cut in the hollow of the knee joint (for the removal of a Baker cyst) should be obsolete since all folds run horizontally (A and B) (Figure 27A is reproduced with permission from Lemperle *et al.*<sup>[10]</sup>)

Since striae do not develop on the feet, “main folding lines” become easily visible during movement of the foot. After bending the foot, the skin can be incised in a horizontal [Figure 28A and B] or oblique direction<sup>[43]</sup>. Both wound edges can then be undermined, bluntly preserving larger nerves and vessels. To





**Figure 28.** A horizontal approach to a ruptured Achilles tendon as in club foot surgery (Cincinnati approach) will prevent frequently seen hypertrophic scars (A and B)

access joints, tendons or fractures through oblique incisions, no fear for the blood supply is indicated, since the arteries are running in random pattern within the skin. Over the dorsum of the foot, shaped incisions will result in improved healing over straight vertical ones.

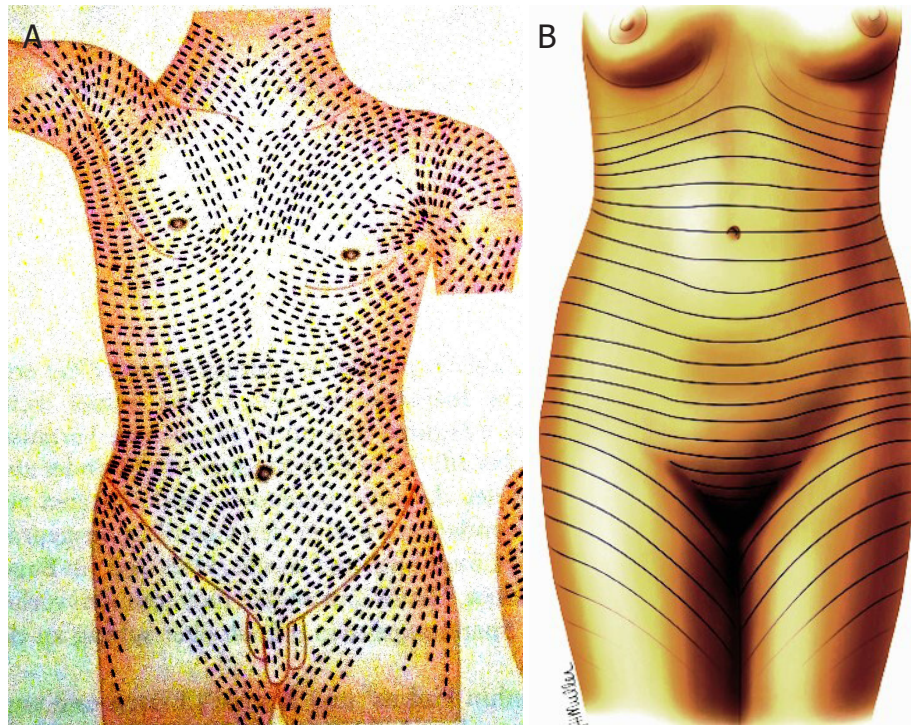
## CONCLUSION

In many areas of the body surface, the described “main folding lines” run closely to the Langer lines, with which they are often confused. Langer lines were created by static forces in the skin of cadavers [Figure 29A]. The normal wrinkle lines are produced by dynamic forces acting on the skin of a living person.

If we exclude Langer lines as historic and not primarily intended as recommendation for the direction of skin incisions, we are left with three well-known publications on skin incisions and optimal scarring. Pinkus described MFL produced by “pinching” skin in 1927<sup>[3]</sup>, and Kraissl recommended incisions perpendicular to the lines running perpendicular to Langer’s lines, especially as they cross joints and facial folds.

In normal folding lines [Figure 29B], the predominant orientation of collagen fibers is parallel to the folds and wrinkles. Collagen bands in scars also form parallel to the wound edges, regardless of scar location. Striae distensae develop perpendicular to the direction of the strongest tension on the skin, and nature reveals that the tension or MFL are perpendicular to the striae.

Surgeons, who have difficulty in identifying skin folds or striae, may use the graphics in this publication as guidance. In elective surgery, most incisions or excisions can be planned in the direction of the MFL [Figure 30A and B], even when the underlying structures such as ribs and other bones suggest the opposite. Even small incisions for minimally invasive surgery should follow these folding lines<sup>[44]</sup>.

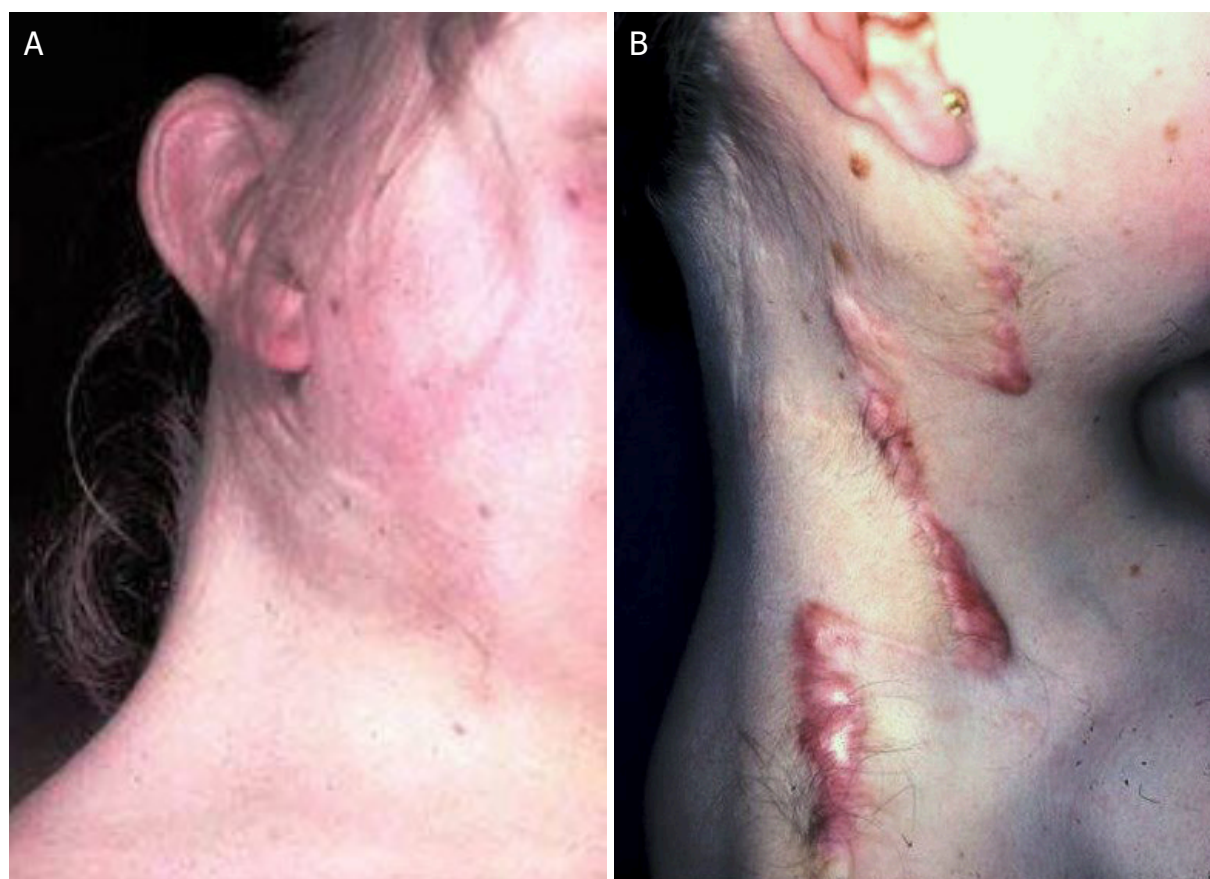


**Figure 29.** Langer's "cleavage lines" were never meant as guidelines for optimal directions for surgical incisions or excisions. Instead, the use of "main folding lines" is advocated in young patients (A and B)



**Figure 30.** Recommended surgical incisions along the main folding lines (MFL), with sparing of major cutaneous nerves and vessels (A and B)





**Figure 31.** Proof of this recommendation in a “pterygium colli” corrected with two Z-plasties on each side. The scars within the neck’s horizontal folding lines are almost invisible; those perpendicular (vertical) to them became hypertrophic (A and B) (Figure 31B is reproduced with permission from Lemperle *et al.*<sup>[11]</sup>)

Besides paying attention to the least damage to the wound edges, due to excessive spreading and squeezing, the use of electrocautery also for the skin incision seems to cause less scarring compared to the usual scalpel<sup>[45]</sup>. Hypertrophic scarring can also be minimized by making the incision long enough to reduce tension. Plastic surgeons are often asked to correct hypertrophic scars after thyroidectomy [Figure 11B] or cholecystectomy [Figure 24B] in patients with very short scars. Consistent and intense traction on the wound edges during an operation may damage soft tissues, which then react with hypertrophic healing. Small hypertrophic scars should be excised only after “maturation” in the direction of the MFL. The normal skin folds should serve as the main guide for the direction of small fusiform skin excisions, as well as for all longer incisions in selected patients [Figure 31A and B].

Non-observance of skin tension lines causes widening or hypertrophy of scars. A linear incision develops a wider gap if it occurs parallel to striae rather than transverse to them. “Relaxed skin tension lines”<sup>[6-8]</sup> seems to be a confusing expression, while “main folding lines”<sup>[3]</sup> are easier to see, imagine and understand. The simplest rule for making optimal incisions in the most favorable direction is to follow natural wrinkle lines: “Proper incisions come together naturally and improper ones tend to gape” (Th. Kocher 1892)<sup>[2]</sup>.

## DECLARATIONS

### Acknowledgments

The author thanks Dr. Mayer Tenenhaus, professor of plastic surgery at the University of California, San Diego, for correcting the former manuscript submitted to “Plast. Reconstruct. Surg.”<sup>[11]</sup>.

### Author's contributions

Study concept and data acquisition, data interpretation and manuscript drafting: Lemperle G

### Availability of data and materials

Some of the photographs herein were found in Google Search without mention of any author or copyright; in others, the authors are named if found. Most of the scar photographs originate from my former Department of Plastic Surgery at the Markus-Hospital in Frankfurt am Main, Germany.

### Financial support and sponsorship

None.

### Conflicts of interest

The author declared that there are no conflicts of interest.

### Ethical approval and consent to participate

A written informed consent to participate in research projects was obtained automatically from all patients of the Plastic Surgery Division of Markus-Hospital in Frankfurt am Main, Germany.

### Consent for publication

A written informed consent to participate in possible publications was obtained automatically from all patients of the Markus-Hospital in Frankfurt am Main, Germany.

### Copyright

© The Author 2020.

## REFERENCES

1. Langer K. On the anatomy and physiology of the skin. I. The cleavability of the cutis. (Translation from German in 1861). *Br J Plast Surg* 1978;31:3-8.
2. Kocher T. Textbook of operative surgery. 3rd English ed. London: Adam and Charles Black; 1911. p. 30.
3. Pinkus F. Die Faltung der Haut. In: Pinkus F, editor. Die normale Anatomie der Haut. Jadassohn's Handbuch der Haut und Geschlechtskrankheiten, Vol 1. Berlin: Springer; 1927. pp. 4-76.
4. Kraissl CJ, Conway H. Excision of small tumours of the skin of the face with special reference to the wrinkle lines. *Surgery* 1949;4:592-600.
5. Kraissl CJ. The selection of appropriate lines for elective surgical incisions. *Plast Reconstr Surg* 1951;8:1-28.
6. Borges AF, Alexander JE. Relaxed skin tension lines, z-plasties on scars, and fusiform excision of lesions. *Brit J Plast Surg* 1962;15:242-54.
7. Borges AF. Elective incisions and scar revision. Boston: Little, Brown; 1973. pp. 5-10.
8. Borges AF. Relaxed skin tension lines (RSTL) versus other skin lines. *Plast Reconstr Surg* 1984;73:144-50.
9. von Torklus D. Atlas orthopädisch-chirurgischer Zugangswege. 4th ed. Auflage Munich: Urban & Fischer Verlag; 2002.
10. Miller MD, Wiesel SW. Operative Techniques in Sports Medicine & Surgery. Baltimore: Lippincott Williams & Wilkins; 2010.
11. Lemperle G, Tenenhaus M, Dieter Knapp D, Lemperle SM. The direction of optimal skin incisions derived from striae distensae. *Plast Reconstr Surg* 2014;134:1424-34.
12. Wilhelmi BJ, Blackwell SJ, Phillips LG. Langer's lines: to use or not to use. *Plast Reconstr Surg* 1999;104:208-14.
13. Carmichael SW. The tangled web of Langer's lines. *Clin Anat* 2014;27:162-8.
14. Russell CJ, Bush JA, Russell GW, Thorlby A, McGrouther DA, et al. Dynamic skin tension in the forearm: effects of pronation and supination. *J Hand Surg Am* 2009;34:423-31.
15. Courtiss EH, Longacre JJ, deStefano GA, Brizio L, Holmstrand K. The Placement of elective skin incisions. *Plast Reconstr Surg* 1963;31:31-44.
16. Barile L, Bufalini C. Incisioni chirurgiche in ortopedia e linee di tensione cutanea. *Arch Putti Chir Organi Mov* 1976;27:127-36.
17. Paul SP, Matulich J, Charlton N. A new skin tensiometer device: computational analyses to understand biodynamic excisional skin tension lines. *Sci Rep* 2016;6:301.
18. Paul SP. Biodynamic excisional skin tension (BEST) lines: revisiting Langer's lines, skin biomechanics, current concepts in cutaneous surgery, and the (lack of) science behind skin lines used for surgical excisions. *J Dermatol Res* 2017;2:77-87.
19. Piérard GE, Lapière CM. Microanatomy of the dermis in relation to relaxed skin tension lines and Langer's lines. *Am J Dermatopathol* 1987;9:219-24.

20. Viennet C, Bride J, Armbruster V, Aubin F, Gabiot AC, et al. Contractile forces generated by striae distensae fibroblasts embedded in collagen lattices. *Arch Dermatol Res* 2005;297:10-7.
21. Arem AJ, Kischer CW. Analysis of striae. *Plast Reconstr Surg* 1980;65:22-9.
22. Alshaiji JM, Handler MZ, Schwartzfarb E, Izakovic J, Schachner LA. Unilateral striae distensae affecting the right axilla in a 16-year-old boy: brief report. *Pediatr Dermatol* 2014;31:617-8.
23. Cho S, Park ES, Lee DH, Li K, Chung JH. Clinical features and risk factors for striae distensae in Korean adolescents. *J Eur Acad Dermatol Venereol* 2006;20:1108-13.
24. Basile FP, Volpe A, Basile AR. Striae distensae after breast augmentation. *Aesth Plast Surg* 2012;36:894-900.
25. Sorensen GW, Odom RB. Axillary and inguinal striae induced by systemic absorption of a topical corticosteroid. *Cutis* 1976;17:355-7.
26. Rotsztejn H, Juchniewicz B, Nadolski M, Wendorff J, Kamer B. The unusually large striae distensae all over the body. *Adv Med Sci* 2010;55:343-5.
27. Salter SA, Batra RS, Rohrer TE, Kohli N, Kimball AB. Striae and pelvic relaxation: two disorders of connective tissue with a strong association. *J Invest Dermatol* 2006;126:1745-48.
28. Watson REB. Stretching the point: an association between the occurrence of striae and pelvic relaxation? *J Invest Dermatol* 2006;126:1688-9.
29. Ashcroft GS, Mills SJ, Lei K, Gibbons L, Jeong MJ, et al. Estrogen modulates cutaneous wound healing by downregulating macrophage migration inhibitory factor. *J Clin Invest* 2003;111:1309-18.
30. Cordeiro RC, Zecchin KG, de Moraes AM. Expression of estrogen, androgen, and glucocorticoid receptors in recent striae distensae. *Int J Dermatol* 2010;49:30-2.
31. Elsaie ML, Baumann LS, Elsaie LT. Striae distensae (stretch marks) and different modalities of therapy: an update. *Dermatol Surg* 2009;35:563-73.
32. Whalen JG, English JC 3rd. Case study on linear focal elastosis. *Dermatol Nurs* 2006;18:469-71.
33. Jeong JS, Lee JY, Kim MK, Yoon TY. Linear focal elastosis following striae distensae: further evidence of keloidal repair process in the pathogenesis of linear focal elastosis. *Ann Dermatol* 2011;23:S141-3.
34. Spitz JL. *Genodermatoses: a full color clinical guide to genetic skin disorders*. 2nd ed. Baltimore: Lippincott, Williams & Wilkins; 2005.
35. Lemperle G, Holmes RH, Cohen SR, Lemperle SM. A classification of facial wrinkles. *Plast Reconstr Surg* 2001;108:1735-50.
36. Lim SY, Kwack WG, Kim Y, Lee YJ, Park JS, et al. Comparison of outcomes between vertical and transverse skin incisions in percutaneous tracheostomy for critically ill patients: a retrospective cohort study. *Crit Care* 2018;22:246.
37. Bedard P, Keon WJ, Brais MP, Goldstein W. Submammary skin incision as a cosmetic approach to median sternotomy. *Ann Thoracic Surg* 1986;41:339-41.
38. Shrotria S. The peri-areolar incision - gateway to the breast! *Eur J Surg Oncol* 2001;27:601-3.
39. Halm JA, Lip H, Schmitz PI, Jekeel J. Incisional hernia after upper abdominal surgery: a randomised controlled trial of midline versus transverse incision. *Hernia* 2009;13:275-80.
40. Bickenbach KA, Karanicolas PJ, Ammori JB, Jayaraman S, Winter JM, et al. Up and down or side to side? A systematic review and meta-analysis examining the impact of incision on outcomes after abdominal surgery. *Am J Surg* 2013;206:400-9.
41. Heller L, Chike-Obi C, Xue AS. Abdominal wall reconstruction with mesh and components separation. *Semin Plast Surg* 2012;26:29-35.
42. Leunig M, Hutmacher JE, Ricciardi BF, Impellizzeri FM, Rüdiger HA, et al. Skin crease 'bikini' incision for the direct anterior approach in total hip arthroplasty: a two- to four-year comparative study in 964 patients. *Bone Joint J* 2018;100-B:853-61.
43. Andermahr J, Jubel A, Elsner A, Schulz-Algie PR, Schiffer G, et al. Die Hautspaltlinien und die Schnittführung bei Fußoperationen. *Orthopäde* 2007; 36:265-72.
44. Pérez-Bustillo A, González-Sixto B, Rodríguez-Prieto MA. Surgical principles for achieving a functional and cosmetically acceptable scar. *Actas Dermosifiliogr* 2013;104:17-28.
45. Ismail A, Abushouk AI, Elmarazy A, Menshawy A, Menshawy E, et al. Cutting electrocautery versus scalpel for surgical incisions: a systematic review and meta-analysis. *J Surg Res* 2017;220:147-63.

Review

Open Access



# Stem cells and periodontal regeneration: present and future

Filippo Citterio, Giacomo Gualini, Ludovica Fierravanti, Mario Aimetti

Department of Surgical Sciences, Periodontology, C.I.R. Dental School, Turin 10126, Italy.

**Correspondence to:** Dr. Filippo Citterio, Department of Surgical Sciences, Periodontology, C.I.R. Dental School, Turin 10126, Italy.  
E-mail: [filippo.citterio@unito.it](mailto:filippo.citterio@unito.it)

**How to cite this article:** Citterio F, Gualini G, Fierravanti L, Aimetti M. Stem cells and periodontal regeneration: present and future. *Plast Aesthet Res* 2020;7:41. <http://dx.doi.org/10.20517/2347-9264.2020.29>

**Received:** 20 Feb 2020 **First Decision:** 6 May 2020 **Revised:** 17 Jun 2020 **Accepted:** 28 Jun 2020 **Published:** 15 Aug 2020

**Academic Editor:** Yi-Lin Cao **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

The ultimate goal of periodontal regeneration is to restore the damaged alveolar bone proper, root cementum, and periodontal ligament with collagen fibers inserted into the root surface. The search for new regenerative strategies is a challenging field of periodontal research, and tissue engineering, using stem cells, has recently been shown as a promising approach. This paper aims at reviewing the current available literature on the use of stem cells for the treatment of periodontitis. Up to now, different mesenchymal stem cells (MSCs) have shown potential for periodontal regeneration in animal studies. The most investigated MSCs for periodontal regeneration are bone marrow MSCs (BMMSCs), periodontal ligament stem cells (PDLSCs), and dental pulp stem cells (DPSCs), which have shown very promising results in animal models. Few studies on humans are available but BMMSCs, PDLSCs, and DPSCs have been proven safe and effective. Clinical trials are sparse, but tend to support the efficacy of MSCs for periodontal regeneration. In the future, more human studies will be required to support the use of MSCs in daily clinical practice, especially in order to identify the best protocol to harvest, process, and graft MSCs. Future perspectives include trans-differentiation of somatic cells to generate induced pluripotent stem cells, homing procedures, the use of exogenous stem cells, and 3D-printed scaffolds.

**Keywords:** Stem cells, regeneration, periodontology, tissue engineering, bone defects

## INTRODUCTION

Stem cells and tissue engineering have recently been introduced into the field of periodontology and they have shown encouraging potential in the treatment of periodontitis, which is a complex immune-



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



inflammatory disease, characterized by the loss of periodontal tissues around teeth and by the formation of periodontal pockets (PD). In susceptible individuals, periodontitis occurs when the inflammatory and immune response to the microbial challenge of dental plaque is dysregulated. If left untreated, periodontitis may progress at different rates and eventually lead to tooth loss, especially in a sub-fraction of patients who may be highly susceptible.

Periodontitis affects the majority of the adult population<sup>[1,2]</sup>, may cause edentulism, and has been listed as a risk factor for major systemic diseases, such as atherosclerosis, cardiovascular diseases<sup>[3]</sup>, diabetes<sup>[4]</sup>, and rheumatoid arthritis<sup>[5,6]</sup>. This may directly influence the general health, social life, and nutritional state of affected individuals, jeopardizing their overall quality of life<sup>[7-10]</sup>. Moreover, the treatment of advanced forms of periodontitis is expensive, with a direct impact on Western countries' productivity, thus making periodontitis a threat for public health<sup>[11]</sup>.

Periodontal treatment aims to control inflammation in the periodontal tissues, avoiding disease progression, preserving natural teeth, and maintaining masticatory function. To achieve these goals, the treatment is focused on reducing or eradicating PDs. Many non-surgical and surgical approaches are currently used. Non-surgical periodontal therapy is the first step for any patient affected with periodontitis. The desegregation of the bacterial biofilm at PD sites provides a reduction of PDs and inflammation, especially in PDs  $\leq 6$  mm<sup>[12,13]</sup>. If the number of residual pockets is limited and inflammation is under control, patients are expected to experience limited tooth loss (0.1 tooth/patient/year<sup>[14]</sup>) throughout a life-long supportive care program, which is generally enough to prevent masticatory dysfunction. However, patients who still present multiple PDs  $\geq 5$  mm, or even one PD  $\geq 6$  mm after non-surgical therapy, take significant risk to experience disease progression and require additional surgical treatment<sup>[15,16]</sup> with the goals: (1) to provide access to root surface; and (2) to arrest disease progression by reducing PDs. Surgical treatment of periodontitis includes non-resective access surgery, resective surgery, and regenerative procedures. Non-resective surgery aims at facilitating root debridement by means of flap elevation. Different techniques have been proposed and minimally-invasive approaches have shown advantages in clinical trials<sup>[17]</sup>. Resective surgery eradicates PD by correcting gingival and bone morphology. Although extremely effective against PDs, it is carried out at the expense of the periodontal support of the involved teeth and invariably causes soft tissue recession<sup>[18,19]</sup>. Periodontal regeneration has the goal to restore the lost periodontium, as it aims at increasing the periodontal attachment, reducing PD, and limiting gingival recession. This makes periodontal regeneration the gold standard for periodontal treatment<sup>[20]</sup>.

Periodontal regeneration has been shown to be effective in the treatment of intrabony and furcation defects with varying degrees of efficacy<sup>[21,22]</sup>; however, regenerative procedures are still exposed to clinical failures or incomplete success due to various limitations, such as patient-specific factors (i.e., smoking, poor plaque control, *etc.*), improper choice of access flaps and biomaterials, and poor periodontal training<sup>[23]</sup>. Alveolar bone proper, root cementum, and periodontal ligament (PL) in the previously damaged periodontium are expected to be regenerated as the ideal treatment outcome, but it has been shown to not always be the case<sup>[24]</sup>. To overcome these limitations, new access flaps<sup>[25-28]</sup> and biological agents<sup>[29,30]</sup> have been developed in recent years; clinical trials, however, have revealed a still controversial efficacy and their histological evidence is generally sparse. Thus, the search for new regenerative procedure is still a challenging field of periodontal research. In this context, tissue engineering<sup>[31]</sup>, cell combination, biomaterials, and growth factors have recently been proposed as promising alternatives for periodontal treatment. In this field, stem cells are attractive. They are undifferentiated cells that possess regenerative potential thanks to their ability to develop into different cell types after proper stimulation<sup>[32]</sup>. In periodontal regeneration, mesenchymal stem cells (MSCs) have been tested *in vitro* and in humans with promising results<sup>[33]</sup>. MSCs from dental and non-dental tissues have been harvested and used<sup>[34]</sup>. Among MSCs from dental tissue, we may list dental pulp stem cells (DPSCs)<sup>[35]</sup>, human exfoliated deciduous teeth cells (SHEDs)<sup>[36]</sup>, periodontal ligament stem



cells (PDLSCs)<sup>[37]</sup>, dental follicle precursor cells (DFPCs)<sup>[38]</sup>, and stem cells from apical papilla (SCAPs)<sup>[39]</sup>. Various non-dental stem cells have been used in periodontal regeneration. Among others, BMMSCs, adipose-derived stem cells (ASCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs) have been the most investigated.

Evidence of which is the most effective protocol is still lacking. Thus, the primary aim of this review is to summarize the available scientific evidence about periodontal regeneration using MSCs. Clinical and histological outcomes of periodontal regeneration are reviewed and synthesized.

## **MESENCHYMAL STEM CELLS FOR PERIODONTAL REGENERATION**

### **Dental stem cells**

Dental Stem Cells (DSCs) are multi-potent, self-renewing MSCs. DSCs may differentiate toward osteogenic, odontogenic, dentinogenic, cementumogenic, adipogenic, chondrogenic, myogenic, and neurogenic lineages. DSCs are easily accessible since they may be found in the human body at all ages. Furthermore, cryopreservation does not affect their properties<sup>[40]</sup>. These characteristics make them accessible and easy to handle.

DPSCs were among the first DSCs isolated. They are easily accessible in large number because they can be obtained from human dental pulp. DPSCs are especially attractive for a plethora of reasons. First, they are found in dental pulp and their harvesting only requires an extracted tooth, including third molars and periodontally compromised teeth. It has also been speculated that they can be obtained from dental pulp with inflammation<sup>[41]</sup> and still show potential to differentiate into osteoblast-like cells. Second, DPSCs share origin, antigenic pattern, and differentiation lineages with periodontal stem cells<sup>[35,40,41]</sup>. Furthermore, they can also differentiate in other cell types including cardio-myocytes, neuron cells, adipocytes, corneal epithelial cells, melanoma cells, and insulin secreting Beta cells<sup>[42]</sup>. Third, they easily interact with biomaterials<sup>[43,44]</sup>.

PDLSCs have been considered ideal candidates for periodontal regeneration since they can be easily recovered by non-invasive procedures after simple tooth extraction. In addition, they can be cultured. PDLSCs are known to possess osteogenic, chondrogenic, and adipogenic potential and exhibit immunosuppressive characteristics similar to those described for bone marrow MSCs (BMMSCs) and DPSCs. They possess the ability to form a cementum/PL complex-like structure.

SHEDs are easy to access by using noninvasive procedures, as they are harvested from deciduous exfoliated teeth. SHEDs exhibit a high rate of proliferation and immuno-modulatory properties, similar to those of BMMSCs, which are comparatively more difficult to harvest. They are able to differentiate into osteoblasts<sup>[45]</sup> and could express an immuno-regulatory potential on T cells, macrophages, and dendritic cells<sup>[46]</sup>. Nakamura *et al.*<sup>[47]</sup> compared the “stemness” of SHEDs to DPSCs and BMMSCs and noticed that SHEDs revealed a higher proliferation rate than DPSCs and BMMSCs and higher expression of genes of cell proliferation and extracellular matrix elements. This makes this cell type an interesting candidate for periodontal regeneration.

DFPCs may act as precursor cells for PDLSCs. DFPCs are able to enhance the proliferation and osteogenic and adipogenic differentiation of PDLSCs to different degrees. Co-culture with DFPCs increases cell layers and extracellular matrix of PDLSC cell sheets *in vitro*<sup>[48]</sup>. However, scientific evidence for this cell type is still limited.

SCAPs are related to developing roots. Their presence in the apical papilla of forming roots has been suggested as a possible explanation of how immature teeth with necrotic pulps are able to undergo

root development. SCAPs have infection-resistant properties too<sup>[49]</sup>, and this may further explain why apexogenesis has been observed even in the presence of apical periodontitis. Despite being difficult to collect, they are a promising tool for regenerative procedures as they have multi-lineage differentiation potential<sup>[50]</sup>.

### Non-dental stem cells

BMMSCs were the first MSCs discovered and have been extensively tested more often on animal models. They have shown osteogenic, adipogenic, chondrogenic, and myogenic differentiation. The major shortcoming of BMMSCs is the pain of the bone marrow harvesting and the limited quantities that can be collected. BMMSCs can differentiate into ameloblast-like cells<sup>[51,52]</sup> and periodontal tissue cells and can enhance periodontal regeneration<sup>[53,54]</sup>. Interestingly, besides periodontal regeneration, BMMSCs may be used for tooth regeneration as they can upregulate the expression of odontogenic genes and contribute to new tooth formation after recombination with embryonic oral epithelium<sup>[55]</sup>.

ASCs are stem cells derived from adipose tissues and are abundant. ASCs allow *in vitro* expansion and have undergone osteogenic, chondrogenic, adipogenic, and neurogenic differentiation in various experimental settings. The harvesting method is less invasive than the method used for BMMSCs, since ASCs may be retrieved in high number from either liposuction or subcutaneous adipose tissue fragments. For this reason, they have been extensively used in regenerative medicine.

ESCs are pluripotent stem cells found in human blastocysts. They show an extraordinary potential for differentiation as they can develop into almost all cell lineages<sup>[56]</sup>. In the context of periodontal research, it has been shown that ESCs can differentiate into odontogenic and periodontal cell lineages, in particular if co-cultured with PDLSCs or embryonic oral epithelium cell<sup>[57,58]</sup>. Ethical concerns have hampered the use of such cells for periodontal regeneration since their harvesting may result in the destruction of human blastocysts. Furthermore, besides their unlimited potential, they have shown major adverse effects such as tumors and unwanted immune responses.

iPSCs were first discovered in 2006 and have since generated substantial interest in regenerative medicine<sup>[59]</sup>. They are a type of pluripotent stem cell that can be generated directly from a somatic cell. They can duplicate indefinitely, as well as give rise to every other cell type in the body. Recently, dental cells including DPSCs, SHEDs, PDLSCs, and SCAPs have been successfully reprogrammed into iPSCs<sup>[60,61]</sup>, and iPSCs have been investigated for periodontal regeneration.

### ANIMAL STUDIES

Before testing the performance of the use of stem cells in humans, its feasibility and safety have always been proven in animal studies. Up to now, more than 2000 articles that used stem cells for regeneration of both tooth and dental supporting tissues on animal models have been listed in MEDLINE. The main animal models used are dogs, mini-pigs, rats, and sheep.

All different types of stem cells have been tested with several approaches (e.g., different isolation processes, different implantation methods, *etc.*) with promising results in terms of regeneration of bone and dental supporting tissues (PL and root cementum)<sup>[62,63]</sup>. Although promising, from all these studies, no clear clinical protocol can be ultimately validated due to the heterogeneity of the investigation designs and because we still do not have a complete understanding of how these cells interact in the healing and regenerative processes [Tables 1 and 2]. Of course, more studies are needed to elucidate these aspects in greater details and better select a stem cell-based protocol for periodontal regeneration. Different results among studies can be ascribed to the different protocols used. The main differences that play an important role in the outcomes are the characteristics of the donor, especially age<sup>[64]</sup>; the isolation and expansion

**Table 1. Summary of available evidence for periodontal regeneration with mesenchymal stem cells**

	<b>Bone regeneration</b>	<b>PL</b>	<b>Cementum</b>
BMMSC	Effective in grade III furcation defects, but bone fill is not complete; Ineffective when used without bone substitutes	Effective in grade III furcation defects; Conflicting results; effective only associated with bone substitutes	Effective in grade III furcation defects
ASC	Effective in extraction sockets	Effective in surgically created intrabony defects	Effective in surgically created intrabony defects
PDLSC	Effective in fenestration; No added benefit associated with non resorbable membranes in fenestrations; Effective in intrabony defects only using bone substitutes	Effective in fenestration; More effective than BMMSC; Effective in intrabony defects with better results using bone substitutes	Effective in fenestration; added benefit associated with non resorbable membranes in fenestrations; Effective in intrabony defects with better results using bone substitutes
DPSC	Effective in extraction sockets; Improve bone regeneration in intrabony defects	Improve regeneration in intrabony defects	Improve regeneration in intrabony defects
DFPC	Improve effects of PDLSC	Improve effects of PDLSC	Improve effects of PDLSC
SHED	Increased bone volume in intrabony defects	Increased PL fibers in intrabony defects	Increased in intrabony defects
SCAP	Increased bone volume in intrabony defects compared to saline	Increased in intrabony defects compared to saline	Increased in intrabony defects compared to saline

BMMSC: bone marrow mesenchymal stem cell; ASC: adipose-derived stem cell; PDLSC: periodontal ligament stem cell; DPSC: dental pulp stem cell; DFPC: dental follicle precursor cell; SHED: stem cell from human exfoliated teeth; SCAP: stem cell from apical papilla

methods<sup>[65]</sup>; the way of delivery and implantation in combination with membranes<sup>[66]</sup>; and biomaterials<sup>[67,68]</sup> or bioactive molecules<sup>[69,70]</sup>.

Briefly, age has no effect on the possibility of isolating and culturing stem cells, but showed a statistically significant effect on the procedure outcome. Cells harvested from donors over 60 years of age had an over 50% failure rate. On the contrary, in younger donors ( $\leq 60$  years old), the failure rate ranged between 14% and 22%. No effect of gender was found, with similar success rates for male and female donors<sup>[64]</sup>.

It has been suggested that the site of harvesting can also influence the outcomes of the cell therapy procedures, according to the differences found in stem cells of the same lineage but taken from different niches. More studies are needed to confirm this finding<sup>[64]</sup>.

The culture and the expansion protocols used to amplify the numbers of transplanted cells are a variable that can alter the “stemness” properties of the stem cells. No clear protocol has been developed yet for the treatment of MSCs, in particular oral ones. We know that under certain conditions (i.e., particular culture mediums) we can alter the differentiation path, favoring a subset of cells that can increase the successful outcomes of our cell treatments<sup>[71]</sup>; however, further research is needed to better understand and guide this process.

Finally, the protocols wherewith these cells are administered can have a positive or negative effect on the final result. Cell therapy can be administered on a scaffold base or on a scaffold-free delivery system. The first one implies the use of a biomaterial, usually calcium-based, a membrane, or a combination of the two. The rationale behind the use of biomaterials and membranes is the need for blood clot stability in order to reach its formation and maturation. When the conformation of the periodontal defect is not firm enough, biomaterials and membranes can make up for the lack of stability and enhance the regeneration process. It has been shown, also *in vivo*, that different materials have different effects, either positive or negative, on the non-cell-based regeneration techniques<sup>[30]</sup>. Similarly, these effects have been found in cell-based regenerative therapies, with promising results from beta-TCP<sup>[46,72-74]</sup>, hyaluronic acid<sup>[75,76]</sup>, and nano designed materials<sup>[77-79]</sup>.

**Table 2. Summary of available evidence for periodontal regeneration with mesenchymal stem cells in animal studies**

Author	Cell type	Biomaterial	Animal model	Defects per group	Defect type	Treatment group	Study period	Analysis	Results
Kawaguchi <i>et al.</i> <sup>[72]</sup> 2004	BMSC	AC	DOG Beagle female 12-20 months old	Unclear	Class III furcation defects 4 mm deep	(a) BMSC + AC (b) AC	1 month	-Histological: HES, Azan staining -Histomorphometric	BMSC group showed neocementum along the denuded dentin with bone e ligament formation. No root resorption or ankylosis reported
Hasegawa <i>et al.</i> <sup>[53]</sup> 2006	BMSC	AC	DOG Beagle female 12-20 months old	6	Class III furcation defects 4 mm deep	(a) BMSC + AC (b) no treatment	1 month	-Histological: HES -Immunohistochemical: GFP, Proliferating Cell Nuclear Antigen (PCNA) -Histological: HES -Histomorphometric	Defects treated with MSC showed newly formed of cementum, PL and alveolar bone All the groups with PRP showed regeneration of bone and cementum. BMSC showed the best potential for periodontal regeneration.
Simsek <i>et al.</i> <sup>[70]</sup> 2012	BMSC	PRP autologues	DOG Mongrel	6 (a, b, c, d, e)	Class II furcation defects 5 mm x 2 mm	(a) BMSC+PRP (b) autogenous cortical bone + PRP (c) PRP (d) autogenous cortical bone (e) no treatment	2 months	-Histological: HES -Histomorphometric	Test group showed higher percentage of cementum and PL regeneration. Bone formation was equal in test and control group
Paknejad <i>et al.</i> <sup>[68]</sup> 2015	BMSC	Deminerlized bovine bone matrix	DOG Mongrel male 14-22 kg	9 (a, b)	Intrabony defects 4 mm x 4 mm	(a) BMSC + Deminerlized bovine bone matrix (b) Deminerlized bovine bone matrix	2 months	-Histological: HES -Histomorphometric	Genetic expression of ASCs and DPSCs are similar. Transplanted ASCs regenerate PL and alveolar bone
Hung <i>et al.</i> <sup>[85]</sup> 2011	rADSCs rDPSCs	Collagen	New Zealand withe RABBIT 2-12 months old	14 (a, b)	Extraction sockets	(a) collagen + rBMP2 (b) collagen	15 weeks	-anatomical -histological -immunohistochemical	Bone, cementum and ligament resulted regenerated in both allogenic and autogenous PDLSC
Ding <i>et al.</i> <sup>[86]</sup> 2010	PDLSC	HA/ $\beta$ -TCP + gelatin membrane	MINIATURE PIG female Wuzhishan, male Guizhou 6-8 months old	6 (a, b, c, d, e)	Intrabony defects 3 mm x 7 mm x 5 mm	(a) PDLSC autologous + HA- $\beta$ -TCP (b) PDLSC allogenic + HA- $\beta$ -TCP (c) PDLSC autologous and allogenic + HA- $\beta$ -TCP (d) HA + $\beta$ -TCP (e) empty defect	3 months	-Clinical: CAL, PD, GR, blood and biochemical tests -Histological: HES -Histomorphometric -Radiological: CT	Both cell types improved regeneration PL and bone tissue had been generated in both groups
Fu <i>et al.</i> <sup>[87]</sup> 2014	PDLSC SHED	HA/ $\beta$ -TCP	MINIATURE PIG female 9-12 months old	6 (a, b, c)	Intrabony defects 5 mm x 7 mm x 7 mm	(a) PDLSC + HA- $\beta$ -TCP (b) SHED + HA- $\beta$ -TCP (c) HA- $\beta$ -TCP	3 months	-Clinical: CAL, PD, GR -Histological: HES -Radiological: CT	
Gao ZH <i>et al.</i> <sup>[91]</sup> 2016	PLSC DPSC	HA/TCP	MINIATURE PIG 18 months old	46 (a) 9 (b)	Root-shaped implant socket 4,1 mm x 10 mm	(a) HA + TCP + DPSC + PDLSC (a) dental implants	6, 12 months	-Clinical: PD, GR, gingivitis, peri-implantitis -Histological: HES, toluidine blue staining -Radiological: CT, micro-CT -Biochemical: compressive strength, modulus of elasticity, torsional force -SEM	

Tsumanuma <i>et al.</i> [88] 2016	PDLSC	PGA/ $\beta$ -TCP/ collagen and absorbable membrane (GTR)	DOG Beagle	8 (a, b, c)	Intrabony defects 5 mm $\times$ 5 mm	(a) PDLSC autologous/PGA + 2 months $\beta$ -TCP + collagen (b) PDLSC allogenic/PGA + $\beta$ -TCP + collagen (c) $\beta$ -TCP + collagen	-Histological: Azan staining -Enzyme-linked immunisorbent assay: CRP, IL-10, IFN- $\gamma$ , CD30 -Histomorphometric -Radiological: micro-CT	No differences were found between autologous and allogenic groups. a) c) showed collagen fibers oblique and parallel to the root. b) had perpendicularly inserted fibers in new cementum
Zhu <i>et al.</i> [89] 2017	PDLSC BMSC	Treated dentine matrix (TDM) and ceramic bone (CA)	MINIATURE PIG 2 years old	10 (a) 8 (b)	Intrabony defects 5.2 mm $\times$ 5 mm	(a) iBMSC + PDLSC + TDM + 3 months CA (b) iBMSC + PDLSC + TDM + CA	-Histological: HES, Masson's trichrome -Immunohistochemical: Col1	PL regeneration was found in both groups
Nuñez <i>et al.</i> [90] 2012	CDC PDLSC	Collagen	DOG Beagle male 1 year old	8 (a, b, c)	Intrabony defects 3 mm $\times$ 4 mm	(a) CDC + collagen (b) PDLSC + collagen (c) collagen	-Histological: Toluidine blue staining -Histomorphometric	Test group showed higher percentages of new cementum, associated with higher attachment gains. No increased bone regeneration was found
Akizuki <i>et al.</i> [95] 2005	PDLC	Hyaluronic acid sheet	DOG Beagle female 3 years-old	5 (a, b)	Dehiscence defects 5 mm $\times$ 5 mm	(a) PDLC + hyaluronic acid sheet (b) hyaluronic acid sheet	-Histological: HES, Masson trichrome staining -Histomorphometric	Group with PDLC showed more cementum, bone and ligament formation
Nakahara <i>et al.</i> [96] 2004	PDLSC	type I (70- 80%) and type III (20- 30%) AC + ePTFE membranes	DOG Beagle female	6 (a, b)	Fenestration defects 6 mm $\times$ 4 mm	(a) PDLSC + atecollagen (b) empty	-Histological: HES, Masson's trichrome staining -Histomorphometric	No differences in bone formation between the groups, but PDLSC showed more cementum formation
Iwata <i>et al.</i> [97] 2009	PDLC	PGA/ $\beta$ -TCP	DOG Beagle male	4 (a, b)	Intrabony defects 5 mm $\times$ 5 mm $\times$ 4 mm	(a) PDLC + PGA + $\beta$ -TCP (b) PGA + $\beta$ -TCP	-Histological: HES, Azan staining -Histomorphometric -Radiological: micro-CT	Control group showed limited new bone formation while the addition of PDLC resulted in bone and cementum regeneration
Liu Y <i>et al.</i> [138] 2008	PDLSC	HA/ $\beta$ -TCP + gelatin membranes	MINIATURE PIG 12 months old	24 (a) 12 (b, c)	Intrabony defects 7 mm $\times$ 3 mm $\times$ 5 mm	(a) PDLSC + HA- $\beta$ -TCP (b) HA- $\beta$ -TCP (c) no treatment	-Clinical: CAL, PI, PD, GR, BOP -Histological: HES, GFP -Radiological: CT	PDLSC showed bone, ligament and cementum regeneration. Good orientation of the anchoring fibers was found
Inukai <i>et al.</i> [98] 2013	PDLC	AC	DOG 18-36 months old	5 (unclear)	Intrabony defects 4 mm $\times$ 5 mm	(a) PDLC + AC (b) AC + PBS (c) no treatment	-Histological: HES -Histomorphometric -Radiological: dental X-ray	Cementum, bone heights and surface were more significant in group a
Khorsand <i>et al.</i> [67] 2013	DPSC	3-4 Demineralized bovine bone matrix granules	DOG Mongrel male 1-2 years old	10 (a, b)	Intrabony defects 3 mm $\times$ 5 mm $\times$ 8 mm	(a) DPSC + Demineralized bovine bone matrix (b) Demineralized bovine bone matrix	-Histologic: HES -Histomorphometric	Bone formation was similar between the groups. Cementum and ligament were regenerated more in the test group



Fu <i>et al.</i> [87] 2014	PD/LSC SHED	HA/ $\beta$ -TCP	MINIATURE PIG 6 (a, b, c) female 9-12 months old	Intrabony defects 5 mm x 7 mm x 7 mm	(a) PD/LSC + HA- $\beta$ -TCP (b) SHED + HA- $\beta$ -TCP (c) HA- $\beta$ -TCP	3 months	-Clinical: CAL, PD, GR -Histological: HES -Radiological: CT	PD/LSC and SHED significantly improved periodontal regeneration Better results were found for groups with GMSC
Fawzy El-Sayed <i>et al.</i> [104] 2012	GMSC	Deminerlized bovine bone matrix + collagen membrane	MINIATURE PIG 8 (a, b, c, d, e, f) 1 female and 7 males 1841 months old	Intrabony defects 3 mm x 7 mm x 5 mm	(a) GMSC + Deminerlized bovine bone matrix (b) GMSC + collagen scaffold (c) Deminerlized bovine bone matrix (d) collagen scaffold (e) mucoperiosteal flap and root planning only (f) without intervention	3 months	-Clinical: CAL, PD, GR, BOP, PI -Histological: HES -Histomorphometric -Radiological: CT	
Ozasa M <i>et al.</i> [139] 2014	ASC	Fibrin gel	DOG Beagle female 50-56 months old	Class II furcation defects 4 mm in depth	(a) ASC + fibrin gel (b) fibrin gel	1, 5 months	-Histological: AZAN -Histomorphometric -Radiological: micro-CT	New alveolar bone, PL and cementum were found at sites treated with ASC
Tobita <i>et al.</i> [103] 2013	ASC	PRP gel	DOG Beagle 9 or 10 months old	Intrabony with class III furcation defects 5 mm in depth	(a) ASC + PRP gel (b) PRP gel (c) empty	2 months	-Histological: HES, Azan or elastic van Gieson staining -Histomorphometric -Immunohistochemical: Osteocalcin -Radiological: X-ray	Periodontal regeneration only occurred in test group

HA: hydroxyapatite; TCP: tricalcium phosphate; PGA: polyglycolic acid; ePTFE: e-polytetrafluoroethylene; PRP: platelet-rich plasma; DPSC: dental pulp stem cells; PD/LSC: periodontal ligament stem cells; PD/LC: periodontal ligament cells; DFSC: dental follicle stem cells; CDC: ce-mentum derived cells; GMSC: gingival margin stem cells; SHED: stem cells from human exfoliated deciduous teeth; BMSC: bone marrow stem cells; ASC: adipose stem cells; MSC: mesenchymal stem cells; AC: atelocollagen; PL: periodontal ligament; CAL: clinical attachment level; PI: plaque index; PD: probing depth; GR: gingival recession; BOP: bleeding on probing; HES: hematoxylin eosin stain; Col: collagen; GFP: green fluorescent protein; CRP: serum c-reactive protein; IL-10: interleukin-10; CD: cluster of differentiation; SEM: scanning electron microscopy

Scaffold-free delivery, instead, points to the transplantation of different cells already organized in particular shapes, from cell sheets to clumps. This approach already has proven potential *in vitro* and *in vivo* models, but it still needs more understanding, experimentation, and better definition to be available for daily use [80,81].

## NON-DENTAL STEM CELLS IN ANIMAL STUDIES

### Bone marrow mesenchymal stem cells

BMSCs seems to enable an improvement in bone, PL, and cementum regeneration [53,70,82]. BMMSCs promoted increased bone formation in fenestration and Grade II furcation defects [54,70,73]. Furcation defects were almost regenerated with cementum, PL, and alveolar bone after BMMSCs transplantation [82]. However, when used in combination with bone substitutes in intrabony defects, it failed to show significant improvement in terms of bone regeneration, compared to bone substitutes alone [68]. Furthermore, BMMSCs had no effect on bone regeneration in three-wall intrabony defects, when used without bone substitutes [69].

BMMSCs had a positive effect on cementum regeneration in Grade II and III furcation defects, but failed to induce increased cementum formation in fenestration defects compared to control group [54], even though it provided improved results in terms of collagen fibers inserted into the root surface. In

Grade III furcation, bone regeneration was not complete. When tested in intrabony defects, BMMSCs provided conflicting results for PL regeneration. One study reported that BMMSCs without bone substitutes did not promote an increased PL regeneration<sup>[82]</sup>. Another study reported that the combination of BMMSCs and bone substitutes provided significantly higher formation of new cementum and PL compared to bone substitutes alone<sup>[68]</sup>. A study which compared the performance of bone graft with PDLSCs or BMMSCs in intrabony defects reported less perpendicularly oriented newly inserted fibers in the BMMSCs group. Conflicting results were also reported in the use of BMMSCs that had undergone *ex vivo* osteogenic differentiation before use. Osteogenically differentiated BMMSCs (oBMMSCs) promoted increased bone formation but not cementum and PL regeneration.

### **Adipose-derived stem cells**

ASCs and DPSCs appeared to have similar genetic expression patterns. ASCs transplanted in periodontal defects have been shown to favor cementum and PL fibers regeneration and to increase periodontal vascularization<sup>[83,84]</sup>. Finally, ASCs in extraction sockets in a rabbit model showed potential for the regeneration of the alveolar bone structure<sup>[85]</sup>.

## **DENTAL STEM CELLS IN ANIMAL STUDIES**

### **Periodontal ligament stem cells**

As for PDLSCs, the majority of the studies showed the positive effect of the use of this type of cells for periodontal regeneration<sup>[86-91]</sup>, as reported in a recent systematic review of pre-clinical studies<sup>[92]</sup>. Importantly, PDLSCs have unique properties to form a cementum/PL complex-like structure when ectopically transplanted in animals<sup>[93,94]</sup>.

In fenestration defects (applied with hyaluronic acid sheet), they showed significantly greater formation of cementum, bone, and periodontal ligament than in control group<sup>[95]</sup>. When PDLSCs were associated with e-PTFE membranes on fenestration defects, it was observed that cementum formation was increased in the test group; however, no difference in bone formation was observed between test and control (e-PTFE membranes alone)<sup>[96]</sup>. In fact, data on bone regeneration with PDLSCs are conflicting, even though their effect on cementum and PL is promising. Another study reported that PDLSCs improved bone formation in circumferential and fenestration defects, but not in three-wall defects treated without bone substitutes<sup>[90]</sup>. In intrabony defects, they provided improved periodontal regeneration with a nearly complete recovery of bone, cementum, and ligament when used in combination with beta tri-calcium phosphate<sup>[86,97]</sup>. The benefits were less pronounced but still maintained when used with non-supportive biomaterials<sup>[90,98]</sup>. PDLSCs' protocols without manipulation before direct implantation have been tested and proven effective, thus making the use of this cell type more sustainable<sup>[95]</sup>.

### **Dental pulp stem cells**

DPSCs are another well studied lineage in the animal model. These cells were isolated almost 20 years ago<sup>[35]</sup> and found to be capable of forming lamellar bone after grafting<sup>[43,99]</sup>. Their use in different ways (injected, organized in sheet, and on different carriers) showed potential in regenerative procedure<sup>[67]</sup>. In the majority of the studies, the regeneration of all periodontal tissues was increased when DPSCs were used. Regenerated cementum was thicker in the group receiving bone substitutes plus DPSCs than in the control group treated with bone substitute alone, and it covered a larger surface of the root<sup>[67]</sup>, even though no noticeable difference in bone formation between the two groups was observed.

### **Dental follicle precursor cells**

DFPCs may improve periodontal regeneration by PDLSCs *in vivo*. DFPCs appear to enhance the self-renewal and multi-differentiation capacity of PDLSCs, which indicates that DFPCs could provide a beneficial microenvironment for periodontal regeneration by using PDLSCs<sup>[100]</sup>.

### Stem cells from human exfoliated deciduous teeth

When injected supra-periostally close to periodontal defects, SHEDs reduced gum bleeding, increased new attachment of PL, and decreased osteoclast differentiation. Micro-CT analysis demonstrated increased bone volume and decreased distance of cementum-enamel junction to alveolar bone crest, compared to control with no treatment<sup>[46]</sup>. Histopathological photomicrographs showed newly regenerated bone and decreased number of inflammatory factors and osteoclasts. In a recent report, SHEDs were compared to PDLSCs for the treatment of intrabony defects. Both treatments were provided in combination with hydroxyapatite and beta tri-calcium phosphate scaffold. The results showed no significant difference between the two groups. SHEDs significantly improved periodontal regeneration compared to the scaffold alone, in a very similar way to PDLSCs<sup>[89]</sup>.

### Stem cells from apical papilla

Local Injection of SCAPs increased CAL and bone volume in periodontal defects compared to injection of 0.9% NaCl. Histopathology results demonstrated remarkable regeneration in the SCAPs group, whereas regeneration of periodontal tissue was hardly found in the 0.9% NaCl group<sup>[101]</sup>.

### Other cells

Other stem cell types are less studied, yet have positive effects, especially APCs<sup>[102]</sup>, ASCs<sup>[103]</sup>, and GMSCs<sup>[104]</sup>. The two latter lineages have another positive characteristic: they can easily be retrieved in large quantity. As it is necessary to implant the highest possible number of cells to have better results, their disposability is definitely an advantage in comparison with other SC types. This advantage is also shared with iPSCs, which can be produced from a series of already differentiated cell types.

## HUMAN STUDIES

### Case reports, case series, and retrospective studies

Periodontal regeneration was investigated in human clinical studies using PDLSCs, DPSCs, and BMMSCs. DPSCs were observed in two case reports<sup>[105,106]</sup> and three case series<sup>[107-109]</sup>. One of the case reports provided positive results for allogenic transplantation of DPSCs<sup>[106]</sup>. In parallel, some initial reports in humans demonstrated the clinical and radiographic efficacy of dental pulp micrografts in post-extraction alveolar defects<sup>[110,111]</sup>. In general, it was found that, in periodontal regeneration, DPSC micrografts associated with surgical procedure were able to reduce PPD and increase CAL.

PDLSCs have been tested for regenerative procedures in intrabony defects and Grade II furcation defects<sup>[112]</sup>. A retrospective study, which included 16 defects in three patients, observed PPD reduction and CAL gain, thus supporting a potential benefit for the use of PDLSCs in periodontal regeneration<sup>[113]</sup>. Later on, in a case report<sup>[114]</sup>, it was observed that periodontal regenerative surgery using PDLSCs, incorporated in a gelatin sponge, produced PPD reduction, CAL gain, and radiographic bone fill. In Grade II furcation defects, PDLSCs provided good clinical results, reducing PPD and improving CAL in six months.

BMMSCs have been tested in four case reports and one case series that reported good clinical outcomes for the periodontal regeneration of intrabony defects<sup>[115-118]</sup> and Grade III furcation defects<sup>[119]</sup>.

### Randomized controlled trials

Once the positive results in the use of stem cells in periodontal regeneration have been proven in animal and humans studies, RCTs are needed to assess the safety and the efficacy of the use of stem cells in periodontal regeneration compared to standard treatments.

Thus far, four RCTs<sup>[120-123]</sup> have been published on assessing the efficacy of stem cells in periodontal regeneration, compared to open flap debridement<sup>[121,123]</sup> or compared to the use of a scaffold alone without

stem cells<sup>[120,122]</sup> in intrabony defects. These RCTs differ in the type of stem cells selected (PDLSCs, DPSCs, or UC-MSCs) and in their application for the regenerative procedure (chairside use or isolation, differentiation, and cell culture in a laboratory). Shailini and coworkers<sup>[121]</sup> applied the concept of “stem cell niche” to periodontal regeneration. In this prospective, randomized, single-blinded, controlled trial with parallel design, 16 patients with one intrabony defect were treated. In the test group, PDLSCs collected from an extracted tooth, together with the soft-tissue adherent to the extracted root surface and to the alveolar socket (PDL tissue niche), were directly mixed to a gelatin sponge and implanted in the intrabony defect. The control group was treated with an open flap debridement. The results after one year showed a greater improvement in PD reduction, CAL gain, and radiographic defect resolution in the group treated with PDLSCs; however, the differences were not statistically significant. No adverse effects were reported, thus suggesting the safety of the procedure.

Similar to Shailini, Chen and coworkers<sup>[120]</sup> studied the efficacy of PDLSCs. PDLSCs harvested from an extracted tooth were isolated, characterized, and grown into sheets in a laboratory. In this single-center randomized trial, 41 intrabony defects were treated with either GTR and PDLSC sheets in combination with demineralized bovine bone matrix or with GTR and demineralized bovine bone matrix alone. The results after one year showed an increased alveolar bone height in both groups, without statistically significant differences between groups. As for the clinical periodontal parameters, no statistically significant differences were found for the increased CAL, PD, or GR between the cell and control groups. No adverse effects in the use of PDL cells sheets were reported.

While Chen and Shailini evaluated the efficacy of PDLSCs, Ferrarotti and coworkers<sup>[122]</sup> evaluated the use of micrografts containing DPSCs delivered into intrabony defects in a collagen scaffold. In this parallel, double-blind, prospective randomized trial, 29 patients with an intrabony defect were treated with either minimally invasive surgical technique (MIST) plus dental pulp micrografts in a collagen sponge biocomplex (test) or MIST plus collagen sponge alone (control). The micrografts enriched in DPSCs were obtained from the pulp chamber of an extracted tooth, dissociated by the use of a biological tissue disaggregator (Rigenera Machine System, Rigenera; HBW, Turin, Italy), and then seeded on a collagen sponge scaffold. After one year, the results showed a statistically significant greater PD reduction, CAL gain, and radiographic bone defect fill in the group treated with DPSCs on a scaffold, compared to the scaffold alone. No adverse effects were reported.

Instead of using dental stem cells, Dhote and coworkers<sup>[123]</sup> tested the efficacy of non-dental stem cells on periodontal regeneration, focusing their attention on the umbilical cord MSCs (UC-MSCs). In this parallel designed RCT, 24 periodontal intrabony defects in 14 patients were treated by either applying allogeneic cord blood MSCs on a beta-tricalcium phosphate (beta-TCP) scaffold in combination with platelet-derived growth factor-BB (rh-PDGF-BB) or by open flap debridement (OFD). The results after six months showed significantly greater CAL gain, PPD reduction and radiographic defect fill in the group treated with a combination of allogeneic UC-MSCs, rh-PDGF-BB, and beta-TCP scaffold compared to the OFD. No adverse effects were reported demonstrating the safety of mesenchymal stem cells derived from umbilical cord for dental tissue engineering.

An interesting field of application for stem cells is represented by defects that cannot be predictably treated with the techniques available today, such as furcation defects or supracrestal regeneration. The application of stem cells to enhance the regeneration of furcations was tested in an RCT by Akbay and coworkers<sup>[113]</sup>, which evaluated the periodontal regenerative potential of PL grafts in Grade II furcation defects. Ten patients were treated in a split mouth design: on one side, a molar was treated with a coronally positioned flap with autogenous PDLSCs grafts obtained from third molars, while, on the other side, with a coronally positioned flap alone. PL remnants attached to cementum and cellular cementum were collected by

scaling the surface of the extracted third molars with sterile curettes and grafted directly into the furcation defect. After six months, a reentry was performed on both sides to assess the defect fill. In one randomly selected patient, gingival biopsies were taken on test and control sites. The results after six months showed improvement in terms of horizontal and vertical defect fill, PD, and CAL in both groups, with significantly better results in PD reduction for the grafted sites. No adverse effects or foreign body reactions were observed in PDL grafts.

In general, the RCTs published thus far suggest safety in the use of stem cells and promising clinical results [Table 3]. The need of a tooth that has to be extracted in order to harvest PDLSCs or DPSCs is a drawback of these cell therapies. This may be considered the main issue in the use of stem cells. Harvest, isolation, and possible differentiation of stem cells are time-consuming, complex, and expensive processes. Protocols with direct use of the harvested cells were proposed and evaluated by Shailini and Ferrarotti<sup>[121,122]</sup>; the former suggested grafting directly the PDL tissues in the defect, while the latter suggested using biological tissue disaggregation to obtain a micrograft. The advantage of these protocols is the reduction of time and costs of treatment; however, the presence and viability of the cells implanted in the defects cannot be proven since no isolation and characterization are performed.

The results of the RCTs available in the literature show promising indirect measurements of the effectiveness of the regeneration process, assessed by means of clinical and radiographic parameters. However, true regeneration can only be proven by histological analysis, impossible to carry out in any of the studies because of ethical limitations.

#### **FUTURE PERSPECTIVES FOR STEM CELLS IN PERIODONTAL REGENERATION**

To overcome some limitation of the present cell therapy and based on the promising results of this animal and human research of stem cells, a further step forward has been proposed by researchers: exogenous human MSCs.

Thus far, autologous use of stem cells has been applied only, using an extracted tooth as the source for either PDLSCs or DPSCs. To overcome this limitation, as well as the limitation of the use of stem cells in elderly people, whose regenerative capacity is limited, the use of exogenous or allogenic stem cells has been proposed<sup>[124]</sup>.

Exogenous human MSCs have already been tested in cases of biologic refractory luminal Crohn's disease with fistulae formation, cranial defects, myocardial regeneration, and patients with aging frailty. Exogenous MSC infusion seemed to be very well tolerated, with only light and short-term effects and frequently no adverse reaction at all. Thus, exogenous MSCs appear to be a feasible technique for periodontal and regenerative treatments in general.

Pluripotent stem cells generated from somatic cells (iPSCs) are a possible stem cell lineage to study for periodontal regeneration<sup>[125]</sup>. They have the potential to differentiate in a spectrum of different cells and tissues. In dental research, iPSCs-derived mesenchymal cells and osseoprogenitor cells were investigated by scientists with great interest. To be used, these cells need to go through a process of transdifferentiation. In this process, mature somatic cells undergo a transformation to a different somatic cell without going through a pluripotent state or a progenitor phase. This process is also called lineage switching or lineage conversion. By means of this process, epigenetic modifications, by directly reprogramming non-osteoblasts cells into functional osteoblasts, have started to be considered as a new therapeutic approach for alveolar bone regeneration. At present, more knowledge for applying these cells to cell-based therapy is needed and preclinical and clinical research will enhance our understanding of these processes. iPSCs reprogrammed from non-dental cells have shown promising results in periodontal regeneration in mice, in combination



**Table 3. Summary of RCT on periodontal regeneration with mesenchymal stem cells**

Author	Study design	Cell type	Stem cells handling	Defect type	Number of patients	Treatment groups	Follow-up	Outcome variables	Results
Akbay <i>et al.</i> <sup>[102]</sup> 2005	RCT with a split mouth design	PDLSC	Direct application of PDL tissue collected from an extracted molar, into the defect	Class II furcation defects	10 patients 20 defects: 10 test/10 control	Test: coronally positioned flap with autogenous PDL grafts that were obtained from third molars Control: coronally positioned flap alone	6 months, with a surgical re-entry	Clinical: PI, GI, PD, GR, CAL Radiographic: linear and volumetric evaluation Volumetric defect fill by impression of the defects Histologic analysis by gingival biopsy from one patient	Sites treated with PDL grafts demonstrated significant improvement in vertical and horizontal defect fill, PD, and CAL at 3 and 6 months compared to pre-surgical values. The difference determined for the PD values of both groups at a statistically significant degree in favor of grafted sites was maintained at all observation periods. No foreign body reaction was observed in PDL grafts
Chen <i>et al.</i> <sup>[120]</sup> 2016	single-center RCT	PDLSC	Collection from an extracted molar; isolation, culture, characterization and engineering into cells sheets in laboratory	Intrabony defects	30 patients 41 defects: 20 test/21 control	Test: GTR and PDLSC sheets in combination with demineralized bovine bone matrix Control: GTR and demineralized bovine bone matrix without stem cells	12 months	Radiographic (main outcome): Increase in alveolar bone height (rx bone fill) Clinical: CAL, PPD, REC Safety assessment: blood and urine examination	Both groups showed a significant increase in the alveolar bone height, without statistically significant differences between groups. Regarding the clinical periodontal parameters, no statistically significant differences were found for the increased CAL, PD or GR between the cell and control groups. No adverse effects on the use of PDL cells sheets were reported
Dhote <i>et al.</i> <sup>[123]</sup> 2015	Parallel designed RCT	UC-MSC	Collection from the hospital in a sterile tube Followed by isolation and culture on $\beta$ -TCP scaffold	Intrabony defects	14 patients 24 defects: 12 test/12 control	Test: ODF applying allogeneic UC-MSCs on a $\beta$ -TCP scaffold in combination rh-PDGF-BB Control: OFD	6 months	Clinical: PI, BPI, CAL, PPD, relative gingival marginal level Radiographic: linear bone growth (LBG)	The test protocol resulted in a significant added benefit in terms of CAL gains, PPD reductions greater radiographic defect fill and improvement in Linear bone growth compared to the OFD alone. No adverse effects, allergy, infection or patients complaints related to the graft material were reported
Ferrarotti <i>et al.</i> <sup>[122]</sup> 2018	Parallel, double-blind, prospective RCT	DPSC	Mechanical dissociation of the dental pulp of an extracted tooth by the use of a biological tissue disaggregator to obtain micrografts rich in autologous DPSC endorsed on a collagen sponge	Intrabony defects	29 patients 29 defects: 15 test/14 control	Test: minimally invasive surgical technique (MIST) plus dental pulp micrografts in a collagen sponge biocomplex Control: MIST plus collagen sponge alone	12 months	Clinical: PI, Bop, PD, REC, CAL Radiographic: bone fill	Test sites exhibited significantly more PD reduction, CAL gain and bone defect fill than controls. Moreover, residual PD < 5 mm and CAL gain $\geq$ 4 mm were significantly more frequent in the test group. No adverse effects were reported

Shalini et al.<sup>[121]</sup>  
2018

Parallel, prospective, single-blinded RCT	PDLSC	"Stem cell niche" concept. PDL tissue adherent to an extracted tooth root and alveolar socket comprised of PDLSCs along with its niche (PDL tissue niche) obtained with curettes and mixed with a gelatin sponge	Intrabony defect	28 patients 28 defects: 14 test/14 control	Test: Open flap debridement followed by direct transplantation of autologous PDLSC niche mixed with a gelatin sponge Control: open flap debridement alone	12 months	Clinical: PI, GBI, PD, CAL, GMP, GT Radiographic: Defect area resolution/bone fill and bone-like tissue density	The result showed a significant reduction of clinical parameters in both groups. A slightly greater improvement in PD reduction, CAL gain and radiographic defect resolution was found in the group treated with PDLSCs than OFD but the differences were not statistically significant. No adverse effects were reported
---	-------	--	------------------	--	--	-----------	--	---

PDLSC: periodontal ligament stem cell; DPSC: dental pulp stem cell; RCT: randomized clinical trial; PI: plaque index; GBI: gingival bleeding index; PD: pocket depth; CAL: clinical attachment level; GMP: gingival margin position; GT: gingival thickness; OFD: open flap debridement

with scaffold or bioactive agents such as EMD, favoring alveolar bone formation, cementum, and PL regeneration<sup>[69]</sup>. In another rat periodontal defect model, iPSCs differentiated into MSCs demonstrated the capacity to enhance periodontal regeneration and the formation of new fibrous tissue, mineralized tissue, and PDL-like tissue<sup>[126,127]</sup>. Furthermore, iPSCs can differentiate into cells that promote tooth regeneration<sup>[128,129]</sup>.

Stem cells homing is another possible solution that has been investigated to overcome the shortcomings of the harvest and the possible immune response against MSCs grafting. Homing is known as the process to recall endogenous cells toward an injured site by means of biochemical signals<sup>[130]</sup>. It is considered the first step of healing in a successful regenerative process. The patient's self-repair capacity and recruitment of stem cells can be stimulated with chemoattractant and growth factors such as stromal cell-derived factor-1 $\alpha$  (SDF-1 $\alpha$ ). The major shortcoming of such molecules is their high expenditure and their very short half-life, especially when injected directly into the injured site. For this reason, combinations with carriers or scaffold for growth factors delivery seem to be a practical strategy<sup>[131]</sup>. SDF-1 $\alpha$ -loaded gelatin sponges have been proven to enhance bone and PL regeneration<sup>[132]</sup>. Emoderivates such as platelet-rich plasma and platelet-rich fibrin are composed of a variety of growth factors and have been used for stem cell homing in periodontal regeneration with promising results<sup>[133,134]</sup>.

The development of 3D printing technology represents an important future perspective as it introduces compartmentalized and hybrid scaffolds with a precise three-dimensional and spatial organization. This makes possible to guide the formation of oriented ligamentous tissues incorporated in the newly formed bone and cementum. Based on the physiological anatomical structure of the periodontium, it has been speculated that particular cell-material designs may enhance the regeneration of the periodontal attachment apparatus. Thus, tissue engineering has been used to recreate a bone-PDL-cementum structure similar to the native one. By stratifying materials and cells in different layers, mimicking nature, researchers have succeeded in regenerating a structure similar to the physiological attachment apparatus<sup>[135]</sup>. Vertical layering of PDLSCs sheets, woven polyglycolic acid, and porous beta-TCP demonstrated the recreation of bone and cementum with inserted collagen fibers<sup>[97]</sup> in intrabony defects. Animal models corroborate these findings and the potential of this customized fiber-guiding scaffold that can be precisely adapt to defects morphology, successfully homing the cell/tissue complex during regeneration processes and resulting in a more stable complex and rapidly maturing matrix<sup>[136]</sup>.

With all these different possible therapeutic approaches, we must not forget the four key factors needed to reach the goal of a “*restitutio ad integrum*” of the periodontal apparatus and have a true regeneration: cells, environment, signals, and time. Stem cells (MSCs, PDLSCs, iPSCs, *etc.*) have to be put or recruited in a favorable and protected environment (e.g., 3D scaffold) and guided in their transformation process by signaling molecules (homing and differentiating factors), for long enough to mature.

A pivotal role is also played by MSCs adhesion capacities and longevity. In fact, cell adhesion is critical for survival, proliferation, and differentiation of MSCs. Similarly, MSCs’ longevity may influence the outcomes of the regeneration therapies. Therefore, to maximize the potential of tissue engineering therapies, researchers have to focus their attention on well-designed scaffolds and precise signaling molecules. The former are meant to favor adhesion and impede anoikis and the latter are to be used both before and during transplantation to stimulate cell proliferation and longevity in order to overcome possible low cells survival rates<sup>[137]</sup>.

These different methods might have to be combined to achieve the maximum result in tissue regeneration. Ideally, an optimum protocol should focus on three key points: (1) the use exogenous or endogenous MSCs pre-treated with bioactive factors; (2) a protective scaffold that favors cells adhesion and spreading; and (3) signaling molecule doping of the scaffold to boost cells’ regenerative abilities, increase their longevity, and recruit nearby already present stem cells.

Finally, the future research is focused on the possible application of cell therapy for regeneration. The fields that are being investigated extend from what is possible at the present time (dentin–pulp regeneration and periodontal regeneration) to future applications (whole-tooth regeneration)<sup>[138]</sup>. Thus far, the evidence for whole-tooth regeneration is limited *in vitro* and in animal models, but research is progressing quickly.

## CONCLUSION

The goal of periodontal tissue engineering is to restore the normal function of the diseased periodontium to support the teeth. To achieve this objective, stem cells, appropriate scaffold, and infection control are required at the diseased site. Even if some studies reported conflicting results and irregular outcomes, the available evidence in animal models supports the applicability of stem cells in periodontal tissue regeneration. We hypothesize that this heterogeneity of results could be due to the different methodologies used. In fact, differences in study models (animal and defect ones), treatment modalities, isolation protocols, and study designs are some of the factors that can influence the final results of the investigation. This leads to the present impossibility to propose a specific protocol or MSC type which may be considered superior to others. The efficacy and safety of cell-based interventions in humans have been proven by case reports and case series, but sparsely by RCTs. PDLSCs and DPSCs have been tested and showed promising results both in animal models and in humans. However, it is yet to be defined which protocol performs better, and, although experimental data have allowed the beginning of clinical trials in periodontal cell therapy, proper consideration of the cell source, material type, and regulatory concerns is crucial to facilitate clinical translation. Furthermore, histological evidence of periodontal regeneration is still lacking and the power of studies is hampered by major limitations. One of the major shortcomings is the defects used in animal models, which may not adequately reflect the complex microbiological and immune-inflammatory environment of the periodontal pocket. Actually, in many cases, the periodontal defects generated in the currently used animal studies do not sufficiently represent those of human periodontitis. Evidence for exogenous MSCs grafting, 3D printed-scaffold, and stem cell homing is promising but still limited. Despite all these shortcomings, tissue engineering in periodontology is fascinating and hopefully will help clinicians to overcome the limitations of present periodontal treatments. In fact, MSCs have provided surprisingly favorable outcomes in conditions in which standard procedures for periodontal

regeneration are unsatisfactory, for instance in furcation defects. In this field, the development of cell-based therapy could provide its greatest benefit in the future.

## DECLARATIONS

### Authors' contributions

Performed data acquisition, as well as provided administrative, technical, and material support: Citterio F, Gualini G, Fierravanti L

Supervision: Aimetti M

Write the final review and tables: Citterio F, Gualini G, Fierravanti L

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Aimetti M, Perotto S, Castiglione A, Mariani GM, Ferrarotti F, et al. Prevalence of periodontitis in an adult population from an urban area in North Italy: findings from a cross-sectional population-based epidemiological survey. *J Clin Periodontol* 2015;42:622-31.
2. Chapple I. Iain Chapple: 'as a Clinician, You Assume That People Know What Periodontitis Is'. Vol 215. *Br Dent J* 2013;431-4.
3. Ide M, Linden GJ. Periodontitis, cardiovascular disease and pregnancy outcome--focal infection revisited? *Br Dent J* 2014;217:467-74.
4. Lalla E, Papapanou PN. Diabetes mellitus and periodontitis: a tale of two common interrelated diseases. *Nat Rev Endocrinol* 2011;7:738-48.
5. Araújo VMA, Melo IM, Lima V. Relationship between periodontitis and rheumatoid arthritis: review of the literature. *Mediators Inflamm* 2015;2015:259074.
6. Loos BG. Systemic effects of periodontitis. *Int J Dent Hyg* 2006;4:34-52.
7. Chapple ILC. Time to take periodontitis seriously. *BMJ* 2014;348:g2645.
8. Chapple IL, Van der Weijden F, Doerfer C, Herrera D, Shapira L, et al. Primary prevention of periodontitis: managing gingivitis. *J Clin Periodontol* 2015;42:S71-6.
9. Petersen PE, Ogawa H. The global burden of periodontal disease: towards integration with chronic disease prevention and control. *Periodontol* 2000 2012;60:15-39.
10. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet* 2005;366:1809-20.
11. Cortellini P, Buti J, Pini Prato G, Tonetti MS. Periodontal regeneration compared with access flap surgery in human intra-bony defects 20-year follow-up of a randomized clinical trial: tooth retention, periodontitis recurrence and costs. *J Clin Periodontol* 2017;44:58-66.
12. Suvan J, Leira Y, Moreno F, Graziani F, Derks J, et al. Subgingival instrumentation for treatment of periodontitis: a systematic review. *J Clin Periodontol* 2019; doi: 10.1111/jcpe.13245.
13. Sanz-Sánchez I, Montero E, Citterio F, Romano F, Molina A, et al. Efficacy of access flap procedures compared to subgingival debridement in the treatment of periodontitis. A systematic review and meta-analysis. *J Clin Periodontol* 2020; doi: 10.1111/jcpe.13259.
14. Trombelli L, FrancESCshetti G, Farina R. Effect of professional mechanical plaque removal performed on a long-term, routine basis in the secondary prevention of periodontitis: a systematic review. *J Clin Periodontol* 2015;42:S221-36.
15. Matuliene G, Pjetursson BE, Salvi GE, Schmidlin K, Brägger U, et al. Influence of residual pockets on progression of periodontitis and tooth loss: results after 11 years of maintenance. *J Clin Periodontol* 2008;35:685-95.

16. Claffey N, Egelberg J. Clinical indicators of probing attachment loss following initial periodontal treatment in advanced periodontitis patients. *J Clin Periodontol* 1995;22:690-6.
17. Graziani F, Gennai S, Cei S, Cairo F, Baggiani A, et al. Clinical performance of access flap surgery in the treatment of the intrabony defect. A systematic review and meta-analysis of randomized clinical trials. *J Clin Periodontol* 2012;39:145-56.
18. Aimetti M, Mariani GM, Ferrarotti F, Ercoli E, Audagna M, et al. Osseous resective surgery with and without fibre retention technique in the treatment of shallow intrabony defects: a split-mouth randomized clinical trial. *J Clin Periodontol* 2015;42:182-9.
19. Ferrarotti F, Giraudi M, Citterio F, Fratini A, Gualini G, et al. Pocket elimination after osseous resective surgery: a systematic review and meta-analysis. *J Clin Periodontol* 2020; doi: 10.1111/jcpe.13281.
20. Cortellini P, Tonetti MS. Clinical performance of a regenerative strategy for intrabony defects: scientific evidence and clinical experience. *J Periodontol* 2005;76:341-50.
21. Kao RT, Nares S, Reynolds MA. Periodontal regeneration - intrabony defects: a systematic review from the AAP Regeneration Workshop. *J Periodontol* 2015;86:S77-104.
22. Avila-Ortiz G, De Buitrago JG, Reddy MS. Periodontal regeneration - furcation defects: a systematic review from the AAP Regeneration Workshop. *J Periodontol* 2015;86:S108-30.
23. Cortellini P, Tonetti MS. Clinical concepts for regenerative therapy in intrabony defects. *Periodontol 2000* 2015;68:282-307.
24. Gottlow J, Nyman S, Karring T, Lindhe J. New attachment formation as the result of controlled tissue regeneration. *J Clin Periodontol* 1984;11:494-503.
25. Cortellini P, Tonetti MS. A minimally invasive surgical technique with an enamel matrix derivative in the regenerative treatment of intrabony defects: a novel approach to limit morbidity. *J Clin Periodontol* 2007;34:87-93.
26. Cortellini P, Tonetti MS. Improved wound stability with a modified minimally invasive surgical technique in the regenerative treatment of isolated interdental intrabony defects. *J Clin Periodontol* 2009;36:157-63.
27. Harrel SK. A minimally invasive surgical approach for periodontal regeneration: surgical technique and observations. *J Periodontol* 1999;70:1547-57.
28. Trombelli L, Farina R, FrancESCshetti G, Calura G. Single-flap approach with buccal access in periodontal reconstructive procedures. *J Periodontol* 2009;80:353-60.
29. Hammarström L. The role of enamel matrix proteins in the development of cementum and periodontal tissues. *Ciba Found Symp* 1997;205:246-60.
30. Trombelli L, Farina R. Clinical outcomes with bioactive agents alone or in combination with grafting or guided tissue regeneration. *J Clin Periodontol* 2008;35:117-35.
31. Palmer RM, Cortellini P. Group B of European Workshop on Periodontology. Periodontal tissue engineering and regeneration: Consensus Report of the Sixth European Workshop on Periodontology. *J Clin Periodontol* 2008;35:83-6.
32. Biehl JK, Russell B. Introduction to stem cell therapy. *J CardioVASC Nurs* 2009;24:98-105.
33. Kobolak J, Dinnyes A, Memic A, Khademhosseini A, Mobasheri A. Mesenchymal stem cells: identification, phenotypic characterization, biological properties and potential for regenerative medicine through biomaterial micro-engineering of their niche. *Methods* 2016;99:62-8.
34. Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. *J Dent Res* 2009;88:792-806.
35. Gronthos S, Mankani M, Brahimi J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A* 2000;97:13625-30.
36. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, et al. SHEDs: stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci U S A* 2003;100:5807-12.
37. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004;364:149-55.
38. Morsczeck C, Moehl C, Götz W, Heredia A, Schäffer TE, et al. In vitro differentiation of human dental follicle cells with dexamethasone and insulin. *Cell Biol Int* 2005;29:567-75.
39. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J Endod* 2008;34:166-71.
40. Gronthos S, Brahimi J, Li W, Fisher LW, Cherman N, et al. Stem cell properties of human dental pulp stem cells. *J Dent Res* 2002;81:531-5.
41. Tziafas D, Kodonas K. Differentiation potential of dental papilla, dental pulp, and apical papilla progenitor cells. *J Endod* 2010;36:781-9.
42. Yu J, He H, Tang C, Zhang G, Li Y, et al. Differentiation potential of STRO-1+ dental pulp stem cells changes during cell passaging. *BMC Cell Biol* 2010;11:32.
43. Graziano A, d'Aquino R, Cusella-De Angelis MG, Francesco FD, Giordano A, et al. Scaffold's surface geometry significantly affects human stem cell bone tissue engineering. *J Cell Physiol* 2008;214:166-72.
44. Papaccio G, Graziano A, d'Aquino R, Graziano MF, Pirozzi G, et al. Long-term cryopreservation of dental pulp stem cells (SBP-DPSCs) and their differentiated osteoblasts: a cell source for tissue repair. *J Cell Physiol* 2006;208:319-25.
45. Su WT, Chiou WL, Yu HH, Huang TY. Differentiation potential of SHEDs using biomimetic periosteum containing dexamethasone. *Mater Sci Eng C Mater Biol Appl* 2016;58:1036-45.
46. Gao X, Shen Z, Guan M, Huang Q, Chen L, et al. Immunomodulatory role of stem cells from human exfoliated deciduous teeth on periodontal regeneration. *Tissue Eng Part A* 2018;24:1341-53.
47. Nakamura S, Yamada Y, Katagiri W, Sugito T, Ito K, et al. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. *J Endod* 2009;35:1536-42.



48. Liu L, Michowski W, Kolodziejczyk A, Sicinski P. The cell cycle in stem cell proliferation, pluripotency and differentiation. *Nat Cell Biol* 2019;21:1060-7.
49. Chrepa V, Pitcher B, Henry MA, Diogenes A. Survival of the Apical papilla and its resident stem cells in a case of advanced pulpal necrosis and apical periodontitis. *J Endod* 2017;43:561-7.
50. Nada OA, El Backly RM. stem cells from the apical papilla (SCAPs) as a tool for endogenous tissue regeneration. *Front Bioeng Biotechnol* 2018;6:103.
51. Hu B, Nadiri A, Kuchler-Bopp S, Perrin-Schmitt F, Peters H, et al. Tissue engineering of tooth crown, root, and periodontium. *Tissue Eng* 2006;12:2069-75.
52. Hu B, Unda F, Bopp-Kuchler S, Jimenez L, Wang XJ, et al. Bone marrow cells can give rise to ameloblast-like cells. *J Dent Res* 2006;85:416-21.
53. Hasegawa N, Kawaguchi H, Hirachi A, Takeda K, Mizuno N, et al. Behavior of transplanted bone marrow-derived mesenchymal stem cells in periodontal defects. *J Periodontol* 2006;77:1003-7.
54. Yang Y, Rossi FM, Putnins EE. Periodontal regeneration using engineered bone marrow mesenchymal stromal cells. *Biomaterials* 2010;31:8574-82.
55. Ohazama A, Modino SA, Miletich I, Sharpe PT. Stem-cell-based tissue engineering of murine teeth. *J Dent Res* 2004;83:518-22.
56. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981;292:154-6.
57. Inanç B, Elçin AE, Elçin YM. In vitro differentiation and attachment of human embryonic stem cells on periodontal tooth root surfaces. *Tissue Eng Part A* 2009;15:3427-35.
58. Ning F, Guo Y, Tang J, Zhou J, Zhang H, et al. Differentiation of mouse embryonic stem cells into dental epithelial-like cells induced by ameloblasts serum-free conditioned medium. *Biochem Biophys Res Commun* 2010;394:342-7.
59. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006;126:663-76.
60. Wada N, Wang B, Lin NH, Laslett AL, Gronthos S, et al. Induced pluripotent stem cell lines derived from human gingival fibroblasts and periodontal ligament fibroblasts. *J Periodontol Res* 2011;46:438-47.
61. Yan X, Qin H, Qu C, Tuan RS, Shi S, et al. iPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. *Stem Cells Dev* 2010;19:469-80.
62. Xu XY, Li X, Wang J, He XT, Sun H-H, et al. Concise review: periodontal tissue regeneration using stem cells: strategies and translational considerations. *Stem Cells Transl Med* 2019;8:392-403.
63. Portron S, Soueidan A, Marsden AC, Rakic M, Verner C, et al. Periodontal regenerative medicine using mesenchymal stem cells and biomaterials: a systematic review of pre-clinical studies. *Dent Mater J* 2019;38:867-83.
64. Shamsul BS, Aminuddin BS, Ng MH, Ruszymah BH. Age and gender effect on the growth of bone marrow stromal cells in vitro. *Med J Malaysia* 2004;59:196-7.
65. Balduino A, Mello-Coelho V, Wang Z, Taichman RS, Krebsbach PH, et al. Molecular signature and in vivo behavior of bone marrow endosteal and subendosteal stromal cell populations and their relevance to hematopoiesis. *Exp Cell Res* 2012;318:2427-37.
66. Suaid FF, Ribeiro FV, Gomes TR, Silvério KG, Carvalho MD, et al. Autologous periodontal ligament cells in the treatment of class III furcation defects: a study in dogs. *J Clin Periodontol* 2012;39:377-84.
67. Khorsand A, Eslaminejad MB, Arabsolghar M, Paknejad M, Ghaedi B, et al. Autologous dental pulp stem cells in regeneration of defect created in canine periodontal tissue. *J Oral Implantol* 2013;39:433-43.
68. Paknejad M, Eslaminejad MB, Ghaedi B, Rohn AR, Khorsand A, et al. Isolation and assessment of mesenchymal stem cells derived from bone marrow: histologic and histomorphometric study in a canine periodontal defect. *J Oral Implantol* 2015;41:284-91.
69. Duan X, Tu Q, Zhang J, Ye J, C Sommer, et al. Application of induced pluripotent stem (iPS) cells in periodontal tissue regeneration. *J Cell Physiol* 2011;226:150-7.
70. Simsek SB, Keles GC, Baris S, Cetinkaya BO. Comparison of mesenchymal stem cells and autogenous cortical bone graft in the treatment of class II furcation defects in dogs. *Clin Oral Investig* 2012;16:251-8.
71. Bakopoulou A, Apatzidou D, Aggelidou E, Gousopoulou E, Leyhausen G, et al. Isolation and prolonged expansion of oral mesenchymal stem cells under clinical-grade, GMP-compliant conditions differentially affects “stemness” properties. *Stem Cell Res Ther* 2017;8:247.
72. Kawaguchi H, Hirachi A, Hasegawa N, Iwata T, Hamaguchi H, et al. Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. *J Periodontol* 2004;75:1281-7.
73. Nagahara T, Yoshimatsu S, Shiba H, Kawaguchi H, Takeda K, et al. Introduction of a mixture of  $\beta$ -tricalcium phosphate into a complex of bone marrow mesenchymal stem cells and type I collagen can augment the volume of alveolar bone without impairing cementum regeneration. *J Periodontol* 2015;86:456-64.
74. Zang S, Jin L, Kang S, Hu X, Wang M, et al. Periodontal wound healing by transplantation of jaw bone marrow-derived mesenchymal stem cells in chitosan/anorganic bovine bone carrier into one-wall infrabony defects in beagles. *J Periodontol* 2016;87:971-81.
75. Fujioka-Kobayashi M, Müller HD, Mueller A, Lussi A, Sculean A, et al. In vitro effects of hyaluronic acid on human periodontal ligament cells. *BMC Oral Health* 2017;17:44.
76. Takeda K, Sakai N, Shiba H, Fujita T, Kajiya M, et al. Characteristics of high-molecular-weight hyaluronic acid as a brain-derived neurotrophic factor scaffold in periodontal tissue regeneration. *Tissue Eng Part A* 2011;17:955-67.
77. Zhang J, Chen Y, Xu J, Wang J, Li C, et al. Tissue engineering using 3D printed nano-bioactive glass loaded with NELL1 gene for repairing alveolar bone defects. *Regen Biomater* 2018;5:213-20.
78. Funda G, TASCshieri S, Bruno GA, Grecchi E, Paolo S, et al. Nanotechnology scaffolds for alveolar bone regeneration. *Materials (Basel)*

- 2020;13:201.
79. Baba S, Yamada Y, Komuro A, Yotsui Y, Umeda M, et al. Phase I/II trial of autologous bone marrow stem cell transplantation with a three-dimensional woven-fabric scaffold for periodontitis. *Stem Cells Int* 2016;2016:6205910.
  80. Motoike S, Kajiya M, Komatsu N, Horikoshi S, Ogawa T, et al. Clumps of mesenchymal stem cell/extracellular matrix complexes generated with xeno-free conditions facilitate bone regeneration via direct and indirect osteogenesis. *Int J Mol Sci* 2019;20:3970.
  81. Takewaki M, Kajiya M, Takeda K, Horikoshi S, Ogawa T, et al. MSCs/ECM cellular complexes induce periodontal tissue regeneration. *J Dent Res* 2017;96:984-91.
  82. Cai X, Yang F, Yan X, Yang W, Yu N, et al. Influence of bone marrow-derived mesenchymal stem cells pre-implantation differentiation approach on periodontal regeneration in vivo. *J Clin Periodontol* 2015;42:380-9.
  83. Lemaitre M, Monsarrat P, BIASco-Baque V, Loubières P, Burcelin R, et al. Periodontal tissue regeneration using syngeneic adipose-derived stromal cells in a mouse model. *Stem Cells Transl Med* 2017;6:656-65.
  84. Tobita M, Uysal AC, Ogawa R, Hyakusoku H, Mizuno H. Periodontal tissue regeneration with adipose-derived stem cells. *Tissue Eng Part A* 2008;14:945-53.
  85. Hung CN, Mar K, Chang HC, Chiang YL, Hu HY, et al. A comparison between adipose tissue and dental pulp as sources of MSCs for tooth regeneration. *Biomaterial* 2011;32:6995-7005.
  86. Ding G, Liu Y, Wang W, Wei F, Liu D, et al. Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. *Stem Cells* 2010;28:1829-38.
  87. Fu X, Jin L, Ma P, Fan Z, Wang S. Allogeneic stem cells from deciduous teeth in treatment for periodontitis in miniature swine. *J Periodontol* 2014;85:845-51.
  88. Tsumanuma Y, Iwata T, Kinoshita A, Washio K, Yoshida T, et al. Allogeneic transplantation of periodontal ligament-derived multipotent mesenchymal stromal cell sheets in canine critical-size supra-alveolar periodontal defect model. *Biores Open Access* 2016;5:22-36.
  89. Zhu B, Liu W, Zhang H, Zhao X, Duan Y, et al. Tissue-specific composite cell aggregates drive periodontium tissue regeneration by reconstructing a regenerative microenvironment. *J Tissue Eng Regen Med* 2017;11:1792-805.
  90. Nuñez J, Sanz-BIASco S, Vignoletti F, Muñoz F, Arzate H, et al. Periodontal regeneration following implantation of cementum and periodontal ligament-derived cells. *J Periodontal Res* 2012;4:33-44.
  91. Gao ZH, Hu L, Liu GL, Wei FL, Liu Y, et al. Bio-root and implant-based restoration as a tooth replacement alternative. *J Dent Res* 2016;95:642-9.
  92. Tassi SA, Sergio NZ, Misawa MYO, Villar CC. Efficacy of stem cells on periodontal regeneration: systematic review of pre-clinical studies. *J Periodont Res* 2017;52:793-812.
  93. Seo BM, Miura M, Gronthos S, Zhang X, Zhu SX, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004;364:149-55.
  94. Gronthos S, Mroczek K, Shi S, Bartold PM. Ovine periodontal ligament stem cells: isolation, characterization, and differentiation potential. *Calcif Tissue Int* 2006;79:310-7.
  95. Akizuki T, Oda S, Komaki M, Tsuchioka H, Kawakatsu N, et al. Application of periodontal ligament cell sheet for periodontal regeneration: a pilot study in beagle dogs. *J Periodontal Res* 2005;40:245-51.
  96. Nakahara T, Nakamura T, Kobayashi E, Kuremoto KI, Matsuno T, et al. In situ tissue engineering of periodontal tissues by seeding with periodontal ligament-derived cells. *Tissue Eng* 2004;10:537-44.
  97. Iwata T, Yamato M, Tsuchioka H, Takagi R, Mukobata S, et al. Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. *Biomaterials* 2009;30:2716-23.
  98. Inukai T, Katagiri W, Yoshimi R, Osugi M, Kawai T, et al. Novel application of stem cell-derived factors for periodontal regeneration. *Biochem Biophys Res Commun* 2013;430:763-8.
  99. d'Aquino R, Graziano A, Sampaoli M, Laino G, Pirozzi G, et al. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. *Cell Death Differ* 2007;14:1162-71.
  100. Liu J, Ruan J, Weir MD, Ren K, Schneider A, et al. Periodontal bone-ligament-cementum regeneration via scaffolds and stem cells. *Cells* 2019;8:537.
  101. Li G, Han N, Zhang X, Yang H, Cao Y, et al. Local injection of allogeneic stem cells from apical papilla enhanced periodontal tissue regeneration in minipig model of periodontitis. *Biomed Res Int* 2018;2018:3960798.
  102. Yamamiya K, Okuda K, Kawase T, Hata K, Wolff LF, et al. Tissue-engineered cultured periosteum used with platelet-rich plasma and hydroxyapatite in treating human osseous defects. *J Periodontol* 2008;79:811-8.
  103. Tobita M, Mizuno H. Adipose-derived stem cells and periodontal tissue engineering. *Int J Oral Maxillofac Implants* 2013;28:e487-93.
  104. Fawzy El-Sayed KM, Paris S, Becker S, Kassem N, Ungefroren H, et al. Isolation and characterization of multipotent postnatal stem/progenitor cells from human alveolar bone proper. *J Craniomaxillofac Surg* 2012;40:735-42.
  105. Aimetti M, Ferrarotti F, Cricenti L, Mariani GM, Romano F. Autologous dental pulp stem cells in periodontal regeneration: a case report. *Int J Periodontics Restorative Dent* 2014;34:s27-33.
  106. Hernández-Monjaraz B, Santiago-Osorio E, Ledesma-Martínez E, Alcauter-Zavala A, Mendoza-Núñez VM. Retrieval of a periodontally compromised tooth by allogeneic grafting of mesenchymal stem cells from dental pulp: a case report. *J Int Med Res* 2018;46:2983-93.
  107. Aimetti M, Ferrarotti F, Mariani GM, Cricenti L, Romano F. Use of dental pulp stem cells/collagen sponge biocomplex in the treatment of non-contained intrabony defects: a case series. *Clin Adv Periodontics* 2015;5:104-9.
  108. Li YE, Zhao S, Nan XI, Wei H, Shi J, et al. Repair of human periodontal bone defects by autologous grafting stem cells derived from inflammatory dental pulp tissues. *Stem Cell Res Ther* 2016;7:141.

109. Aimetti M, Ferrarotti F, Gamba MN, Giraudi M, Romano F. Regenerative treatment of periodontal intrabony defects using autologous dental pulp stem cells: a 1-year follow-up case series. *Int J Periodontics Restorative Dent* 2018;38:51-8.
110. d'Aquino R, De Rosa A, Lanza V, Tirino V, Laino L, et al. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. *Eur Cell Mater* 2009;18:75-83.
111. Monti M, Graziano A, Rizzo S, Perotti C, Fante CD, et al. In vitro and in vivo differentiation of progenitor stem cells obtained after mechanical digestion of human dental pulp. *J Cell Physiol* 2017;232:548-55.
112. Akbay A, Baran C, Günhan O, Özmeriç N, Balış K. Periodontal regenerative potential of autogenous periodontal ligament grafts in Class II furcation defects. *J Periodontol* 2005;76:595-604.
113. Feng F, Akiyama K, Liu Y, Wang TM, Chen JH, et al. Utility of PDL progenitors for in vivo tissue regeneration: a report of 3 cases. *Oral Dis* 2010;16:20-8.
114. Kl V, Ryana H, Dalvi PJ. Autologous periodontal stem cell assistance in periodontal regeneration technique (SAI-PRT) in the treatment of periodontal intrabony defects: A case report with one-year follow-up. *J Dent Res Dent Clin Dent Prospects* 2017;11:123-6.
115. Koo S, Alshihri A, Karimbux NY, Maksoud M. Cellular allograft in the treatment of a severe periodontal intrabony defect: a case report. *Clinic Adv Periodontics* 2012;2:35-9.
116. McAllister BS. Stem cell-containing allograft matrix enhances periodontal regeneration: case presentations. *Int J Periodontics Restorative Dent* 2011;31:149-55.
117. Yamada Y, Ueda M, Hibi H, Baba S. A novel approach to periodontal tissue regeneration with mesenchymal stem cells and platelet-rich plasma using tissue engineering technology: a clinical case report. *Int J Periodontics Restorative Dent* 2006;26:363-9.
118. Yamada Y, Hara K, Nakamura S, Ueda M, Ito K, et al. Minimally invasive approach with tissue engineering for severe alveolar bone atrophy case. *Int J Oral Maxillofac Surg* 2013;42:260-3.
119. Rosen PS. A case report on combination therapy using a composite allograft containing mesenchymal cells with an amnion-chorion barrier to treat a mandibular class III furcation. *Clin Adv Periodontics* 2013;3:64-9.
120. Chen FM, Gao LN, Tian BM, Zhang XY, Zhang YJ, et al. Treatment of periodontal intrabony defects using autologous periodontal ligament stem cells: a randomized clinical trial. *Stem Cell Res Ther* 2016;7:33.
121. Shalini HS, Vandana KL. Direct application of autologous periodontal ligament stem cell niche in treatment of periodontal osseous defects: a randomized controlled trial. *J Indian Soc Periodontol* 2018;22:503-12.
122. Ferrarotti F, Romano F, Gamba MN, Quirico A, Giraudi M, et al. Human intrabony defect regeneration with micrografts containing dental pulp stem cells: a randomized controlled clinical trial. *J Clin Periodontol* 2018;45:841-50.
123. Dhote R, Charde P, Bhongade M, Rao J. Stem cells cultured on beta tricalcium phosphate (beta-TCP) in combination with recombinant human platelet-derived growth factor - BB (rh-PDGF-BB) for the treatment of human infrabony defects. *J Stem Cells* 2015;10:243-54.
124. Ledesma-Martínez E, Mendoza-Núñez VM, Santiago-Osorio E. Mesenchymal stem cells for periodontal tissue regeneration in elderly patients. *J Gerontol A Biol Sci Med Sci* 2019;74:1351-8.
125. Cho YD, Kim KH, Ryoo HM, Lee YM, Ku Y, et al. Recent advances of useful cell sources in the periodontal regeneration. *Curr Stem Cell Res Ther* 2019;14:3-8.
126. Hynes K, Menicanin D, Han J, Marino V, Mrozik K, et al. Mesenchymal stem cells from iPS cells facilitate periodontal regeneration. *J Dent Res* 2013;92:833-9.
127. Cai X, Yang F, Yan X, Yang W, Yu N, et al. Influence of bone marrow-derived mesenchymal stem cells pre-implantation differentiation approach on periodontal regeneration in vivo. *J Clin Periodontol* 2015;42:380-9.
128. Wen Y, Wang F, Zhang W, Li Y, Yu M, et al. Application of induced pluripotent stem cells in generation of a tissue-engineered tooth-like structure. *Tissue Eng Part A* 2012;18:1677-85.
129. Hynes K, Menicanin D, Bright R, Ivanovski S, Huttmacher DW, et al. Induced pluripotent stem cells: a new frontier for stem cells in dentistry. *J Dent Res* 2015;94:1508-15.
130. Pacelli S, Basu S, Whitlow J, Chakravarti A, Acosta F, et al. Strategies to develop endogenous stem cell-recruiting bioactive materials for tissue repair and regeneration. *Adv Drug Deliv Rev* 2017;120:50-70.
131. Lee K, Silva EA, Mooney DJ. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. *J R Soc Interface* 2011;8:153-70.
132. Cai X, Yang F, Walboomers XF, Wang Y, Jansen JA, et al. Periodontal regeneration via chemoattractive constructs. *J Clin Periodontol* 2018;45:851-60.
133. Miron RJ, Zhang Y. Autologous liquid platelet rich fibrin: a novel drug delivery system. *Acta Biomater* 2018;75:35-51.
134. Miron RJ, Zucchelli G, Pikos MA, Salama M, Lee S, et al. Use of platelet-rich fibrin in regenerative dentistry: a systematic review. *Clin Oral Investig* 2017;21:1913-27.
135. Park CH, Kim KH, Lee YM, Seol YJ. Advanced engineering strategies for periodontal complex regeneration. *Materials (Basel)* 2016;9:57.
136. Park CH, Rios HF, Taut AD, Miguel MP, Flanagan CL, et al. Image-based, fiber guiding scaffolds: a platform for regenerating tissue interfaces. *Tissue Eng Part C Methods* 2014;20:533-42.
137. Lee S, Choi E, Cha MJ, Hwang KC. Cell adhesion and long-term survival of transplanted mesenchymal stem cells: a prerequisite for cell therapy. *Oxid Med Cell Longev* 2015;2015:632902.
138. Hu L, Liu Y, Wang S. Stem cell-based tooth and periodontal regeneration. *Oral Dis* 2018;24:696-705.
139. Ozasa M, Sawada K, Iwayama T, Yamamoto S, Morimoto C, et al. Murakami, periodontal tissue regeneration by transplantation of adipose tissue-derived multi-lineage progenitor cells. *Inflamm Regen* 2014;34:109-16.

Review

Open Access



# Major upper limb replantation: a review of clinical pearls

Margaret Luthringer, Margaret Dalena, Haripriya S. Ayyala

Division of Plastic and Reconstructive Surgery, Rutgers-New Jersey Medical School, Newark, NJ 08873, USA.

**Correspondence to:** Dr. Haripriya S. Ayyala, Division of Plastic and Reconstructive Surgery, Rutgers-New Jersey Medical School, 140 Bergen St, Suite E1620, Newark, NJ 08873, USA. E-mail: ha289@njms.rutgers.edu

**How to cite this article:** Luthringer M, Dalena M, Ayyala HS. Major upper limb replantation: a review of clinical pearls. *Plast Aesthet Res* 2020;7:42. <http://dx.doi.org/10.20517/2347-9264.2020.35>

**Received:** 11 Mar 2020 **First Decision:** 22 May 2020 **Revised:** 3 Jul 2020 **Accepted:** 20 Jul 2020 **Published:** 15 Aug 2020

**Academic Editor:** A Thione **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Replantation of major segments of the extremities can be a formidable task. Adequate debridement of crushed tissues is a prerequisite for successful major limb replantation. This article serves to elucidate the important situational and patient factors a surgeon must consider when choosing between replantation or revision amputation for upper limb salvage.

**Keywords:** Limb salvage, major limb replantation, microsurgery, upper extremity, reconstruction

## INTRODUCTION

Since Malt and McKhan's first successful upper extremity replantation in 1962, ever-evolving surgical techniques have redefined outcomes for patients with these life-altering injuries<sup>[1]</sup>. Still, these cases remain a challenge for surgeons who must make difficult decisions regarding replant candidacy and surgical options. In 2005, approximately 170,000 people in the United States were living with a wrist-proximal upper extremity amputation. Young males are predominantly affected; trauma remains the leading cause of injury in this group<sup>[2]</sup>. Though prosthetic options for upper extremity amputation have significantly improved in recent years, rejection rates of devices have been described to be as high as 30%<sup>[3]</sup>. Further, post-operative functionality can be unpredictable. Upper limb replantation continues to yield better overall subjective results over revision amputation<sup>[3]</sup>. This article serves to review the important situational and patient factors a surgeon must consider when choosing between replantation or revision amputation for



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



upper limb salvage. We also emphasize technical points that may lead to successful upper extremity major limb replantation.

## EVALUATION AND ASSESSMENT

Surgeons considering upper extremity replantation must take into account the injury's devastating effect on patient quality of life, functionality, and possible financial burden<sup>[4,5]</sup>. Despite the profound nature of this decision process, few substantiated algorithms delineating replantation versus revision amputation exist in the current literature. Märdian *et al.*<sup>[5]</sup> proposed an algorithm that took into account injury pattern, patient risk factors, ischemia time, and contamination. Larson *et al.*<sup>[6]</sup> expounded upon variables that were significantly associated with choosing replantation: severity of systemic conditions, mechanism and level of injury, ischemia time, and patient motivation comprised the foremost imperative factors involved in management choice. Regardless, all algorithms should include a full initial patient evaluation per conventional trauma standards. After going through the advanced trauma life support protocol, a thorough exam, appropriate studies, and radiographic images of the proximal appendage and amputated segment should be included in the initial assessment. Patients suffering high acuity systemic trauma may demonstrate compromised ability for wound healing which is incompatible with replantation. Life threatening injuries may absolutely preclude patients from undergoing long operations with sustained general anesthesia<sup>[6,7]</sup>. Larson *et al.*<sup>[6]</sup> described in his retrospective study, spanning 11 years with 62 upper extremity amputation patients, a significant association with the choice of revision amputation and an Injury Severity Score of 16 or greater, indicating severe trauma. Further, those higher acuity patients who did undergo replantation were more likely to experience replant failure<sup>[6]</sup>.

The mechanism and level of injury plays a large role in anticipating the salvage probability of soft tissue structures at amputation sites. Sharp or penetrating injuries, especially “guillotine” amputations, demonstrate less extensive damage to sharply divided soft tissues. These injuries are associated with significantly higher rates of attempted replantation over crush or avulsion events<sup>[6]</sup>. Injuries that are distally located may also encourage replantation attempts. On the other hand, proximal injuries may be prone to increased effects of ischemia as more muscle mass and neuronal tissue are devoid of perfusion. Additionally, this tissue is subsequently exposed to greater oxidative stress and reperfusion injury that can sabotage microsurgical replantation efforts<sup>[6,8-10]</sup>. Therefore, the upper extremity amputation injury must be considered when constructing an operative plan.

Furthermore, a close relationship between the surgeon and occupational therapist is invaluable to obtain optimal function outcomes. Frequent communication of plans allows for real-time feedback, rehabilitative protocol revision when needed, and can increase a patient's faith in the treatment plan. Having a trusted and reliable team of therapists available is vital when evaluating a patient for successful replantation. It is also critical the patient be evaluated holistically to ensure appropriate candidacy for replantation. Patients with injuries caused by multiple self-harm attempts may discourage replantation attempts unless post-operative mental health care and constant monitoring are in place. When possible, the surgeon must assess the patient's occupation, physical demands, and motivation to endure a long and arduous rehabilitative course.

## PROGNOSTIC FACTORS FOR UPPER EXTREMITY LIMB VIABILITY

One of the most crucial variables affecting limb salvage after replantation is the ischemia time of the amputated segment. Replantation for wrist-proximal amputations should be attempted before 12 h of cold ischemia time or 6 h of warm ischemia time<sup>[11]</sup>. Prolonged warm ischemia time is particularly associated with increased changes in cellular metabolism of susceptible muscle tissue. Damaging oxidative stress renders the muscles dysfunctional, increases vessel permeability, and predisposes the patient to reperfusion syndrome<sup>[11]</sup>. Emergency personnel should be instructed to wrap the amputated extremity in gauze and



cool it on ice as soon as possible<sup>[1]</sup>. Inflow and outflow shunts may be utilized in anticipation of long transport times. A large catheter may be used between a single intact vein if present; arterial flow is re-established with a carotid shunt or intravascular catheter<sup>[1]</sup>. A prospective study of 186 complete upper extremity amputations by Waikakul *et al.*<sup>[8]</sup> described post-operative reperfusion syndrome in 5.4% of patients. Though not statistically significant, patients with a total ischemic time between 6-8 h had a higher rate of failure when compared to those with 0-6 h. Tantry *et al.*<sup>[9]</sup> presented a series of 14 upper extremity amputation patients, all of whom had total limb ischemia times of more than 6 h. The 4 patients who developed intraoperative/early post-operative events all had 8 or more hours of total ischemia. Reperfusion events observed included hypotension, bronchospasm, acidosis, and atrial fibrillation.

The severity, mechanism, and level of injury to the upper extremity is associated with the probability of successful limb salvage. Severity of the injury can manifest in several ways; large crush injuries may damage multiple functional muscle units, portending a poor prognosis for post-operative function. These extensive crush injuries are also typically plagued with severe tissue contamination. Meticulous debridement, vigorous irrigation, and antimicrobial therapy can mitigate the deleterious effects of initial contamination on functional outcomes after replantation<sup>[9]</sup>. A crush injury may be debrided in order to convert it to a sharp injury, facilitating ease and functional healing of the repair. Avulsion events may themselves obviate functional recovery, as axonal and vascular damage to nefariously proximal levels may underlie seemingly distal amputations. In fact, rotary machine injuries portend the worse outcomes of all injury types<sup>[12]</sup>. Clean guillotine injuries are more amenable to successful replantation and subsequent function<sup>[6,8]</sup>. Replantation of more distal amputations tends to be associated with better function post-operatively. This may be in part due to the increased length of axonal regeneration required for higher injuries<sup>[10]</sup>.

Most patient demographic variables have little bearing on replantation success, with gender and age showing no significant difference in salvage rates. History of smoking may be associated with higher rates of failed replantation efforts<sup>[8]</sup>.

## PEARLS FOR UPPER EXTREMITY REPLANTATION

### Debridement and soft tissue injury

While some studies use severe contamination as a contraindication to replantation surgery, contamination has no significant association with adverse events or limb survival after thorough irrigation and debridement<sup>[6,9,12]</sup>. Aggressive debridement combined with appropriate antibiotic coverage decreases the risk of post-operative infections and is imperative in the management of upper extremity injuries<sup>[6]</sup>. Meticulous inspection of the amputated segment is crucial and can be done prior to the operation itself to maximize efficiency<sup>[13]</sup>. Exposed vascular structures should be carefully examined under loupe magnification or the operating microscope. Signs of vessel damage, such as a “red line” or corkscrew “ribbon sign”, can signify traction injury and separation of intima from media. Any anastomosis attempted in regions afflicted with this type of damage are prone to thrombosis<sup>[14,15]</sup>. Thus, with prior inspection of the amputated segment, the zone of injury of the amputated limb can be properly estimated and the need for vein grafts and a safe location for neurovascular anastomoses can be considered before incising the proximal stump. Any ischemic or contaminated tissue of the segment can be sharply debrided at this time. Aggressive excision of any tissue that appears compromised is advocated, as soft tissue complications are the main cause of failure in major limb replantation<sup>[16]</sup>.

Tissue sepsis and necrosis can lead to neurovascular exposure and desiccation and may incite an inflammatory cascade that promulgates a thrombogenic environment<sup>[16]</sup>. Sharp excision and copious wound irrigation with an antibiotic solution are encouraged; pulse lavage or high-pressure irrigation is not often recommended as this can push fluid and bacteria into the tissues causing significant edema and deep infection<sup>[17]</sup>. As stated previously, ischemic time should be at the forefront of the surgeon's mind during this

process. Shunting should be utilized as necessary during the debridement process, which may preclude separate preparation of the amputated segment. As serial debridement may be required until definitive coverage, temporary dressings are often utilized to keep the wounds clean and to prevent desiccation<sup>[18]</sup>.

### Bone shortening

In addition to thorough debridement of soft tissue, bone shortening is a technique that is highly advantageous in replantation surgeries. Bone shortening is critical for removing any devitalized bone and other necrotic structures, thereby promoting bony union and decreasing the risk of osteomyelitis, deep infections, and necrosis<sup>[19]</sup>. Additionally, bone shortening presents the opportunity to convert crush and avulsion injuries to “guillotine” type injuries, facilitating reconstruction by converting damaged nerves and vessels to clean and vitalized structures suitable for coaptation and anastomosis<sup>[19,20]</sup>. Bone shortening may also allow physicians to avoid vein and nerve grafts. Shortening also decreases tension on anastomoses and skin repair and reduces soft tissue defect size, decreasing the need for flap coverage<sup>[19,21,22]</sup>. The radius and ulna may tolerate from 2.5 cm to 5.0 cm of shortening and the humerus may tolerate up to 8 cm of shortening with acceptable functional results<sup>[13]</sup>. The benefits of bone shortening, including decreased risk of wound infections and replantation failure, outweigh the risks of a shortened limb with potentially diminished functionality. The literature has demonstrated that shortening within the aforementioned parameters has a negligible effect on subsequent function<sup>[15,19]</sup>. Appropriate tensioning of the involved muscles and tendons and meticulous nerve repair, with or without the use of transfers, is paramount in this setting. When possible, the patient should be informed of the expected cosmetic implications of arm length discrepancy as well as the arduous post-operative rehabilitative course to gain back strength and range.

### Ectopic banking

Though rarely employed, the amputated segment may be temporarily banked if a patient's critical condition or complex injury at the proximal stump precludes immediate replantation. Godina *et al.*<sup>[23]</sup> first described this method in 1986 for the ectopic implantation and subsequent replantation of an amputated hand. This technique allows for expeditious temporary salvage of the amputated segment as only skin and vascular reconstruction need be undertaken<sup>[24]</sup>. Recipient vessel site choice is crucial and must be well outside the zone of injury. Though Godina initially favored the thoracodorsal artery at the axilla as the recipient vessel in banking, microsurgeons have generally shifted their preference to the contralateral distal radial artery. This allows for ease of serial debridement and physical therapy of the ectopic segment. Further, the contralateral radial artery can be reconstructed with a vein graft at the same time as the replant, decreasing operative time<sup>[24]</sup>. The banked segment should be replanted as soon as possible, preferably within 1-2 weeks. This allows for a more stable position for the segment and for nerves and tendons to be repaired rapidly<sup>[24,25]</sup>. When the ectopic segment is harvested, the previous anastomosis should be preserved. A significant amount of the length of the recipient vessel should be taken to avoid the zone of injury during replantation<sup>[24]</sup>.

### Secondary procedures

Secondary procedures are commonplace following upper extremity limb replantation. Fufa *et al.*<sup>[26]</sup> described a series of patients that underwent an average of three secondary procedures. Axelrod *et al.*<sup>[19]</sup> demonstrated an average of 4.2 secondary procedures per patient. Prior to any attempts, patients should understand the arduous road of replantation, including the need for multiple reconstructive surgeries to improve functional results. Secondary procedures commonly include soft tissue coverage with vascularized flaps, free functioning muscle transfer, tenolysis, tendon transfer, and nerve grafts<sup>[6,19,26,27]</sup>. Certain secondary procedures are more common depending on the location of the replantation as well as the mechanism of amputation sustained.

Distal and wrist level amputations commonly require tenolysis to improve range of motion, while proximal-level amputations often require soft tissue coverage due to high intensity mechanisms of injury

typically sustained at this amputation level<sup>[27]</sup>. Soft tissue coverage may include skin grafting or vascularized tissue transfer; however, flaps are typically preferred as skin grafts may cause contracture limiting muscle excursion<sup>[26,27]</sup>.

Free functioning muscle transfer is an important secondary procedure commonly performed in avulsion injuries of the forearm and elbow. Avulsion injuries of this area severely disrupt the long flexor and extensor tendons of the hand. These tendons may be too damaged for direct repair and essential musculature may be removed after thorough debridement. When tendon transfers are not an option, the replanted limb can suffer severely diminished functional capacity, requiring free functional neurotized muscle transfer<sup>[26,27]</sup>.

### Anticoagulation

Intraoperative and post-operative anticoagulation therapy are controversial topics among microsurgeons performing replantation. Ideally, the goals of anticoagulation for microvascular anastomoses are to prevent thrombosis and improve vascular patency by decreasing platelet function, increasing blood flow or decreasing blood viscosity, and counteracting the effects of thrombin on platelets and fibrinogen<sup>[28,29]</sup>. However, these effects may also lead to undue complications such as hemorrhage or hematoma which may further compromise the vascular anastomoses within replants<sup>[28,30]</sup>.

We recommend a post-operative regimen similar to other free tissue transfer procedures, consisting of low molecular weight heparin (LMWH) at prophylactic deep vein thrombosis dosing starting on post-operative day 1 for the duration of the patient's inpatient stay as well as 325 mg of Aspirin for 1 month post-operatively. Other commonly used anticoagulants include heparin and dextran; however, LMWH is preferred over unfractionated heparin as LMWH has better defined therapeutic and prophylactic dosages and carries a decreased risk of heparin induced thrombocytopenia<sup>[28]</sup>. Dextran decreases platelet activity and increases blood volume which thereby decreases blood viscosity; however, it is largely avoided in the current literature as it may induce serious complications such as anaphylaxis, pulmonary edema, adult respiratory distress syndrome, cerebral edema, acute renal failure, or congestive heart failure<sup>[31]</sup>. Additionally, some microvascular surgeons performing free flaps and digit and limb replantation choose to not use any pre-, intra-, or post-operative anticoagulation while still reporting favorable results<sup>[32]</sup>. Ultimately, the controversy surrounding this topic remains and prospective, randomized controlled trials are required to define recommendations for anticoagulation therapy following major upper limb replantation.

### REPLANTATION VS. AMPUTATION WITH AND WITHOUT PROSTHETIC FITTING

Patients who undergo successful replantation report significantly improved outcomes and satisfaction as compared to patients who undergo revision amputation with prosthetic rehabilitation<sup>[33]</sup>. However, prosthetic technology, including targeted muscle reinnervation, myoelectric prostheses and osseointegration, continues to advance and holds the potential for superior limb dexterity and functionality<sup>[33,34]</sup>. Limb replantation is currently superior to revision amputation and prosthetic rehabilitation. As prosthesis technology improves, future research will be required into this topic.

### Cost-effectiveness analysis

As compared to revision amputation, upper extremity replantation is associated with longer rehabilitation, increased time to return to work, and increased costs overall<sup>[12]</sup>. However, upper extremity replantation has been demonstrated to be a cost-effective approach as compared to revision amputation, particularly for highly motivated patients and patients of younger age at time of injury<sup>[35]</sup>.

Other than patient reported outcomes and functional scoring systems, there is no definitive cost-benefit analysis of major upper extremity replantation as compared to revision amputation with prosthetic fitting.

Patients' baseline functional status, occupation, type of injury sustained, and type of prosthetic used is unique to each patient, rendering a definitive cost benefit analysis difficult to produce<sup>[7]</sup>. Initial costs of limb replantation exceed the cost of revision amputations<sup>[33,35]</sup>. However, advanced prostheses such as those involving targeted muscle reinnervation and myoelectric prosthesis can be incredibly expensive<sup>[12,33]</sup>. While pain and difficulty of prosthetic use are some of the most common reasons for prosthesis disuse by patients, financial burden is another commonly cited reason for prosthesis abandonment<sup>[33]</sup>. Intensive rehabilitation is required for both replantation and prosthesis use in order to regain the most function<sup>[36]</sup>.

## CONCLUSION

Upper extremity amputations are devastating injuries. However, with appropriate management strategies in place, major limb replantation is a potentially life changing option for those who have suffered these injuries. While major limb replantation is not a viable option for every patient, the authors emphasize the importance of appropriate patient selection and surgical management strategies, including aggressive soft tissue and bone debridement in order to improve outcomes in these injuries.

## DECLARATIONS

### Acknowledgments

The authors would like to acknowledge Dr. Ramazi Datiashvili for his expert advice.

### Authors' contributions

Wrote the manuscript: Luthringer M, Dalena M

Approved the final version of the manuscript: Luthringer M, Dalena M, Ayyala HS

Conceived the study: Ayyala HS

Provided critical revisions that are important for intellectual content: Ayyala HS

Supervision and project administration: Ayyala HS

Contributed equally to this article: Luthringer M, Dalena M, Ayyala HS

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Hanel DP, Chin SH. Wrist level and proximal-upper extremity replantation. *Hand Clin* 2007;23:13-21.
2. Ziegler-Graham K, MacKenzie EJ, Ephraim PL, Travison TG, Brookmeyer R. Estimating the prevalence of limb loss in the United States: 2005 to 2050. *Arch Phys Med Rehabil* 2008;89:422-9.

3. Yueh JH, Bar-Meir ED, Liao EC, Lee BT. Major limb replantation. *Eplasty* 2011;11:ic1.
4. Prucz RB, Friedrich JB. Upper extremity replantation: current concepts. *Plast Reconstr Surg* 2014;133:333-42.
5. Märdian S, Krapohl BD, Roffeis J, Disch AC, Schaser KD, et al. Complete major amputation of the upper extremity: early results and initial treatment algorithm. *J Trauma Acute Care Surg* 2015;78:586-93.
6. Larson JV, Kung TA, Cederna PS, Sears ED, Urbanchek MG, et al. Clinical factors associated with replantation after traumatic major upper extremity amputation. *Plast Reconstr Surg* 2013;132:911-9.
7. Chung KC, Alderman AK. Replantation of the upper extremity: indications and outcomes. *J Am Soc Surg Hand* 2002;2:78-94.
8. Waikukul S, Vanadurongwan V, Unnanuntana A. Prognostic factors for major limb re-implantation at both immediate and long-term follow-up. *J Bone Joint Surg Br* 1998;80:1024-30.
9. Tantry TP, Kadam D, Shenoy SP, Bhandary S, Adappa KK. Perioperative evaluation and outcomes of major limb replantations with ischemia periods of more than 6 hours. *J Reconstr Microsurg* 2013;29:165-72.
10. Atkins SE, Winterton RIS, Kay SP. (v) Upper limb amputations: where, when and how to replant. *Curr Orthopaed* 2008;22:31-41.
11. Carden DL, Korthuis RJ. Mechanisms of postischemic vascular dysfunction in skeletal muscle: implications for therapeutic intervention. *Microcirc Endothelium Lymphatics* 1989;5:277-98.
12. Wolfe VM, Wang AA. Replantation of the upper extremity: current concepts. *J Am Acad Orthop Surg* 2015;23:373-81.
13. Woo SH. Practical tips to improve efficiency and success in upper limb replantation. *Plast Reconstr Surg* 2019;144:878e-911.
14. Pet MA, Ko JH. Indications for replantation and revascularization in the hand. *Hand Clin* 2019;35:119-30.
15. Maricevich M, Carlsen B, Mardini S, Moran S. Upper extremity and digital replantation. *Hand (N Y)* 2011;6:356-63.
16. Cavadas PC. Salvage of replanted upper extremities with major soft-tissue complications. *J Plast Reconstr Aesthet Surg* 2007;60:769-75.
17. Dzwierzynski WW. Replantation and revascularization. In: Neligan P, Chang J, editors. *Plastic Surgery*. Vol 6. 3 ed. Elsevier; 2017.
18. Ahuja NK, Datiashvili RO. Biobrane in the management of critical microsurgical wounds of the upper extremity. *Microsurgery* 2012;32:196-200.
19. Axelrod TS, Buchler U. Severe complex injuries to the upper extremity: revascularization and replantation. *J Hand Surg Am* 1991;16:574-84.
20. Daoutis NK, Gerostathopoulos N, Efsthathopoulos D, Misitzis D, Bouchlis G, et al. Major amputation of the upper extremity. Functional results after replantation/revascularization in 47 cases. *Acta Orthop Scand Suppl* 1995;264:7-8.
21. Battiston B, Tos P, Clemente A, Pontini I. Actualities in big segments replantation surgery. *J Plast Reconstr Aesthet Surg* 2007;60:849-55.
22. Leclère FM, Mathys L, Juon B, Franz T, Unglaub F, et al. Macroréplations of the upper extremity: a series of 11 patients. *Arch Orthop Trauma Surg* 2012;132:1797-805.
23. Godina M, Bajec J, Baraga A. Salvage of the mutilated upper extremity with temporary ectopic implantation of the undamaged part. *Plast Reconstr Surg* 1986;78:295-9.
24. Higgins JP. Ectopic banking of amputated parts: a clinical review. *J Hand Surg Am* 2011;36:1868-76.
25. Li J, Ni GH, Guo Z, Fan HB, Cong R, et al. Salvage of amputated thumbs by temporary ectopic implantation. *Microsurgery* 2008;28:559-64.
26. Fufa D, Lin CH, Lin YT, Hsu CC, Chuang CC, et al. Secondary reconstructive surgery following major upper extremity replantation. *Plast Reconstr Surg* 2014;134:713-20.
27. Venkatramani H, Bhardwaj P, Sabapathy SR. Role of free functioning muscle transfer in improving the functional outcomes following replantation of crush avulsion amputations of the forearm. *Injury* 2019;50 Suppl 5:S105-10.
28. Askari M, Fisher C, Weniger FG, Bidic S, Lee WP. Anticoagulation therapy in microsurgery: a review. *J Hand Surg Am* 2006;31:836-46.
29. Ketchum LD. Pharmacological alterations in the clotting mechanism: use in microvascular surgery. *J Hand Surg Am* 1978;3:407-15.
30. Hemker HC, Béguin S, Kakkar VV. Can the haemorrhagic component of heparin be identified? Or an attempt at clean thinking on a dirty drug. *Haemostasis* 1996;26:117-26.
31. Levin LS, Cooper EO. Clinical use of anticoagulants following replantation surgery. *J Hand Surg Am* 2008;33:1437-9.
32. Veravuthipakorn L, Veravuthipakorn A. Microsurgical free flap and replantation without antithrombotic agents. *J Med Assoc Thai* 2004;87:665-9.
33. Pet MA, Morrison SD, Mack JS, Sears ED, Wright T, et al. Comparison of patient-reported outcomes after traumatic upper extremity amputation: replantation versus prosthetic rehabilitation. *Injury* 2016;47:2783-8.
34. Pierrie SN, Gaston RG, Loeffler BJ. Current concepts in upper-extremity amputation. *J Hand Surg Am* 2018;43:657-67.
35. Yoon AP, Mahajani T, Hutton DW, Chung KC; Finger Replantation and Amputation Challenges in Assessing Impairment, Satisfaction, and Effectiveness (FRANCHISE) Group. Cost-effectiveness of finger replantation compared with revision amputation. *JAMA Netw Open* 2019;2:e1916509.
36. Cancio JM, Ikeda AJ, Barnicott SL, Childers WL, Alderete JF, et al. Upper extremity amputation and prosthetics care across the active duty military and veteran populations. *Phys Med Rehabil Clin N Am* 2019;30:73-87.



Review

Open Access



# Metoidioplasty as a one-stage phallic reconstruction in transmen

Marta Bizic<sup>1,2</sup>, Borko Stojanovic<sup>1,2</sup>, Marko Bencic<sup>2</sup>, Noemi Bordas<sup>2,3</sup>, Miroslav Djordjevic<sup>1,2</sup>

<sup>1</sup>Department of Urology, Faculty of Medicine, University of Belgrade, Belgrade 11000, Serbia.

<sup>2</sup>Belgrade Center for Urogenital reconstructive Surgery, Belgrade 11000, Serbia.

<sup>3</sup>Department of Urology, Kiskunhalasi Semmelweis Kórház, Kiskunhalas 6400, Hungary.

**Correspondence to:** Dr. Marta Bizic, Department of Urology, Faculty of Medicine, University of Belgrade, Tirsova 10, Belgrade 11000, Serbia. E-mail: martabizic@uromiros.com

**How to cite this article:** Bizic M, Stojanovic B, Bencic M, Bordas N, Djordjevic M. Metoidioplasty as a one-stage phallic reconstruction in transmen. *Plast Aesthet Res* 2020;7:43. <http://dx.doi.org/10.20517/2347-9264.2020.80>.

**Received:** 17 Apr 2020 **First Decision:** 18 May 2020 **Revised:** 7 Jun 2020 **Accepted:** 19 Jun 2020 **Published:** 15 Aug 2020

**Academic Editors:** Marlon E. Buncamper, Stan J. Monstrey **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Gender dysphoria is a condition where there is a discrepancy between the gender assigned at birth and the desired gender, leading the patient to pursue surgical intervention. Reconstruction of the neophallus for transmen is still challenging, even though there are many surgical techniques with satisfying results. The aim of neophallic reconstruction in gender affirmation surgery (GAS) for transmen is to provide stand-up voiding, erotic sensation, orgasm and penetration ability, and acceptable donor site morbidity with minimal scarring and complications. Metoidioplasty as a variant of phalloplasty for transmen is a one-stage procedure that results in male-like external genitals, with minimal scarring, ability of standing micturition, and full erogenous sensation with the ability to achieve orgasm during sexual intercourse. Metoidioplasty is a method of choice for those transmen who wish to have GAS in one procedure without multi-staged procedures to create the adult-male-sized neophallus.

**Keywords:** Clitoris, gender affirmation surgery, metoidioplasty, neophallus, genital reconstruction, transmen

## INTRODUCTION

Gender affirmation surgeries usually represent the final step in the transition process of an individual suffering from gender dysphoria. Genital reconstructive surgeries, known as “bottom surgeries”, are performed according to Standards of Care (SOC) of the World Professional Association for Transgender Health (WPATH): they require two letters of recommendation by two board certified mental health



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



professionals and must come after at least 12 months of substitution hormonal therapy<sup>[1]</sup>. Preoperative consultation with the selected surgeon performing gender affirmation surgery (GAS) is welcome in order to reconcile the patients' expectations of surgery outcomes and the possibilities of modern medicine, to prevent any postoperative disappointments<sup>[2]</sup>. Even though under testosterone therapy transmen's body encounters a lot of changes, some individuals still require facial and body masculinization procedures or frontline hair procedures.

It is still difficult to assess the real prevalence of gender dysphoria, but the latest studies report an increase in prevalence for individuals assigned male at birth to 0.014-0.015, while, for individuals assigned female at birth, it is 0.002-0.003. The self-report of transgender identity in children, adolescents, and adults, ranging from 0.5% to 1.3%, has also increased according to recent studies<sup>[3,4]</sup>.

Phalloplasty, as previously said, is still a challenging procedure, reserved for highly specialized institutions and surgeons. The first phalloplasty was performed in the 1930s by Bogoras, followed by years of refining surgical techniques to satisfy patients' expectations of functionality and esthetics. However, there is no replacement for erectile, urethral tissue, and nerves that would provide ideal male genitals for males requiring genital reconstruction<sup>[5,6]</sup>. The reconstruction of the neophallus should be performed as a one-stage procedure, yielding sensation (tactile and erogenous), functional neourethra (ability of stand-up voiding), penetrative sexual intercourse, and minimal scarring of the donor site<sup>[5,7]</sup>. Unfortunately, to this day, there is no single surgical technique to satisfy all these goals of male genitalia reconstruction<sup>[8]</sup>.

Male genitalia reconstruction in transmale individuals can be performed by two surgical approaches: phalloplasty and metoidioplasty (a variant of phalloplasty). Phalloplasty involves the creation of an adult-sized neophallus using local or outlying tissue flaps, as either pedicled or free flaps with microvascular anastomosis. Rigidity for penetration during sexual intercourse is obtained after penile prosthesis implantation. Metoidioplasty involves creation of the neophallus using the hormonally-hypertrophied clitoris, with or without urethroplasty, and scrotoplasty, with or without testicular prostheses implantation. In the majority of patients, metoidioplasty enables voiding in standing position and full erogenous sensation, but penetration during sexual intercourse is possible in only rare cases (by self-report)<sup>[5,9]</sup>.

The first report of using clitoris in male genitalia reconstruction was in 1973, and as a term "metoidioplasty" was first introduced by Lebovic and Laub, originally from the Greek words "meta" (change), "aidion" (female genitalia), and "plasty" (formation)<sup>[10,11]</sup>. Metoidioplasty can be considered as the method of choice, for those individuals requiring male genitalia reconstruction in single surgery to complete their transition and who do not wish to have stigma scars outside the genital area.

This narrative review aims to evaluate all available techniques of metoidioplasty and to report the postoperative results and complications. The paper was approved by the Institutional Review Board (No. 2-1-1/2020).

### **Preoperative evaluation**

Transmale individuals undergoing genital reconstruction in GAS are required to have spent at least one year on hormonal substitution therapy according to the WPATH SOC<sup>[1]</sup>. For those who have chosen metoidioplasty as the surgical technique, additional preoperative short-term use of vacuum pump in combination with local application of dihydrotestosterone gel is recommended to provide better postoperative results<sup>[5,12]</sup>.

Knowledge of female and male anatomy and embryology is of essential importance for surgeons performing transgender genital reconstructive surgeries. Female and male external genitals, i.e., the clitoris and penis,

are homologous organs and are both responsible for sexual pleasure<sup>[13]</sup>. The clitoris, similarly to the penis, has a glans, prepuce, two corpora cavernosa, crura, bulbs, suspensory ligaments, and root. Unlike the penis, it does not contain corpus spongiosum with urethra. In cis-females, clitoral glans and prepuce are the only visible parts of the clitoris, while the clitoral body is curved, hidden, and attached with two parts of suspensory ligament (superficial and deep) to the pubic bones and fatty tissue of the mons, preventing its protrusion during arousal<sup>[14]</sup>. Due to the presence of androgen receptors, the clitoris enlarges under testosterone therapy, so that one part of the clitoral body becomes visible as well. As reported by several different studies, the preoperative clitoral size in transmen varies from 2.5 to 4.6 cm<sup>[5,15,16]</sup>. Its dissection and the division of suspensory ligaments during metoidioplasty will allow for straightening and additional lengthening of the clitoris and enable voiding while standing. The labia minora are paired, hairless mucocutaneous structures, rich in nerve endings and sensory receptors with very good vascularization, which makes them good tissue for genital reconstruction. The labia majora are paired fibroadipose structures homologous to the scrotum in cis-males and are used for scrotum reconstruction in transmen GAS<sup>[5,17]</sup>.

Even though GASs have been performed for more than 50 years, little is known about their effects on the sexual experiences of transpeople, especially of transmen. Preoperative counseling with a sexologist, the treating surgeon, and a psychotherapist is very important to reveal the patient's sexuality and sexual functioning before the surgery, but will also be of essential importance after the GA<sup>[18]</sup>.

### Operative techniques

From the time it was first introduced as a genital reconstruction procedure using hypertrophied clitoris, metoidioplasty has been refined by several authors in order to gain neophallic length, achieve more natural-looking male-like genitalia, and to provide voiding while standing<sup>[10,19]</sup>. Lebovic and Laub performed ventral chordee release with urethral reconstruction in two stages, wherein the patient was not able to void while standing after the first stage<sup>[10,20]</sup>. Bouman's refinement consisted of urethral lengthening to the tip of the clitoris using the vaginal mucosal flap, but without ventral chordee release<sup>[10,21]</sup>. Gilbert's modification of the technique included clitoral release and complete urethral reconstruction using only local flaps originating from the labia minora<sup>[10,22]</sup>. Hage *et al.*<sup>[7]</sup> used the combination of the abovementioned techniques (Bouman's and Laub's) to obtain the best possible results for their patients, who set standing micturition as their main goal<sup>[7,10]</sup>. Later, Hage and van Turnhout<sup>[23]</sup>, in their long-term follow-up study, reported that, on average, 2.6 procedures were needed to achieve satisfying results after performed metoidioplasty. For the purpose of continuous improvement of surgical techniques, Perovic and Djordjevic<sup>[24]</sup> reported very high success rate in their series of patients who underwent metoidioplasty related to standing micturition and esthetic appearance.

Nowadays, there are three major subtypes of metoidioplasty that can be considered as distinct procedures: simple metoidioplasty, ring metoidioplasty, and complete metoidioplasty (Belgrade metoidioplasty).

Simple metoidioplasty involves the release of clitoral ligaments and urethral plate, but without urethral reconstruction. It is usually selected by the patients who fear postoperative complications related to urethroplasty. It results in male-like genitals, with a sensate small phallus, scrotum with or without testicular implants, and native urethral opening without the possibility of voiding while standing. The complication rate with simple metoidioplasty is rather small and acceptable by the patients and their surgeons (less than 5%) and is usually related to the skin (dehiscence, local infection, and rotation of the neophallus)<sup>[25,26]</sup>.

Ring metoidioplasty includes urethral reconstruction along with the dissection of the suspensory ligaments of the clitoris and ventral urethral plate division. The urethral ring flap is harvested from the vaginal

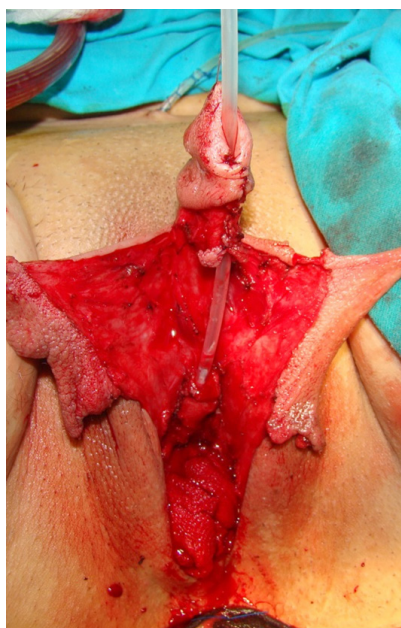


**Figure 1.** Preoperative appearance. Hypertrophied clitoris under hormonal therapy. Foley catheter inserted into the bladder

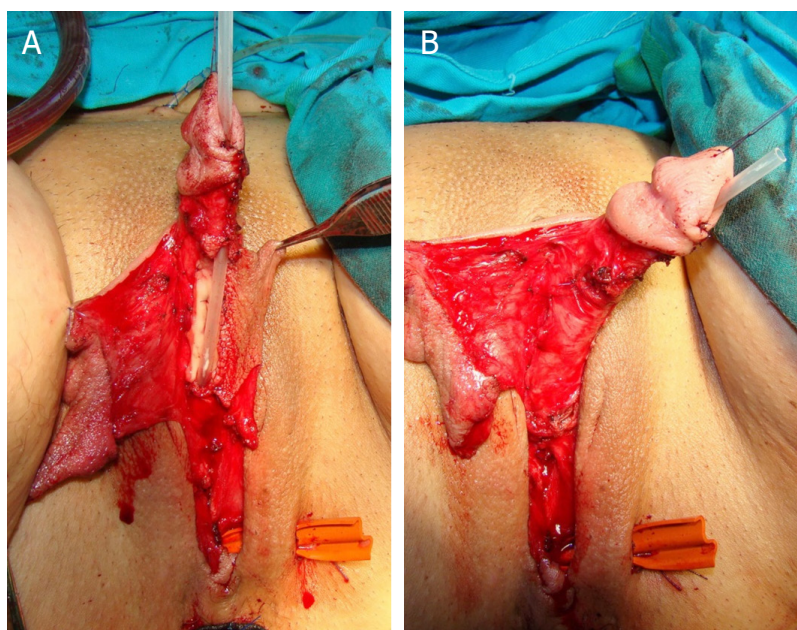
introitus, attached ventrally to the clitoral body, and tubularized, thus creating the neophallic urethra. Final urethroplasty is performed by joining the tubularized ring flap and the flap from the anterior vaginal wall in an oblique fashion, while the remaining labial and clitoral skin is used for neophallus shaft reconstruction using multiple Z-plasties to avoid ventral scar contracture<sup>[27]</sup>. Complication rates occurring after ring metoidioplasty vary from 3%-5% for urethral strictures to 10%-26% for urethral fistulae. Ring metoidioplasty is performed as a one-stage procedure, except the scrotoplasty, which is always performed as an additional procedure in one or two stages<sup>[25]</sup>.

Complete metoidioplasty (Belgrade metoidioplasty) is based on the experience in dealing with the most severe forms of hypospadias and disorders of sex development in children<sup>[24,28]</sup>. The latest modification of the original technique involves simultaneous removal of internal female organs, vaginectomy (colpocleisis), complete clitoral lengthening and straightening with the urethroplasty to the tip of the glans, and scrotoplasty with bilateral testicular implants insertion as a one-stage procedure. The current technique relies on the embryological and anatomical homology between the clitoris and penis, confirming the clitoris as a smaller version of the penis with impaired urethral development<sup>[2,14]</sup> [Figure 1]. The procedure involves laparoscopically-assisted hysterectomy with bilateral salpingo-oophorectomy, if not performed prior to metoidioplasty, and complete vaginal mucosa removal by colpocleisis, with male-like perineoplasty, except for one small portion close to the native urethral meatus. Further, clitoral degloving is performed by a circular incision between the inner and outer layers of the clitoral prepuce downwards to the urethral plate and continued with complete dissection of the superficial and deep portions of the suspensory ligament. Additional straightening and lengthening are obtained by urethral plate dissection to correct ventral chordee [Figure 2]. Urethroplasty is performed using all available hairless skin and/or mucosal grafts over the urethral stent size 12-14Fr so that standing micturition would be possible, and a suprapubic urinary catheter is introduced to the bladder for urine derivation<sup>[5,26,29,30]</sup> [Figure 3A and B]. Scrotoplasty is performed by joining two labia minora flaps in the midline and inserting two silicone prostheses [Figure 4]. Postoperative care includes administration of broad-spectrum antibiotics and anticholinergic drugs while the suprapubic catheter is in place. Vacuum pump use, in combination with phosphodiesterase Type-5 inhibitors, for a period of six months postoperatively, is advised to prevent retraction of the





**Figure 2.** Clitoral lengthening and straightening by urethral plate dissection and suspensory ligaments dissection



**Figure 3.** Urethral reconstruction using buccal mucosa graft quilted to the corpora cavernosa and vascularized skin flap originating from the labia minora over the urethral stent (A); All suture lines are covered with vascularized tissue to prevent fistula formation (B)

neophallus<sup>[5,31,32]</sup>. Complications occurring after complete metoidioplasty can be classified as minor or major and vary from 10% to 37% depending on different literature data<sup>[5,9,33]</sup>. Minor complications are usually managed conservatively (hematoma, skin infection, urinary tract infection, partial skin necrosis, and dribbling and spraying during voiding). Major complications are usually related to urethroplasty and include either urethral fistulae or stricture, problems with testicular implants (displacement and rejection), and persistent vaginal cavity; these require surgical repair<sup>[2,5,26,33,34]</sup>. In our latest study, we reported overall complications in 46.8% of our 793 patients. Minor complications occurred in 17.7% and were solved





**Figure 4.** Appearance at the end of surgery. Neophallic skin is reconstructed using available skin from the labia minora and dorsal clitoral skin. Two testicular silicone prostheses are inserted into the scrotum created from the labia majora. Drain is placed in the vaginal vault

**Table 1. Postoperative outcomes after metoidioplasty**

Author/year	No. of patients	Follow-up (years, mean)	Aesthetic satisfaction (%)	Voiding while standing (%)	Erogenous sensation (%)	Sexual intercourse (%)	Complications (%)	Urethral fistula (%)	Urethral stricture (%)
Perovic and Djordjevic <sup>[24]</sup> 2003	22	3.9	77.3	NA	NA	NA	22.7	13.6	9
Hage and van Turnhout <sup>[23]</sup> 2006.	70	8	75.7	NA	NA	NA	88.6	37.1	35.7
Takamatsu and Harashina <sup>[27]</sup> 2009	43	0.6	88.4	67.4	100	2.3	34.9	27.9	7
Djordjevic <i>et al.</i> <sup>[30]</sup> 2009	38	2.2	100	100	100	36.8	39.5	5.3	0
Djordjevic and Bizic <sup>[29]</sup> 2013	207	5.3	100	91.8	100	NA	47.8	7.7	2.9
Vukadinovic <i>et al.</i> <sup>[16]</sup> 2014	97	2.5	95.9	100	100	20.6	27.8	6.2	2.1
Stojanovic <i>et al.</i> <sup>[34]</sup> 2017	79	3.7	96.2	100	100	69.6	25.3	5.1	3.8
Bizic <i>et al.</i> <sup>[5]</sup> 2019†	793	NA	94.7	100	100	100	46.8	8.8	1.4
van de Grift <i>et al.</i> <sup>[18]</sup> 2019	38*	2.7	68	NA	36	63.5	NA	NA	NA

\*Twenty-nine patients received phalloplasty and nine patients received metoidioplasty; †Personal experience in review study. NA: not applicable

conservatively and spontaneously, while 29.1% of our patients required surgical repair because of major complications<sup>[5]</sup>. The majority of patients undergoing metoidioplasty, up to 88% or more according to recent reports, are satisfied with the appearance of their genitals<sup>[5,9,26]</sup>. Voiding in standing position was possible for the vast majority of patients in recent studies, up to 93.2% (range 67.4%-100%). Recent studies confirmed increased positive association among gender affirmation, body satisfaction, and sexual outcomes after the performed GAS, which was reported for 63.5% of the patients undergoing metoidioplasty. It may be associated to completely preserved erogenous sensation of the neophallus, which is more prominent. Patients after metoidioplasty report sexual intercourse in a broader sense than just penetration, with increased sexual initiative and pleasure<sup>[18]</sup>. Metoidioplasty, as a one-stage genital gender affirmation surgery, brings about 40% overall complication rate, the most common complications being related to urethral reconstruction. Urethral fistulae are more common than urethral strictures, and most heal spontaneously<sup>[15]</sup> [Table 1]. However, between 1% and 24% of patients who have undergone metoidioplasty decide to pursue

some other available phalloplasty procedure in order to obtain the adult-sized neophallus as their final goal<sup>[33]</sup>.

## CONCLUSION

The number of gender affirmation surgeries is increasing worldwide. Specifically, genitourinary surgeries are of vital importance in GAS for transmen. The creation of “ideal” male external genitals is still a great challenge, and no surgical approach can fulfill all the criteria to meet this goal. It is important to offer pre- and postoperative counseling to patients, to discuss their expectations from the surgery in order to prevent disappointment and improve their subsequent psychosexual functioning.

The neophallus created by metoidioplasty is often shorter when compared with other phalloplasty techniques, and thus inadequate for penetration during sexual intercourse. In some individuals, this may be a limiting factor for upright voiding. On the other hand, individuals who decide to have metoidioplasty as the final option in their transition are more likely to have a single-stage procedure, to keep erogenous sensation, and to avoid multiple surgeries and complications.

In a sense, the majority of metoidioplasty patients get what is considered “ideal” male genitalia in a one-stage and time-saving procedure with reduced overall treatment costs and low postoperative complication rate.

## DECLARATIONS

### Authors' contributions

Made substantial contribution to conception and design of the study: Bizic M, Djordjevic M

Performed data analysis and interpretation: Stojanovic B, Bordas N

Performed data acquisition and provided technical support: Stojanovic B, Bencic M

Performed supervision and had responsibility for the organization and course of the project and the manuscript preparation: Bizic M, Djordjevic M

Performed writing of the manuscript: Bizic M, Bordas N, Bencic M

Performed critical review of the manuscript: Djordjevic M

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

This study is supported by Ministry of Education, Science and Technological Development, Republic of Serbia (175048).

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

The study was approved by Institutional Review Board (No. 2-1-1/2020) and an informed consent to participate is obtained from the patients prior the surgery.

### Consent for publication

A written informed consent for publication is obtained from patients.

### Copyright

© The Author(s) 2020.

## REFERENCES

- Coleman E, Bockting W, Botzer M, Cohen-Kettenis P, DeCuypere G, et al. Standards of Care for the health of transsexual, transgender and gender non-conforming people. 7th ed. *Int J Transgenderism* 2012;13:165-232.
- Djordjevic ML. Novel surgical techniques in female to male gender confirming surgery. *Transl Androl Urol* 2018;7:628-38.
- Zucker KJ. Epidemiology of gender dysphoria and transgender identity. *Sex Health* 2017;14:404-11.
- American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 5th ed. Arlington: VA American Psychiatric Association; 2013. pp. 451-9.
- Bizic MR, Stojanovic B, Joksic I, Djordjevic ML. Metoidioplasty. *Urol Clin North Am* 2019;46:555-66.
- Morrison SD, Chen ML, Crane CN. An overview of female-to-male gender-confirming surgery. *Nat Rev Urol* 2017;14:486-500.
- Hage JJ, Bout CA, Bloem JJ, Megens JA. Phalloplasty in female-to-male transsexuals: what do our patients ask for? *Ann Plast Surg* 1993;30:323-6.
- Lee WG, Christopher N, Ralph DJ. Penile reconstruction and the role of surgery in gender dysphoria. *Eur Urol Focus* 2019;5:337-9.
- Frey JD, Poudrier G, Chiodo MV, Hazen A. A systematic review of metoidioplasty and radial forearm flap phalloplasty in female-to-male transgender genital reconstruction: is the “ideal” neophallus an achievable goal? *Plast Reconstr Surg Glob Open* 2016;4:e1131.
- Hage JJ. Metaidoplasty: an alternative phalloplasty technique in transsexuals. *Plast Reconstr Surg* 1996;97:161-7.
- Laub DR, Eicher W, Laub DR II, Hentz VR. Penis construction in female-to-male transsexuals. In: Eicher W, Kubli F, Herms V, editors. *Plastic surgery in the sexually handicapped*. Berlin: Springer; 1989. pp. 113-28.
- Djordjevic ML, Stanojevic D, Bizic M, Kojovic V, Majstorovic M, et al. Metoidioplasty as a single stage sex reassignment surgery in female transsexuals: belgrade experience. *J Sex Med* 2009;6:1306-13.
- Mazloomdoost D, Pauls RN. A comprehensive review of the clitoris and its role in female sexual function. *Sex Med Rev* 2015;3:245-63.
- Stojanovic B, Djordjevic ML. Anatomy of the clitoris and its impact on neophalloplasty (metoidioplasty) in female transgenders. *Clin Anat* 2015;28:368-75.
- Hadj-Moussa M, Agarwal S, Ohl DA, Kuzon WM Jr. Masculinizing genital gender confirmation surgery. *Sex Med Rev* 2019;7:141-55.
- Vukadinovic V, Stojanovic B, Majstorovic M, Milosevic A. The role of clitoral anatomy in female to male sex reassignment surgery. *ScientificWorldJournal* 2014;2014:437378.
- Clerico C, Lari A, Mojallal A, Boucher F. Anatomy and aesthetics of the labia minora: the ideal vulva? *Aesthetic Plast Surg* 2017;41:714-9.
- van de Grift TC, Pigot GLS, Kreukels BPC, Bouman MB, Mullender MG. Transmen’s experienced sexuality and genital gender-affirming surgery: findings from a clinical follow-up study. *J Sex Marital Ther* 2019;45:201-5.
- Durfee R, Rowland W. Penile substitution with clitoral enlargement and urethral transfer. In: Laub DR, Gandy P, editors. *Proceedings of the second interdisciplinary symposium on gender dysphoria syndrome*. Palo Alto: Stanford University Press; 1973. pp. 181-3.
- Lebovic GS, Laub DR. Metoidioplasty. In: Ehrlich RM, Alter GJ, editors. *Reconstructive and plastic surgery of the external genitalia: adult and pediatric*. Philadelphia: WB Saunders Co.; 1999. pp. 355-60.
- Bouman FG. The first step in phalloplasty in female transsexuals. *Plast Reconstr Surg* 1987;79:662-4.
- Gilbert DA, Winslow BH, Gilbert DM, Jordan GH, Horton CE. Transsexual surgery in the genetic female. *Clin Plast Surg* 1988;15:471-87.
- Hage JJ, van Turnhout AA. Long-term outcome of metaidoplasty in 70 female-to-male transsexuals. *Ann Plast Surg* 2006;57:312-6.
- Perovic SV, Djordjevic ML. Metoidioplasty: a variant of phalloplasty in female transsexuals. *BJU Int* 2003;92:981-5.
- Bowers ML, Stojanovic B, Bizic M. Female-to-male gender affirmation metoidioplasty. In: Salgado CJ, Monstrey SJ, Djordjevic ML, editors. *Gender affirmation: medical and surgical perspectives*. New York: Thieme Medical Publishers Inc; 2017. pp. 109-18.
- Djordjevic ML, Stojanovic B, Bizic M. Metoidioplasty: techniques and outcomes. *Transl Androl Urol* 2019;8:248-53.
- Takamatsu A, Harashina T. Labial ring flap: a new flap for metaidoplasty in female-to-male transsexuals. *J Plast Reconstr Aesthet Surg* 2009;62:318-25.
- Djordjevic ML, Majstorovic M, Stanojevic D, Bizic M, Kojovic V, et al. Combined buccal mucosa graft and dorsal penile skin flap for repair of severe hypospadias. *Urology* 2008;71:821-5.
- Djordjevic ML, Bizic MR. Comparison of two different methods for urethral lengthening in female to male (metoidioplasty) surgery. *J Sex Med* 2013;10:1431-8.
- Djordjevic ML, Bizic M, Stanojevic D, Bumbasirevic M, Kojovic V, et al. Urethral lengthening in metoidioplasty (female-to-male sex reassignment surgery) by combined buccal mucosa graft and labia minora flap. *Urology* 2009;74:349-53.
- Djordjevic ML, Bizic MR, Stanojevic D. Phalloplasty in female-to-male transsexuals. In: Djordjevic M, Santucci R, editors. *Penile reconstructive surgery*. Saarbrücken: LAP Lambert Academic Publishing; 2012. pp. 279-304.
- Bizic MR, Stojanovic B, Djordjevic ML. Genital reconstruction for the transgendered individual. *J Pediatr Urol* 2017;13:446-52.
- Nikolavsky D, Hughes M, Zhao LC. Urologic complications after phalloplasty or metoidioplasty. *Clin Plast Surg* 2018;45:425-35.
- Stojanovic B, Bizic M, Bencic M, Kojovic V, Majstorovic M, et al. One-stage gender-confirmation surgery as a viable surgical procedure for female-to-male transsexuals. *J Sex Med* 2017;14:741-6.

Review

Open Access



# Complications after cosmetic periocular filler: prevention and management

Mike Zein<sup>1</sup>, Ryan Tie-Shue<sup>2</sup>, Nathan Pirakitikulr<sup>3</sup>, Wendy W. Lee<sup>3</sup>

<sup>1</sup>Mcknight Vision Research Center, Bascom Palmer Eye Institute, University of Miami-Miller School of Medicine, Miami, FL 33136, USA.

<sup>2</sup>Department of Biomedical Research, Yale University, New Haven, CT 06520, USA.

<sup>3</sup>Division of Oculofacial Plastic and Reconstructive Surgery, Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami-Miller School of Medicine, Miami, FL 33136, USA.

**Correspondence to:** Dr. Wendy W. Lee, Division of Oculofacial Plastic and Reconstructive Surgery, Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami-Miller School of Medicine, 900 NW 17th St, Miami, FL 33136, USA.  
E-mail: wlee@med.miami.edu

**How to cite this article:** Zein M, Tie-Shue R, Pirakitikulr N, Lee WW. Complications after cosmetic periocular filler: prevention and management. *Plast Aesthet Res* 2020;7:44. <http://dx.doi.org/10.20517/2347-9264.2020.133>

**Received:** 10 Jun 2020 **First Decision:** 30 Jun 2020 **Revised:** 8 Jul 2020 **Accepted:** 15 Jul 2020 **Published:** 15 Aug 2020

**Academic Editor:** Wen-Guo Cui **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Soft tissue fillers are a mainstay in contemporary, minimally invasive facial rejuvenation procedures owing to timely results and minimal recovery period. Although associated with a low complication rate, soft tissue fillers are not without risk. Complications range from mild superficial skin irregularities to granuloma formation to vascular occlusion leading to skin necrosis or even blindness. Fillers vary in composition, elasticity, hydrophilicity and duration of effect that is tailored to specific cosmetic indications. Selecting the right product for the desired effect can cut down on unwanted outcomes. Severe adverse events can be avoided with safe injection technique, early recognition of symptoms and a thorough knowledge of the local anatomy. This review outlines several complications all providers should recognize and discusses strategies for their prevention and management.

**Keywords:** Fillers, hyaluronic acid, rejuvenation, periorbital, aesthetic, skin necrosis, complications, blindness

## INTRODUCTION

Soft tissue fillers have become an increasingly popular intervention for facial rejuvenation over the past two decades with quick results, minimal recovery time and relatively low complication rate. Since their approval



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



by the FDA in 2004, the number of hyaluronic acid (HA) filler injections performed in the United States has steadily risen to nearly 2.7 million procedures per year as of 2018<sup>[1]</sup>. As a naturally occurring component of skin and connective tissue, HA is highly biocompatible and non-immunogenic. HA is a favored choice for patients with little to no history with injectables as its effects are temporary, lasting between 6 to 24 months with natural degradation. This process can be accelerated with the use of hyaluronidase providing some ability to reverse unwanted effects.

While soft tissue fillers and HA in particular are non-incisional and less invasive than other interventions for facial rejuvenation, they still carry a number of risks when performed without proper precautions. In the United States, soft tissue filler injections are performed by a wide variety of health care providers, including but not limited to facial plastic surgeons, dermatologists, oculoplastic surgeons, plastic surgeons and the nurse practitioners and physician assistants working under their supervision. While such providers may be fully licensed to perform these procedures, there are considerable differences in training, familiarity with relevant anatomy, and ability to manage complications. Furthermore, the black market in cosmetic fillers and ready availability of unlicensed injectors provides a steady source of complications that licensed providers should be prepared to encounter<sup>[2,3]</sup>. Between 2013 and 2017 over 2800 reported adverse events occurred in the United States according to FDA databases<sup>[4]</sup>.

Complications vary and range from the mild, self-resolving ecchymoses to the more persistent irregular surface contours, festoons, and the bluish hue (Tyndall effect) seen with superficial filler placement. Severe granulomas have also been seen long after filler injection. The most severe complications are due to filler vascular occlusion, which can result in skin necrosis and sometimes irreversible vision loss<sup>[5]</sup>. This review focuses on filler-associated complications that are most commonly encountered by ophthalmologists and oculoplastic surgeons, and addresses various preventative and management strategies.

## AVOIDING VASCULAR COMPLICATIONS

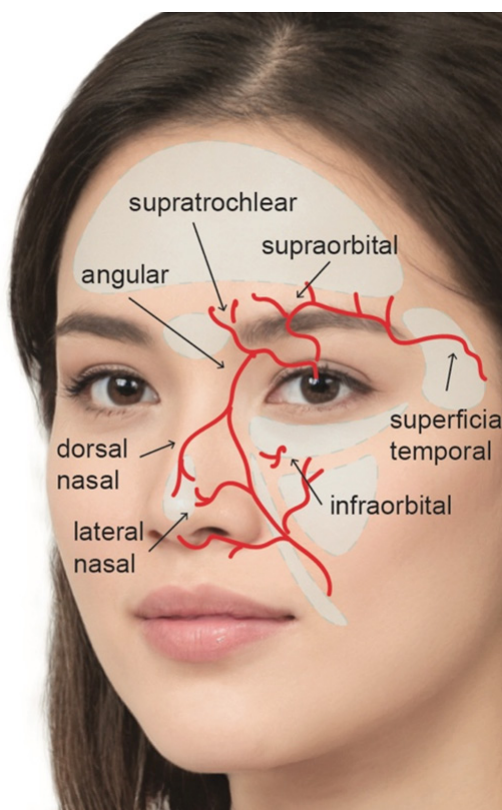
A thorough knowledge of the local anatomy, use of safe injection techniques and timely recognition of symptoms can help minimize the risk of the most severe complications. The face and periorbital area are supplied by a rich network of blood vessels that communicate through complex anastomoses. Iatrogenic perforation or cannulation of the arterial wall during filler injection can introduce emboli that may cause vaso-occlusion either up- or downstream of the site of injection<sup>[6]</sup>.

### Pertinent anatomy

Particularly high-risk zones for vascular complications include the glabella, temporalis fossa, tear trough, midface, nasolabial grooves, and nasal dorsum owing to the large vessels in these areas [Figure 1]<sup>[7]</sup>. In the glabellar region, the supratrochlear branch of the ophthalmic artery exits along the orbital rim approximately 2 cm lateral to midline superficial to the corrugator and deep to the orbicularis and frontalis, before becoming more superficial and entering the subcutaneous plane 2 cm above the orbital rim. The supraorbital branch of the ophthalmic artery exits along the orbital rim through the supraorbital notch approximately 3 cm lateral to midline. Similar to the supratrochlear artery, the supraorbital artery courses deep to the orbicularis and frontalis before entering the subcutaneous plane anywhere from 2 to 6 cm above the orbital rim<sup>[8,9]</sup>.

The temporalis fossa consists of skin, subcutaneous fat, temporoparietal fascia, superficial and deep temporal fascia surrounding loose areolar tissue, temporalis and periosteum. The frontal branch of the superficial temporal artery courses within the temporoparietal fascia approximately 2 cm superior and lateral to the peak of the brow. The middle temporal vein and the temporal branch of the facial nerve also course near this region, posing the additional risk of pulmonary embolism and nerve injury<sup>[10]</sup>. There is also a connection between the temporal fossa and the orbit. The zygomatico-temporal artery connects the





**Figure 1.** Vascular anatomy relevant to cosmetic filler injection and their relation to commonly targeted zones including the forehead, glabella, temporalis fossa, tear trough, midface, nasolabial groove and nasal dorsum

anterior deep temporal artery to the lacrimal artery and runs through the zygomatico-temporal foramen. This foramen is located on the posterior surface of the zygomatic bone. Filler injected in the region of this foramen could potentially travel directly into the orbit via this route.

In the tear trough and midface regions, the infraorbital artery and nerve emerge through the infraorbital foramen approximately 3 cm lateral to midline just inferior to the orbital rim<sup>[11,12]</sup>. Along the nasolabial groove, the facial artery and its branches course in a highly variable pattern. The facial artery can be found medial, lateral or crossing the nasolabial folds. On average it is found 1.7 mm medial to the folds at the upper middle third and 0.3 mm medial at the lower middle third<sup>[13]</sup>. The inferior alar artery and lateral nasal artery branch off from the facial artery at the level of the ala. At the takeoff of the lateral nasal artery, the facial artery becomes more superficial and continues as the angular artery, which crosses the nasojugal groove medially where it is prone to injury during tear trough injections.

After branching into the inferior alar branch and lateral nasal artery, the angular artery continues towards the medial canthus and connects to the dorsal nasal arterial system. The nasal dorsum contains a larger arterial and venous system superficial to nasal musculature in the subcutaneous plane. Sparse vascular networks are located within the areolar layer, including a marginal artery, which courses over the lower lateral cartilage caudal border, and the dorsal nasal artery, a terminal branch of the ophthalmic artery that courses above the muscular layer. Both of these arteries course superficially towards the nasal tip. Each of the arteries listed above have a connection with the ophthalmic and central retinal arteries so injection in each of these areas carries the risk of blindness.

## Principles of safe injection

A safe injection begins with selecting the appropriate needle. We advocate the use of smaller needles such as 30 G for injecting into the superficial and mid-dermis, and 27 G for deeper injections. Some injectors recommend the use of large (25 or 22 G) blunt cannulas as they may provide a lower risk of iatrogenic vessel puncture. After the patient has been properly cleansed and anesthetized, digital pressure can be used to mark and occlude vessels at anatomic landmarks to prevent inadvertent puncture and back flow<sup>[14,15]</sup>. Needles should be introduced perpendicular or parallel to vessels with the tip pointed towards the direction of arterial flow. If a cannula is being used, it should always be parallel to the vessel. Prior to injecting, the syringe is gently aspirated to ensure no blood return, though the absence does not guarantee a vessel has not been punctured<sup>[16]</sup>. The product is then introduced at low flow to avoid overcoming mean arterial pressure should intra-arterial cannulation occur. Keeping the needle in constant motion during injection will also help prevent product from depositing inside a vessel. If resistance is felt, then the needle should be repositioned.

As product is injected, the needle tip should be maintained at a depth to minimize contact with any vessels. The appropriate depth depends on the region being treated. In the glabella the needle tip is kept either superficially in the dermis or deep to the frontalis muscle to avoid vessels in the subcutaneous plane. In the temporalis fossa, filler is placed under the temporalis muscle to avoid vessels coursing in the temporoparietal fascia<sup>[17]</sup>. As an alternative, a cannula can be used in more superficial tissues of the temple. In the tear trough and midface, filler should be placed just above the periosteum with special care medially where the infraorbital canal is located. If filler is needed medially, it can be injected laterally then massaged into place.

Safely injecting along the nasolabial groove can be challenging due to the variable course of the facial artery. In the lower two-thirds, the facial artery generally runs at the muscular plane or deeper. We therefore advocate keeping the needle tip in the superficial subcutaneous plane in this region and then moving deeper to the preperiosteal plane near and above the alar base. At the nasal dorsum the vascular network is located entirely in the subcutaneous plane so injections are kept deep in the preperichondrial and preperiosteal planes.

Vascular compromise must be treated urgently once recognized. All filler procedures must be performed with a “filler crash cart” in the immediate vicinity. This kit should contain at minimum 10 vials of unexpired hyaluronidase (e.g., Hylenex, Halozyme Therapeutics, San Diego, CA; Vitrase, Bausch & Lomb, Irvine, CA). Other items recommended by many providers include aspirin to prevent clot propagation, and warm compresses and nitroglycerin to promote vasodilation. A quick referral system for ophthalmic care should be in place in the event the ophthalmic artery is injured.

## Signs and symptoms of vascular injury

Vascular injury can cause a range of complications. Early signs that an artery has been punctured include the appearance of blood upon aspiration, formation of a hematoma, skin blanching or discoloration, and intense pain at the injection site<sup>[18]</sup>. Should any of these occur, the procedure must be aborted immediately. If not promptly recognized, continued injection after intra-arterial cannulation can lead to vascular occlusion resulting in skin necrosis or worse, vision loss. Skin necrosis occurs in < 0.001%-0.5% of patients but accounts for 43% of serious complications related to soft tissue filler injections in the MAUDE database<sup>[19]</sup>. Early on the skin takes on a blanched then mottled and dusky appearance in the distribution of the injured vessel [Figure 2]. The surrounding area may also appear erythematous.

Emboli can also travel retrograde up through the ophthalmic artery resulting in ocular complications<sup>[20]</sup>. The first reported case of vision loss due to cosmetic soft tissue filler injection occurred in 1988 and was



**Figure 2.** A 38-year old female developed pain, redness and swelling of the left cheek hours after Radiesse injection to the zygoma for midface augmentation. She came to our care two days later. Note the erythema and dusky appearance to the skin in the distribution of the infraorbital artery and facial artery. The patient developed pustules and sloughing the following day. She was treated with aspirin, topical nitroglycerin, oral prednisone, intradermal sodium thiosulfate, hyperbaric oxygen and manual debridement with gradual improvement in tissue perfusion over two weeks and minimal scar tissue buildup

due to retinal artery occlusion<sup>[21]</sup>. These patients report sudden profound unilateral vision loss, but may have preservation of central vision if the cilioretinal artery is spared<sup>[22]</sup>. Emboli traveling to other branches of the ophthalmic artery can result in ischemic optic neuropathy presenting with altitudinal vision loss<sup>[23,24]</sup> or oculomotor nerve palsy presenting with diplopia and in some cases ptosis<sup>[25-27]</sup>. Complete occlusion of the ophthalmic artery results in orbital infarction syndrome which is characterized by severe orbital pain, vision loss, loss of extraocular motility and ptosis. On ophthalmic exam these patients also demonstrate hypotony, corneal edema and persistent ocular inflammation owing to poor perfusion of all ocular structures<sup>[28-30]</sup>. The number of ophthalmic complications related to cosmetic filler continues to rise with the growing demand for these procedures. In the period between January 2015 and September 2018, there were 48 published cases of filler induced ophthalmic complications; the majority were related to vision loss<sup>[31]</sup>.

Certain fillers are associated with a greater risk of vascular compromise owing to differences in particle size, viscosity, cohesivity and effect on inflammation and clotting [Table 1]<sup>[16,32-35]</sup>. The incidence of vascular occlusion from hyaluronic acid fillers has been reported to be approximately one tenth the overall incidence from all fillers, which includes polymethyl-methacrylate microspheres (Bellafill, Suneva Medical, San Diego, CA), calcium hydroxylapatite (e.g., Radiesse, Merz Pharmaceuticals, Greensboro, North Carolina) and poly-L-lactic acid (e.g., Sculptra, Valeant Aesthetics, Bridgewater, New Jersey)<sup>[36]</sup>. The risk is also highest from injections of the glabellar and nasolabial regions<sup>[19,37]</sup>. If a hyaluronic acid product was injected, the area should be immediately flooded with injections of hyaluronidase. While allergic reactions to hyaluronidase have been reported, in the acute setting the benefits of this therapy far outweigh the risks<sup>[38]</sup>. For other products topical nitroglycerin can be considered and warm compresses can

**Table 1. Summary of hyaluronic acid dermal fillers currently commercially available in the United States**

Product	Site	[HA] (mg/mL)	G' (Pa)	Duration (months)
Belotero Balance	Superficial - mid-dermis	22.5	30	6
Restylane-L	Superficial - mid-dermis	20	565	6
Restylane	Medium - deep	20	544	9
Restylane Silk	Superficial - sub-mucosal	20	344	6
Restylane Lyft	Medium - deep	20	545	9
Restylane Refyne	Medium - deep	20	47	12
Restylane Defyne	Medium - deep	20	260	12
Restylane Kysse	Superficial- sub-mucosal	20	156	12
Juvéderm Ultra XC	Superficial - medium	24	207	12
Juvéderm Volbella XC	Superficial - medium	15	274	12
Juvéderm Vollure XC	Medium - deep	17.5	317	18
Juvéderm Voluma XC	Medium - deep	20	353	24
Juvéderm Ultra Plus XC	Medium - deep	24	244	12
Revanesse Versa Plus	Medium - deep	28	130	12
Teosyal RHA 2	Superficial - mid-dermis	23	144	15
Teosyal RHA 3	Medium - deep	23	184	15
Teosyal RHA 4	Deep - sub-cutaneous	23	296	15

[HA]: concentration of hyaluronic acid, G': elastic modulus. Values were obtained from manufacturer documentation and published sources<sup>[56-60]</sup>. Exact G' values varied between sources, but the relative differences between listed products were similar

be rapidly applied, and if available the patient can be referred for hyperbaric oxygen. Few reports suggest sodium thiosulfate can be used to dissolve calcium hydroxylapatite<sup>[39,40]</sup>. However, apart from injection of hyaluronidase, no other therapy has been proven effective<sup>[41]</sup>. There is no consensus on how to treat vision loss from filler-associated vascular occlusion, though anterior chamber paracentesis, ocular massage, hyperbaric oxygen and retrobulbar hyaluronidase injection have all been tried<sup>[37,42]</sup>. Fortunately, with proper attention to anatomy vascular complications are exceedingly rare.

## AVOIDING BAD COSMETIC OUTCOMES

### Strategies for filler placement

There are four basic injection techniques associated with dermal fillers. The most basic technique is threading, which involves the application of a continuous line of filler injected in a retrograde fashion to correct discrete rhytids. The crosshatching technique builds upon this and involves continuous overlapping horizontal and vertical lines to build volume. The third technique is fanning, which involves drawing filler lines in a fan-shaped projection. Finally, serial puncture involves the injection of discreet aliquots of product to correct deep deformities.

### Irregular surface contours

Superficial irregularities are commonly encountered in regions with minimal subcutaneous fat. To avoid this complication, filler must be placed deeply. In the tear trough region, we use a serial puncture technique to advance the needle to the periosteum along the inferior orbital rim and deliver small boluses of product working from medial to lateral. Similarly, in the temporalis fossa, we inject deeply under the fascia of the temporalis muscle in the area of maximal volume loss attempting to avoid vessels and nerves while providing a nice contour. In areas that require more superficial injection such as along the nasolabial groove, using hyaluronic acid fillers with higher cohesivity will diminish surface irregularities<sup>[43]</sup>. Any lumps or bumps noticed early on are addressed with manual massage, while persistent irregularities generally must be dissolved with hyaluronidase.

### Festoons

Festoons or chronic fluid collections often become more noticeable following filler injection with particular hydrophilic hyaluronic acids. Tear trough filler is particularly prone to this complication and it

is therefore important to select a product with low water affinity such as Restylane-L (Galderma, Lausanne, Switzerland).

### **Tyndall effect**

The Tyndall effect is characterized by a blue-grey discoloration of filler placed superficially under the skin. This occurs primarily in the tear trough region where there is minimal subcutaneous fat and the overlying skin is very thin. The phenomenon is due to the scattering of blue light as it passes through small particles in suspension<sup>[44,45]</sup>. As all fillers are similarly prone to this complication, the primary preventative measure is deep injection. Belotero Balance (Merz, Frankfurt, Germany) is made with varying particle sizes, a product characteristic thought to decrease the chances of the Tyndall effect.

## **OTHER COMPLICATIONS**

### **Granulomas**

Granulomas may appear anywhere from 6 to 24 months after injection, and is estimated to occur in 0.1%-1% of patients<sup>[46]</sup>. Presentation usually consists of a constellation of swelling, tenderness, erythema and possible suppuration<sup>[19,47]</sup>. The foreign body reaction results in prolonged inflammation that results in the formation of a nodule comprised of macrophages producing inflammatory products<sup>[48]</sup>. HA fillers reinforced with hydroxyethyl-methacrylate fragments have been associated with late-onset granuloma formation<sup>[49]</sup>. It is postulated that impurities and surface irregularities associated with formulations containing particles < 20 µm in size, are phagocytosed which propagates granuloma formation<sup>[47,50-52]</sup>. Granulomas typically resolve within two years without the need for intervention, but if the lesion persists can be treated with intralesional corticosteroids or surgical excision. Supplemental laser resurfacing or dermabrasion can help improve superficial surface irregularities<sup>[53]</sup>. Attempt at dissolving the granulomas with hyaluronidase is also a good option.

### **Infections**

Infections, while rare, can present in a variety of forms, ranging from erythematous nodules to abscesses. The most common culprits are usually bacterial skin flora (*Streptococcus pyogenes* and *Staphylococcus aureus*), or in some cases herpes simplex virus<sup>[18,48,54]</sup>. In some cases, latent infections or atypical bacteria can be explained by biofilm formation<sup>[55]</sup>. In the event of an abscess, incision and drainage is indicated followed by empiric antibiotics to cover the previously mentioned bacteria. Prophylaxis for herpes simplex infections is indicated for patients with a history of cold sore outbreaks.

## **CONCLUSION**

With the growing number of filler injections used, awareness and prevention of ocular and facial complications is paramount. While the majority are self-resolving, such as ecchymoses at the injection site, ocular complications such as irreversible blindness and orbital and ocular ischemia are detrimental to patients' visual prognosis and general quality of life. Through a strong grasp of the local anatomy, periodicity and presentation of complications, safe technique and knowledge of the available formulations on the market, these adverse events can be mitigated or prevented completely.

## **DECLARATIONS**

### **Authors' contributions**

Conceptualization, writing, and editing of this manuscript: Zein M, Tie-Shue R, Pirakitikulr N, Lee WW

### **Availability of data and materials**

Not applicable.



### Financial support and sponsorship

This work was supported in part by the NIH Center Core (P30EY014801). The sponsor or funding organizations had no role in the design or conduct of this research.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

All photographs were obtained and used with consent.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. American Society of Plastic Surgeons: 2018 Plastic Surgery Statistics Report. Available from: <https://www.plasticsurgery.org/documents/News/Statistics/2018/plastic-surgery-statistics-full-report-2018.pdf>. [Last accessed on 5 Aug 2020]
2. Chayangs O, Wanitphakdeedecha R, Pattanaprichakul P, Hidajat IJ, Evangelista KER, et al. Illegal injectable fillers: the adverse effects comparison study. *J Cosmet Dermatol* 2020; doi: 10.1111/jocd.13492.
3. Brennan R, Wells JSG, Van Hout M. "Saving face": an online study of the injecting use of diy botox and dermal filler kits. *Plast Surg (Oakv)* 2018;26:154-9.
4. Beauvais D, Ferneini EM. Complications and litigation associated with injectable facial fillers: a cross-sectional study. *J Oral Maxillofac Surg* 2020;78:133-40.
5. Rayess HM, Svider PF, Hanba C, Patel VS, DeJoseph LM, et al. A cross-sectional analysis of adverse events and litigation for injectable fillers. *JAMA Facial Plast Surg* 2018;20:207-14.
6. Tansatit T, Apinuntrum P, Phetudom T. A dark side of the cannula injections: how arterial wall perforations and emboli occur. *Aesthetic Plast Surg* 2017;41:221-7.
7. Scheuer JF 3rd, Sieber DA, Pezeshk RA, Campbell CF, Gassman AA, et al. Anatomy of the facial danger zones: maximizing safety during soft-tissue filler injections. *Plast Reconstr Surg* 2017;139:50e-8.
8. Kleintjes WG. Forehead anatomy: arterial variations and venous link of the midline forehead flap. *J Plast Reconstr Aesthet Surg* 2007;60:593-606.
9. Erdogmus S, Govsa F. Anatomy of the supraorbital region and the evaluation of it for the reconstruction of facial defects. *J Craniofac Surg* 2007;18:104-12.
10. Jang JG, Hong KS, Choi EY. A case of nonthrombotic pulmonary embolism after facial injection of hyaluronic acid in an illegal cosmetic procedure. *Tuberc Respir Dis (Seoul)* 2014;77:90-3.
11. Aziz SR, Marchena JM, Puran A. Anatomic characteristics of the infraorbital foramen: a cadaver study. *J Oral Maxillofac Surg* 2000;58:992-6.
12. Ercikti N, Apaydin N, Kirici Y. Location of the infraorbital foramen with reference to soft tissue landmarks. *Surg Radiol Anat* 2017;39:11-5.
13. Yang HM, Lee JG, Hu KS, Gil YC, Choi YJ, et al. New anatomical insights on the course and branching patterns of the facial artery: clinical implications of injectable treatments to the nasolabial fold and nasojugal groove. *Plast Reconstr Surg* 2014;133:1077-82.
14. Scheuer JF 3rd, Sieber DA, Pezeshk RA, Gassman AA, Campbell CF, et al. Facial danger zones: techniques to maximize safety during soft-tissue filler injections. *Plast Reconstr Surg* 2017;139:1103-8.
15. Tansatit T, Moon HJ, Apinuntrum P, Phetudom T. Verification of embolic channel causing blindness following filler injection. *Aesthetic Plast Surg* 2015;39:154-61.
16. DeLorenzi C. Complications of injectable fillers, part 2: vascular complications. *Aesthet Surg J* 2015;34:584-600.
17. Breithaupt AD, Jones DH, Braz A, Narins R, Weinkle S. Anatomical basis for safe and effective volumization of the temple. *Dermatol Surg* 2015;41 Suppl 1:S278-83.
18. Sciafani AP, Fagien S. Treatment of injectable soft tissue filler complications. *Dermatol Surg* 2009;35 Suppl 2:1672-80.
19. Daines SM, Williams EF. Complications associated with injectable soft-tissue fillers: a 5-year retrospective review. *JAMA Facial Plast Surg* 2013;15:226-31.
20. Li X, Du L, Lu JJ. A novel hypothesis of visual loss secondary to cosmetic facial filler injection. *Ann Plast Surg* 2015;75:258-60.
21. Shin H, Lemke BN, Stevens TS, Lim MJ. Posterior ciliary-artery occlusion after subcutaneous silicone-oil injection. *Ann Ophthalmol* 1988;20:342-4.

22. Doguizi S, Sekeroglu MA, Anayol MA, Yilmazbas P. Central retinal artery occlusion with double cilioretinal artery sparing. *Retin Cases Brief Rep* 2019;13:75-8.
23. Chen Y, Wang W, Li J, Yu Y, Li L, et al. Fundus artery occlusion caused by cosmetic facial injections. *Chin Med J (Engl)* 2014;127:1434-7.
24. Kim A, Kim SH, Kim HJ, Yang HK, Hwang JM, et al. Ophthalmoplegia as a complication of cosmetic facial filler injection. *Acta Ophthalmol* 2016;94:e377-9.
25. Bae IH, Kim MS, Choi H, Na CH, Shin BS. Ischemic oculomotor nerve palsy due to hyaluronic acid filler injection. *J Cosmet Dermatol* 2018;17:1016-8.
26. Dagi Glass LR, Choi CJ, Lee NG. Orbital complication following calcium hydroxylapatite filler injection. *Ophthalmic Plast Reconstr Surg* 2017;33:S16-7.
27. Myung Y, Yim S, Jeong JH, Kim BK, Heo CY, et al. The classification and prognosis of periocular complications related to blindness following cosmetic filler injection. *Plast Reconstr Surg* 2017;140:61-4.
28. Kim YJ, Kim SS, Song WK, Lee SY, Yoon JS. Ocular ischemia with hypotony after injection of hyaluronic acid gel. *Ophthalmic Plast Reconstr Surg* 2011;27:e152-5.
29. Ramesh S, Fiaschetti D, Goldberg RA. Orbital and ocular ischemic syndrome with blindness after facial filler injection. *Ophthalmic Plast Reconstr Surg* 2018;34:e108-10.
30. Silva MT, Curi AL. Blindness and total ophthalmoplegia after aesthetic polymethylmethacrylate injection: case report. *Arq Neuropsiquiatr* 2004;62:873-4.
31. Beleznyay K, Carruthers JDA, Humphrey S, Carruthers A, Jones D. Update on avoiding and treating blindness from fillers: a recent review of the world literature. *Aesthet Surg J* 2019;39:662-74.
32. Kim YK, Jung C, Woo SJ, Park KH. Cerebral angiographic findings of cosmetic facial filler-related ophthalmic and retinal artery occlusion. *J Korean Med Sci* 2015;30:1847-55.
33. Park KH, Kim YK, Woo SJ, Kang SW, Lee WK, et al. Iatrogenic occlusion of the ophthalmic artery after cosmetic facial filler injections: a national survey by the Korean Retina Society. *JAMA Ophthalmol* 2014;132:714-23.
34. Barbucci R, Lamponi S, Magnani A, Poletti LF, Rhodes NP, et al. Influence of sulfation on platelet aggregation and activation with differentially sulfated hyaluronic acids. *J Thromb Thrombolysis* 1998;6:109-15.
35. Braverman IM, Keh-Yen A. Ultrastructure of the human dermal microcirculation. IV. Valve-containing collecting veins at the dermal-subcutaneous junction. *J Invest Dermatol* 1983;81:438-42.
36. Beleznyay K, Humphrey S, Carruthers JD, Carruthers A. Vascular compromise from soft tissue augmentation: experience with 12 cases and recommendations for optimal outcomes. *J Clin Aesthet Dermatol* 2014;7:37-43.
37. Narins RS, Jewell M, Rubin M, Cohen J, Strobos J. Clinical conference: management of rare events following dermal fillers--focal necrosis and angry red bumps. *Dermatol Surg* 2006;32:426-34.
38. Landau M. Hyaluronidase caveats in treating filler complications. *Dermatol Surg* 2015;41 Suppl 1:S347-53.
39. Rullan PP, Olson R, Lee KC. The use of intralesional sodium thiosulfate to dissolve facial nodules from calcium hydroxylapatite. *Dermatol Surg* 2019; doi: 10.1097/DSS.000000000000223.
40. Robinson DM. In vitro analysis of the degradation of calcium hydroxylapatite dermal filler: a proof-of-concept study. *Dermatol Surg* 2018;44 Suppl 1:S5-9.
41. Hwang CJ. Periorbital injectables: understanding and avoiding complications. *J Cutan Aesthet Surg* 2016;9:73-9.
42. Chesnut C. Restoration of visual loss with retrobulbar hyaluronidase injection after hyaluronic acid filler. *Dermatol Surg* 2018;44:435-7.
43. Sundaram H, Rohrich RJ, Liew S, Sattler G, Talarico S, et al. Cohesivity of hyaluronic acid fillers: development and clinical implications of a novel assay, pilot validation with a five-point grading scale, and evaluation of six u.s. food and drug administration-approved fillers. *Plast Reconstr Surg* 2015;136:678-86.
44. DeLorenzi C. Complications of injectable fillers, part I. *Aesthet Surg J* 2013;33:561-75.
45. Rootman DB, Lin JL, Goldberg R. Does the tyndall effect describe the blue hue periodically observed in subdermal hyaluronic acid gel placement? *Ophthalmic Plast Reconstr Surg* 2014;30:524-7.
46. Christensen L. Normal and pathologic tissue reactions to soft tissue gel fillers. *Dermatol Surg* 2007;33 Suppl 2:S168-75.
47. Lemperle G, Gauthier-Hazan N, Wolters M, Eisemann-Klein M, Zimmermann U, et al. Foreign body granulomas after all injectable dermal fillers: part I. Possible causes. *Plast Reconstr Surg* 2009;123:1842-63.
48. Funt D, Pavicic T. Dermal fillers in aesthetics: an overview of adverse events and treatment approaches. *Clin Cosmet Investig Dermatol* 2013;6:295-316.
49. Capodiferro S, Sportelli P, Limongelli L, Dell'Olio F, Tempesta A, et al. Delayed sclerosing granulomatous reaction to dermal filler injection of poly-hydroxyethyl-methacrylate suspended in hyaluronic acid: histochemical and confocal laser scanning microscopical analysis. *Clinical Case Rep* 2019;7:2215-9.
50. El-Khalawany M, Fawzy S, Saied A, Al Said M, Amer A, et al. Dermal filler complications: a clinicopathologic study with a spectrum of histologic reaction patterns. *Ann Diagn Pathol* 2015;19:10-5.
51. Ledon JA, Savas JA, Yang S, Franca K, Camacho I, et al. Inflammatory nodules following soft tissue filler use: a review of causative agents, pathology and treatment options. *Am J Clin Dermatol* 2013;14:401-11.
52. Lemperle G, Morhenn V, Charrier U. Human histology and persistence of various injectable filler substances for soft tissue augmentation. *Aesthetic Plast Surg* 2003;27:354-66; discussion 67.
53. Lafaille P, Benedetto A. Fillers: contraindications, side effects and precautions. *J Cutan Aesthet Surg* 2010;3:16-9.

54. Alijotas-Reig J, Fernandez-Figueras MT, Puig L. Inflammatory, immune-mediated adverse reactions related to soft tissue dermal fillers. *Semin Arthritis Rheum* 2013;43:241-58.
55. Dumitrascu DI, Georgescu AV. The management of biofilm formation after hyaluronic acid gel filler injections: a review. *Clujul Med* 2013;86:192-5.
56. Rohrich RJ, Bartlett EL, Dayan E. Practical approach and safety of hyaluronic acid fillers. *Plast Reconstr Surg Glob Open* 2019;7:e2172.
57. Bogdan Allemann I, Baumann L. Hyaluronic acid gel (Juvederm) preparations in the treatment of facial wrinkles and folds. *Clin Interv Aging* 2008;3:629-34.
58. Lee W, Hwang SG, Oh W, Kim CY, Lee JL, et al. Practical guidelines for hyaluronic acid soft-tissue filler use in facial rejuvenation. *Dermatol Surg* 2020;46:41-9.
59. Ballin AC, Cazzaniga A, Brandt FS. Long-term efficacy, safety and durability of Juvederm(R) XC. *Clin Cosmet Investig Dermatol* 2013;6:183-9.
60. Öhrlund Å. Evaluation of rheometry amplitude sweep cross-over point as an index of flexibility for ha fillers. *J Cosmet Dermatol Sci Appl* 2018;8:47-54.

Original Article

Open Access



# Midline raphe scroti artery flap for penile shaft reconstruction

Ursula Mirastschijski<sup>1,2</sup>, Carla Schwenke<sup>3</sup>, Igor Schwab<sup>4</sup>, Andreas Buchhorn<sup>5</sup>, Andreas Schmiedl<sup>5</sup>

<sup>1</sup>Mira-Beau gender esthetics, Berlin 10777, Germany.

<sup>2</sup>Wound Repair Unit, CBIB, Faculty of Biology and Chemistry, University of Bremen, Bremen 28359, Germany.

<sup>3</sup>Department of Urology, Josef-Hospital, Delmenhorst 27753, Germany.

<sup>4</sup>Department of Plastic, Reconstructive and Aesthetic Surgery, Klinikum Bremen-Mitte, Bremen 28205, Germany.

<sup>5</sup>Institute of Anatomy, Hannover Medical School, Hannover 30625, Germany.

**Correspondence to:** Prof. Dr. Ursula Mirastschijski, Wound Repair Unit, CBIB, Faculty of Biology and Chemistry, University of Bremen, Leobener Str./NW2, Bremen 28359, Germany. E-mail: mirastsc@uni-bremen.de

**How to cite this article:** Mirastschijski U, Schwenke C, Schwab I, Buchhorn A, Schmiedl A. Midline raphe scroti artery flap for penile shaft reconstruction. *Plast Aesthet Res* 2020;7:45. <http://dx.doi.org/10.20517/2347-9264.2020.44>

**Received:** 20 Mar 2020 **First Decision:** 20 Jul 2020 **Revised:** 26 Jul 2020 **Accepted:** 4 Aug 2020 **Published:** 21 Aug 2020

**Academic Editor:** Marlon E. Buncamper **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

**Aim:** To investigate a novel method for penile shaft reconstruction.

**Methods:** Penile tissue loss is caused by injury, infections, obesity or cancer resection. Reconstructive techniques comprise skin grafts with the risk of scarring and tissue rigidity. To develop an alternative reconstructive procedure, the pertinent vascular anatomy was studied on fresh cadavers instilled with red latex, which permitted the design of the midline raphe scrotal artery flap (MiRA). After anatomical proof-of-feasibility, penile reconstruction was performed in adult patients with classic buried penis or after cancer resection.

**Results:** Anatomical studies revealed a novel finding of two scrotal septa, each with the terminal branch of the internal pudendal artery. Pedicled on both arteries, a neurovascular island flap could be harvested. In the presence of excess scrotal tissue, the entire circumference of the penile shaft could be covered by this flap. Patients with penile skin defects and excess scrotal tissue were eligible for flap harvest. The flap was raised either as an extended island flap pedicled on both septal arteries for complete penile shaft coverage, or as a VY-flap for partial reconstruction; the donor site was closed primarily. Post-operative complications included swelling or partial wound dehiscence. There were no flap losses or perfusion problems. Patients reported full sensitivity to the penile shaft skin and sufficient skin elasticity for erection.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Conclusion:** The MiRA flap is a technically safe neurovascular flap suitable for the reconstruction of partial or full defects of the penile shaft, such as after type III buried penis surgery, and provides sufficiently elastic and sensitive skin for functional penile reconstruction.

**Keywords:** Scrotal anatomy, septal artery, penile shaft coverage, penile reconstruction, buried penis, scrotal island flap, midline raphe scrotal artery flap

## INTRODUCTION

The penile skin is unique in that it has usually no hair and no subcutaneous fat layer. It is highly elastic and flexible, allowing for around a 2-fold increase in penile length and a 1.5-fold increase in girth during erection<sup>[1]</sup>. Hence, reconstruction of the penile envelope is difficult when most parts of the skin are lost.

Common causes for penile defects include infections such as Fournier's gangrene<sup>[2]</sup>, inflammatory reactions with tissue induration and granuloma formation after injection of various substances<sup>[3]</sup>, tumour resections<sup>[4]</sup>, trauma (e.g., burns<sup>[5]</sup>, combat<sup>[6]</sup> or other related accidents<sup>[7]</sup>) or penile invagination into the pre-pubic fat apron in highly obese men<sup>[8]</sup>. With the rising incidence of morbid obesity, this phenomenon, also called buried penis, is increasingly seen in men with resulting impairment of micturition and sexual intercourse<sup>[9]</sup>.

Full- or split-thickness skin grafts are the current gold standard for the reconstruction of penile skin defects<sup>[10-12]</sup>. Draw-backs of this technically simple and straightforward procedure are the tendency for scarring and the rigidity of the skin graft once it has taken. Penile deviation or pain during erection with loss of penile length are commonly reported. Another reconstructive possibility is the use of scrotal skin. Despite the similarity of scrotal and penile skin - both are devoid of a subcutaneous fat layer and highly elastic - the reach of scrotal skin flaps is limited to the base of the penile shaft without the possibility of covering the entire shaft length<sup>[13]</sup>. Hence, temporary burying of the penis in the scrotum with delayed release, or local flaps for defects at the penile base are currently practised as well<sup>[14,15]</sup>.

The concept of plastic-reconstructive surgery is based on intimate anatomical knowledge of tissue structure and perfusion. The course of vessels enables the design of pedicled or free flaps to reach distant defects for closure. Despite its primitive function as a testicular bag, the scrotal sac has a pivotal role in maintenance of a man's reproductivity. The delicate regulation of temperature by the scrotum is orchestrated by an interplay of vessel width, muscle and skin contraction. As a consequence, vessels from different sources enter the scrotal sac to build a dense network. It is a well-known fact that branches from the external pudendal artery, also called rami scrotales laterales and anteriores, provide blood to the lateral and ventral scrotal skin, respectively. The internal pudendal artery sends branches to the dorsal part of the scrotum (rami scrotales posteriores), interacting closely with the branches of the external pudendal artery. Until recently, there was no information on arterial perfusion of the scrotal septum<sup>[16]</sup>.

In order to find novel surgical means to reconstruct the penile shaft, scrotal and penile anatomy and vessel distribution were anatomically studied on deceased body donors instilled with red ink into pelvic vessels first. After demonstration of the vascular anatomy of a scrotal island flap based on the arteries of both scrotal septa, patients with penile shaft defects were reconstructed with the novel neurovascular midline raphe scrotal artery flap (MiRA) island flap.



## METHODS

### Anatomical studies

Initially, dissection studies were performed on cadavers at Hannover Medical School. Perfusion fixation was carried out via the femoral artery with a solution containing 2.7% paraformaldehyde ( $n = 4$ ). Because the scrotal sac is positioned in the lower, dorsal parts of the supine laying body, a lot of liquid was found in the tissue which made vessel studies difficult. Furthermore, this fixation technique turned the normally soft and flexible genital tissue into a stiff matrix with rigid skin such that flap studies were impossible. Consequently, for flap surgery, fresh cadavers within 24 h of death were chosen. Male pelvic parts of fresh cadavers including the genitals ( $n = 4$ ) were instilled with red silicone dye S 10 (KSK02A15.0 BIODUR® S 10; Biodur® Products GmbH, Heidelberg, Germany). Before vessel injection, the dye was mixed with the hardener S 6 (KSH03A1.0 BIODUR® Härter S 6) in a ratio of 100:5. The abdominal aorta was dissected and incised above the bifurcation. An anterograde tube was then inserted into the abdominal aorta above the bifurcation into both common iliac arteries. Using a 500 mL perfusion syringe, the colour suspension was pressed through the tube into the aorta by hand. The femoral artery was clamped below the exit of the profunda femoris artery. About 500 mL was injected. The solution was distributed over the internal iliac artery into the internal pudendal artery and over the external iliac artery into the external pudendal artery. After one-day of hardening, preparation of the vessels was started. Pictures were taken with a Nikon D5100 and a Nikon D800E camera.

For studies of the genital vasculature, the main vessels were prepared and branches to the scrotal sac and penis were followed. Of note, the testicular vasculature (funiculus spermaticus) was not dissected or investigated. First, the external pudendal artery was dissected at its origin in the groin or upper medial thigh and its course followed until the point of branching at the lateral aspect of the scrotal skin. Interestingly, there were variations with regard to its origin: directly from the femoral artery ( $n = 3$ ), from the profound femoral artery ( $n = 2$ ) and from the inferior epigastric artery ( $n = 1$ ). Two donors had femoral artery surgery previously such that anatomical studies of the thigh vessels were impossible due to severe scarring and the presence of vascular implants.

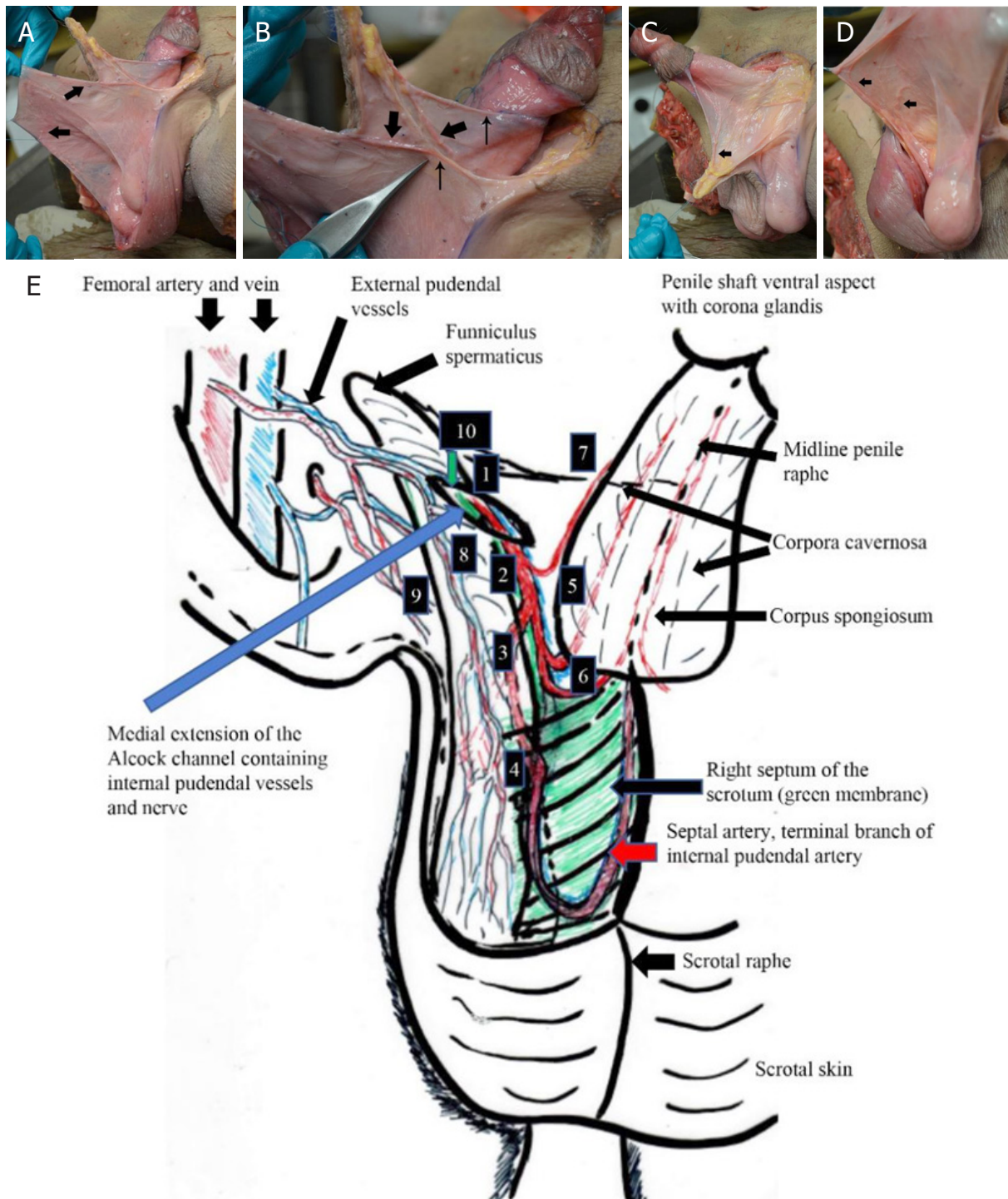
For flap anatomy, incision markings were positioned as shown in [Figure 1A-C](#), parallel to the midline scrotal raphe. After incision, the scrotal fasciae were dissected carefully starting from the most caudal point of the flap [[Figure 1D-F](#)]. The septum was visualized. Due to the prominent visibility of the septal vessels, with the terminal branch running from the dorsal aspect of the septum like an arch to the ventral plane of the scrotum, a second vessel was noticed with the same course but in a different plane. After further dissection, it became clear that the scrotal midline is separated beneath the raphe by two septa each containing one terminal branch of the internal pudendal artery on each side. Considering the embryology and the fact that the scrotal sac is formed by the fusion of two embryonal swellings, this observation is not the least surprising but rather, a logical consequence of embryological development. We could also demonstrate a dual septum with separate vessels which were communicating with each other. Next, the flap was pedicled on both septa and septal arteries, and lateral branches communicating with the central branches were dissected, retaining those branches that inserted directly into the flap. The septa at the dorsal aspect of the scrotum were released up to its cranial root and until sufficient mobility of the flap for penile shaft coverage was achieved. The flap was wrapped around the penile shaft and closed at its dorsal aspect [[Figure 1G-I](#)]. Because scrotal tissue is highly elastic, and due to pre-existing excessive cutaneous tissue, the donor site was closed primarily after orchidopexy [[Figure 1H and I](#)]. Because the flap and its vascular pedicle were designed around the scrotal midline, which is also called raphe, the flap was named the MiRA flap.

The novel finding of the scrotal anatomy with presence of two scrotal septa including an arch forming terminal branch of the internal pudendal artery is shown in detail in [Figure 2](#). Since the scrotum is



**Figure 1.** Representative images of anatomical studies on fresh cadavers are presented. A-C: pre-operative anatomical markings of the external genital region with a circumcised penis, excessive scrotal tissue and peno-scrotal webbing (B, arrow); D-F: flap mobilization and measurements of approximately 11 cm in length (E) and 7 cm in width (F); G-I: penile defect coverage (G) and donor site closure (H, I) are demonstrated





**Figure 2.** Septal anatomy with demonstration of the dual presence of scrotal septa including an arch forming terminal branch of the internal pudendal artery that is running from the dorsal to the ventral aspect of the septum. A: lateral view with the flap folded behind the septa. Arrows depicting the septal artery of each septum; B: close-up of A with the septal arteries (thick arrows) and anastomoses with lateral scrotal branches derived from the external pudendal artery (thin arrows); C: septal artery with anastomoses to the lateral border of the flap; D: close-up of the left septum with upper arrow depicting the septal artery. Lower arrow pointing to the artery of the right septum that is visible shining through the tissue layer of the left septum; E: graphical overview of the vascular anatomy of the scrotum. The scrotum is vascularized by the anterior, lateral and posterior branches of different arteries. The main vessels of the scrotum are the internal and external pudendal artery. After leaving the Alcock channel (ischio-rectal fascia, blue long arrow), the internal pudendal vessels (1) divide into the rectal (not shown) and perineal artery (2). The dorsal scrotal branches (3) originate from the perineal artery which continues in the midline as the septal artery (4, red arrow). Further branches of each internal pudendal artery are the central arteries - providing the ipsilateral cavernosal bodies (5) and the corpus spongiosum (6) - and the dorsal penile artery (7). The anterior (8) and lateral (9) scrotal branches derive from the external pudendal artery originating from the femoral artery. Of note, the pudendal nerve (10, green arrow) accompanies the internal pudendal artery and its branches throughout the scrotum and penis.

**Table 1. Demographic data of patients included in the study**

Patient number	Diagnosis	Co-morbidities	Previous genital operations	Age (years)*	BMI (kg/m <sup>2</sup> )	Follow up (months)
1	Buried penis Grade III	Diabetes type II, hypertension, benign prostatic hyperplasia	Circumcision	74	35.1	6
2	Buried penis Grade III	Diabetes type II, hypertension, morbid obesity	Circumcision	63	40.5	12
3	Buried penis Grade III	Diabetes type II, hypertension	Circumcision, recurrent phimosis	63	38.5	6
4	Buried penis Grade III	hypertension, hyperuricemia, morbid obesity	Circumcision due to LSC**, recurrent phimosis	56	50.7	12
5	Penile cancer	Compensated cardiac insufficiency, hypertension	Cancer resection (Buschke-Lowenstein-Tumor)	80	n.d.	0

\*at the time of operation; \*\*LSC: Lichen sclerosus et atrophicus; BMI: body mass index; n.d.: no data. Classification of the buried penis according to Mirastschijski<sup>[9]</sup> 2018

vascularized by the anterior, lateral and posterior branches of different arteries, a schematic overview of the vascular perfusion is shown in [Figure 2E](#).

### Patient selection for reconstructive surgery

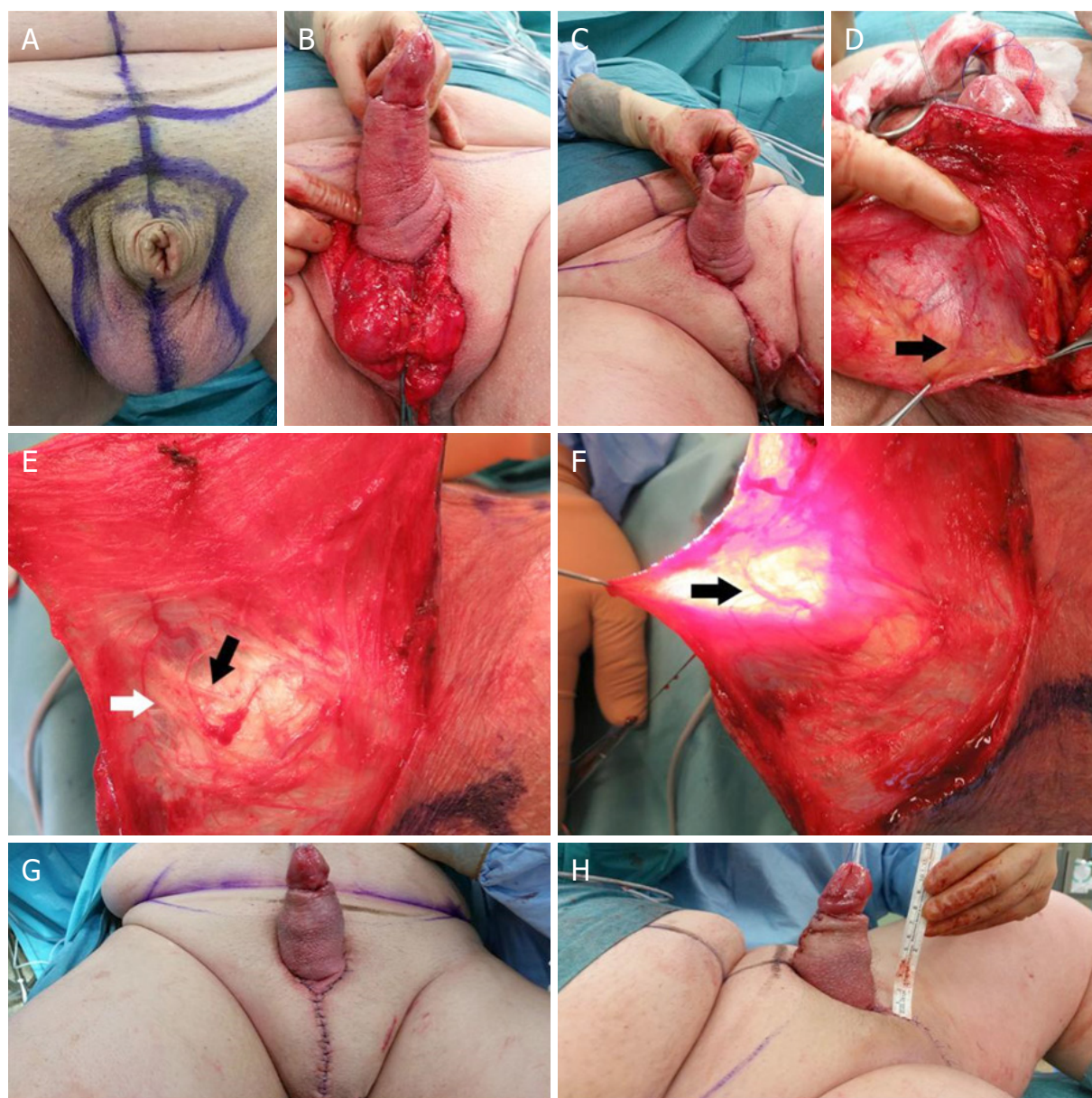
Male patients ( $n = 5$ ) with genital defects after tumour or with classic buried penis type III according to the previously published classification<sup>[9]</sup> were selected for penile shaft reconstruction with the novel MiRA flap. After thorough discussion and informed consent was obtained, elective reconstructive penile surgery took place at the Department of Plastic, Reconstructive and Aesthetic Surgery or the Department of Urology at Klinikum Bremen-Mitte, Bremen, Germany, in the years 2016 and 2017. The prerequisite for choosing patients suited for this type of surgery was: (1) a penile shaft defect of the entire penile length; (2) sufficient scrotal tissue and; and (3) written consent for the operation and photo-documentation.

The mean follow-up was around 7 months. The post-operative follow-up of patients 1 to 3 was uneventful except for initial swelling and minor wound dehiscence. Patient 4 needed partial flap removal due to recurrent LSC which had formed beneath the neo-preputium of the flap. Patient 5 received out-patient care for penile cancer with his urologist and was lost from follow-up. For more detailed information see [Table 1](#).

### Surgical technique MiRA flap

Preoperatively, incision lines were marked on patients in the upright and supine position [[Figure 3A](#)]. The midline of the scrotal sac, the raphe scroti, is the central part of the flap because it depicts the insertion point of the scrotal septa and the position of the supplying vessels, i.e., the end branches of the internal pudendal artery. Incision lines are marked bilaterally from the midline/the raphe scroti with a V- or W-shape at its distal mobilization point. A scrotal examination was performed to confirm the presence of both testes in the scrotal sac and absence of inguinal hernias. Furthermore, the presence of excess scrotal tissue, also called peno-scrotal-webbing, was marked for flap harvest. Diagnosis of the stage of the adult buried penis was performed according to the adult buried penis classification<sup>[17]</sup>. Prior to surgery, the genital area was shaved. Patients were placed supine, washed with medical soap and disinfected. First, diseased tissue was excised, e.g., sclerotic Lichen sclerosus skin lesions or in one case, a penile carcinoma of the glans. All excised tissue was submitted for histopathological analysis. Patients presenting with adult buried penis were treated according to the previously published algorithm<sup>[17]</sup>. In short, after liposuction, the pre-pubic fat apron was excised. The buried penis was retrieved and the area for flap reconstruction measured with a ruler. The size of the MiRA flap was designed accordingly [[Figure 3B and C](#)]. After





**Figure 3.** The surgical technique of the midline raphe scrotal artery flap is presented. A: preoperative markings; B: flap harvest and positioning around the penile shaft with complete coverage; C-F: the terminal branch of the pudendal artery runs in an arch-formed way from the dorsal plane of the septum to the ventral side. The black arrow depicts the artery (D-F), the white arrow depicts the concomitant nerve (E); G, H: postoperative result after wound closure. Visualization of the septal artery by diaphanoscopy in F

incision, the flap was raised on both septal arteries preserving both septa underneath the island flap [Figure 3D-F]. Vascular branches anastomosing with lateral vessels were sealed using LigaClips®. The flap was mobilized, freeing both septa from its caudal fixation in the scrotum up to the cranial fixation point at the penile base so that it could be easily wrapped around the entire penile shaft. Closure of the scrotal sac was performed after bilateral orchidopexy, thorough hemostasis and drain placement. Due to excessive and elastic scrotal tissue, the donor site defect was closed primarily after mobilization of the wound edges with subcutaneous absorbable single knots and intradermal running sutures after inserting a Penrose drain [Figure 3G-H]. The prepubic wound was closed according to plastic-surgical standards with fascial anchoring sutures after Baroudi and Ferreira<sup>[18]</sup> to avoid seroma formation and recurrence of penile retraction into the prepubic tissue. For penile shaft reconstruction, the MiRA scroti island flap, pedicled



on both septal arteries, was wrapped around the penile shaft and closed with subcutaneous resorbable (e.g., 3.0 Vicryl) and cutaneous non-absorbable (e.g., 3.0 Ethilon) single knots sutures. Because of post-operative swelling, single knot sutures are preferred to avoid wound dehiscence. The reconstructed penis is then dressed using a fatty gauze to protect the skin of the flap. A stabilizing sponge is positioned around the penile shaft and secured with staplers at the penile base and with sutures (3.0 Prolene, blunt needle) to the glans. Aside from stabilizing the flap to the penile shaft, the sponge inhibits excessive post-operative swelling and lymphedema as well.

## RESULTS

Five patients were included in the study after providing informed consent to the operation and to anonymous publication of their photographs. Demographic data of the patients is listed in [Table 1](#). Mean post-operative follow-up was around 7 months. The surgical method was performed in each of the patients without any complications. In each patient, the scrotal septum consisted of two membranes as found in the anatomical study. In each septum, an arch-shaped terminal branch of the internal pudendal artery was identified exactly underneath the scrotal raphe, which served as a surgical landmark. Therefore, all island flaps were supplied by a dual arterial system. In all cases, excessive scrotal tissue was present to provide sufficient material for the island flap. In four cases of patients with adult buried penis, the complete penile shaft was grafted with the MiRA flap. [Figure 4](#) depicts an example of a patient with buried penis grade III [[Figure 4A and B](#)]. Initial swelling was reduced after 4 weeks post-operatively [[Figure 4 C and D](#)]. After one year, the patient was very satisfied with the result including the neo-foreskin [[Figure 4E and F](#)]. No recurrence of the buried penis was noticed in any of the monitored cases. In one case, a partial ventral shaft defect was covered with the flap after excision of a carcinoma of the glans that had grown into the ventral skin [[Figure 5](#)]. The surgical procedure was performed by two independent surgeons. Post-operative complications included a moderate hematoma ( $n = 1$ ) without the need for intervention and lymphedematous swelling which is very common for the genitalia. In two cases, a wound dehiscence occurred followed by secondary suture after reduction of the swelling. Long-term results included lymphedematous swelling that resolved after six to eight weeks and the recurrence of pre-existing Lichen sclerosus et atrophicus in one patient with the need to excise part of the flap which had formed a neo-foreskin covering the glans completely. Patients were highly satisfied and reported immediate sensation to the penile shaft after the operation, and of enhanced erectile activity - which might have derived from the penile anchoring sutures.

## DISCUSSION

Penile shaft reconstructive surgery had been hitherto limited to skin grafts with loss of tissue elasticity and sensitivity or to part-time burying the penis into scrotal tissue. Local flaps were restricted to the penile base with insufficient tissue to cover the complete length of the shaft. Here, we present a double artery pedicled midline scrotal island flap with complete cutaneous and sensitive restoration of the penile shaft.

The current gold standard for surgical penile shaft reconstruction are full-<sup>[17]</sup> or split-thickness skin grafts<sup>[12]</sup> with the disadvantages of post-operative shrinkage<sup>[10]</sup>, scarring and loss of elasticity and, as a consequence, reduced penile excursion which can impair erection and sexual intercourse. Major drawbacks of skin grafts are the complete loss of sensitivity to the penile shaft and partial reduction of the penile length in cases of graft shrinkage. The ultimate goal of our study was to find a surgical technique for full functional restoration of the penile shaft while at the same time remaining a simple surgical procedure suitable for patients with critical co-morbidities.

The use of scrotal tissue for penile shaft reconstruction is not novel. Scrotal skin is similar to penile skin in that it does not contain a subcutaneous fat layer and has a higher amount of elastic fibers than that found in non-genital skin<sup>[19]</sup>. In contrast to penile skin, it is thicker and contains hair and sebaceous

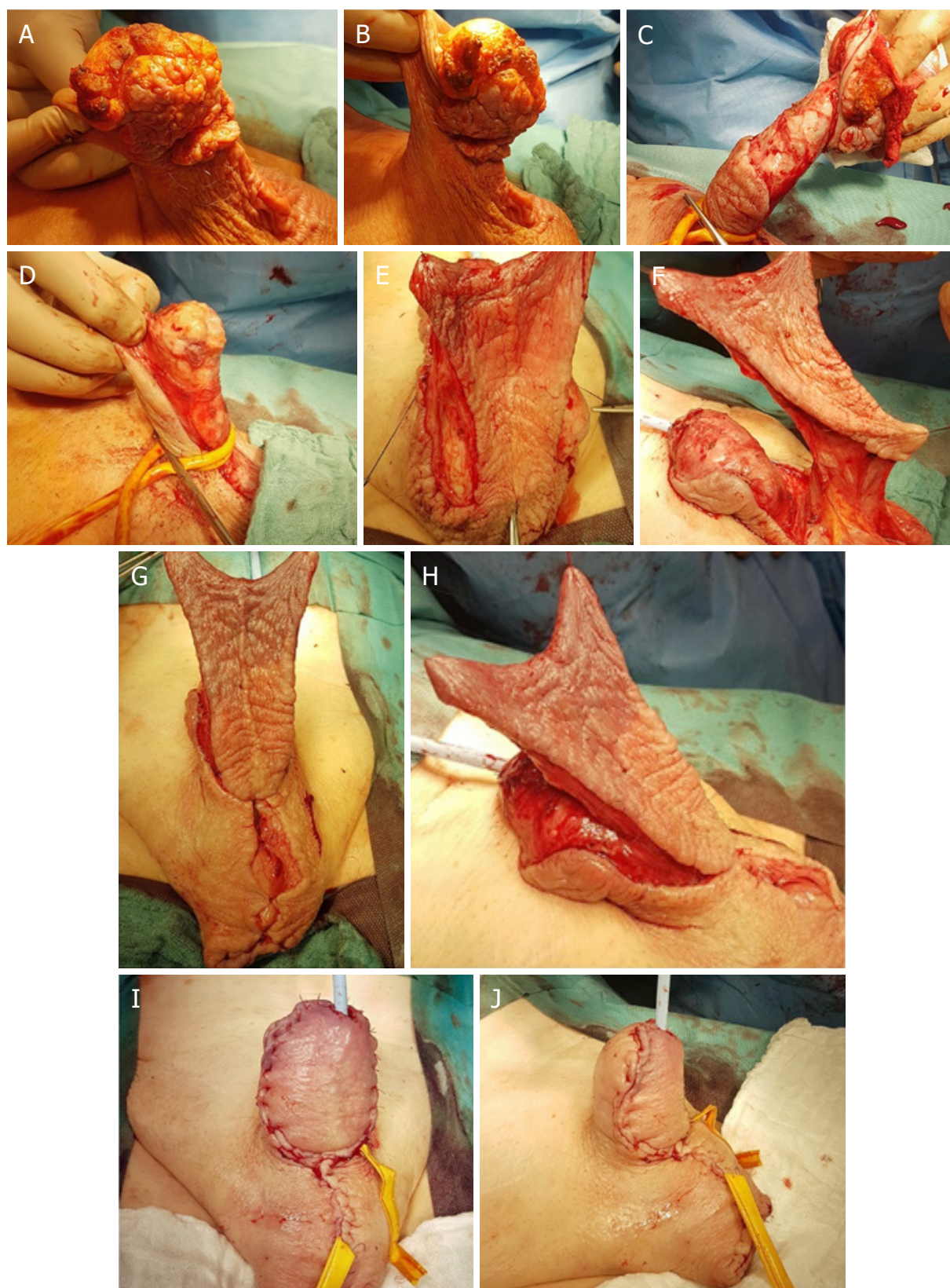


**Figure 4.** Pre- and postoperative example of a patient with buried penis type III and penile reconstruction with the MiRA island flap. A, B: preoperative view with completely invaginated penile shaft of a classic type III buried penis patient; C, D: postoperative result after 4 weeks; E, F: stable result after one year without recurrence of the buried penis and with neo-foreskin

glands. Several techniques were described to use scrotal skin for penile reconstruction, e.g., a scrotal dartos flap<sup>[20]</sup>, advancement flaps<sup>[13,21]</sup> or a ventral slit scrotal flap<sup>[22]</sup>. The drawback of local advancement flaps is the limited reach with coverage of the penile base and parts of the penile shaft but not the entire length of the penis. The current urological golden standard for complete penile coverage is temporal burying of the penile shaft in the scrotal sac<sup>[14,23]</sup>. After 3 weeks, the flap is revascularized through penile vessels which permit retrieval of the penis with full shaft coverage by scrotal skin. Because the scrotal skin is completely released, no nervous continuity is provided and, as a consequence, there is no sensitivity to the shaft.

Anatomical studies on fresh cadavers were the prerequisite for the design of the novel flap presented in this study. The male genital anatomy is poorly delineated specifically with regard to scrotal vessels. Here, we describe for the first time the existence of two scrotal septa, each equipped with the terminal branch of the internal pudendal artery that runs from the dorsal wall of the scrotum within the septum to the ventral wall and anastomoses with lateral branches deriving from the external pudendal artery. Carrera *et al.*<sup>[16]</sup> described in 2009 the central perfusion of the scrotal sac by two terminal branches of the internal pudendal





**Figure 5.** The MiRA flap is suitable for partial penile reconstruction in a patient presenting with a Buschke-Lowenstein tumor that extended onto the ventral side of the penile shaft. A, B: preoperative setting; C, D: tumor resection including total glansectomy; E-H: intraoperative view with flap harvest (E, F) and closure of the donor site (G, H); I, J: postoperative result with partial preservation of the penile shaft enabling micturition in the upright position

artery, however, they did not discern that these arteries were running in parallel in two separate septa. A study by Angspatt *et al.*<sup>[24]</sup> showed that the anterior scrotal artery supplies 62.5%-100% (mean 75.9%) of the scrotal skin in the anteroposterior dimension and 66%-100% (mean 88%) in the superoinferior dimension. The main blood supply runs through the branches of the external pudendal artery. The remaining tissue was supplied by the posterior scrotal artery. Seemingly, the dominant vascular system perfusing the scrotal tissue derives from the external pudendal artery with the anterior and lateral scrotal branches. The internal pudendal artery provides branches to the dorsal part of the scrotum and the septum.

During embryonal development, the external genitalia in both sexes originate from the genital tubercles and swellings. Under the influence of male or female hormones, genital swellings differentiate into the scrotum (dihydrotestosterone) or labia majora (estrogen)<sup>[25]</sup>. In females, the process continues with separation of the genital swellings that surround the vaginal vestibulum; in the male embryo, both genital swellings fuse in the midline to become the scrotal sac<sup>[25]</sup>. The scrotal septum depicts the fusion line of both genital swellings, and it is not surprising that it consists of two thin membranes with identical anatomical structure. Genital development is indeed common knowledge, however, the existence of two scrotal septa including a mirroring vasculature has not been described so far.

An island flap with dual arterial blood supply implies a safe surgical technique for penile shaft coverage. Both occlusion or disruption of the blood supply via one vessel will not jeopardize flap survival due to redundant blood supply by the twin artery. Our assumption is based on angiosome studies by Angspatt *et al.*<sup>[24]</sup> and previous reports on the anatomy of the posterior scrotal artery<sup>[26]</sup>. The septal scrotal artery is a branch of the posterior scrotal artery deriving from the perineal artery [Figure 2E]. In our anatomical studies, we could not clearly detect such angiosomes. Because the posterior scrotal artery anastomoses with branches of the lateral scrotal artery, it is conceivable that the angiosome may include the skin area around the raphe scroti along the septum scroti.

Interestingly, there are two further reports on scrotal island flaps with pedicles different from ours. Karim *et al.*<sup>[27]</sup> used a dorsal scrotal island flap supplied by the dorsal scrotal vessels for perianal defect coverage. Fakin *et al.*<sup>[28]</sup> reconstructed patients with penile granuloma with a ventral scrotal flap pedicled on the deep external pudendal artery. Abundant bilateral cutaneous perfusion from different vascular sources with highly elastic and excessive tissue renders the scrotum an ideal donor area for reconstructive surgery. To avoid testicular torsion or ascension due to diminished volume of the scrotal sac after skin and septal resection, bilateral orchidopexy is recommended and should be routinely performed.

Nerves concomitant to the septal arteries provide cutaneous sensation over the ventral aspect of the scrotum in proximity to the midline where the flap is harvested. This unexpected finding was reported by our patients who were puzzled to locate the sensation of the neo-penile skin to the scrotal sac [Table 2]. In fact, the brain's plasticity enables even elderly patients to learn the new location of the skin within a few weeks, a well-known feature from reconstructive hand surgery<sup>[29]</sup>. In larger patient cohorts, objective measurement tools such as the Semmes Weinstein test for sensitivity analysis are recommended to evaluate clinical findings in a standardized way.

A general drawback of island flaps is the cessation of lymphatic drainage due to complete cutaneous excision with loss of lymphatic continuity [Table 2]. Consequently, a prolonged lymphedema is noticed until new lymphatic vessels have developed. To minimize swelling and the risk of wound dehiscence, a protective and stabilizing dressing is positioned circumferentially around the penis with fixation to the perineum and glans. After removal, manual lymphatic drainage and compression therapy are recommended. The highly elastic scrotal skin provides sufficient tissue to cover the entire penile shaft and can form a neo-prepuce as well. In case of patients with Lichen sclerosus et atrophicus (balanitis xerotica

**Table 2. Summary of outcomes with midline raphe scroti artery island flap**

Pros	Cons
Simple surgical technique, sufficient perfusion due to the presence of two arteries, intact sensibility due to the presence of cutaneous nerves	Penile lymphedema, wound dehiscence
Highly elastic tissue well-suited for size changes during erection	Postoperative compression therapy
Restoration of micturition	Bulkiness,
Rehabilitation of sexual function	presence of hair
Prevention of recurrent buried penis	Scrotal size reduction,
Reduction of excessive scrotal tissue	orchidopexy
Neo-foreskin	Contra-indication: LSC**
	Recurrence due to neo-foreskin

\*\*LSC: Lichen sclerosus et atrophicus

obliterans), a recurrence of the disease was noticed due to restoration of the moist and predisposing milieu for this skin disease. In this particular case, a revision with partial excision of the novel flap was indicated and the defect was covered with a skin graft.

In conclusion, the novel neurovascular MiRA island flap is well suited to reconstruct the entire penile shaft after tissue loss with restoration of a fully sensitive cutaneous envelope. Due to its abundant vascular perfusion via two septal arteries, it is a safe and easily performed surgical technique for a wide range of tissue defects of the male genitalia.

## DECLARATIONS

### Authors' contributions

Invention of the flap, surgical design in anatomical studies: Mirastschijski U

Surgical procedures in patients: Mirastschijski U, Schwenke C

Made substantial contributions to the conception and design of the study, performed data analysis, interpretation and acquisition, provided administrative, technical and material support, writing the manuscript, corrections and advice: Mirastschijski U, Schwenke C, Schwab I, Buchhorn A, Schmiedl A

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

An informed consent to participate in the study was obtained from each patient. For variation of standard surgical procedures, no ethical approval is needed.

### Consent for publication

A written informed consent for anonymous publication of photographs was obtained from each patient.

### Copyright

© The Author(s) 2020.



## REFERENCES

1. Schill S, Panfilov D, Mirastschijski U. Intimchirurgie beim Mann. In: Mirastschijski U, Rimmel E, editors. Intimchirurgie. Berlin: Springer; 2019. pp. 49-67.
2. Sorensen MD, Krieger JN. Fournier's gangrene: epidemiology and outcomes in the general US population. *Urol Int* 2016;97:249-59.
3. Dellis AE, Arkoumanis T, Kyprianou C, Papatsoris AG. Paraffinoma, siliconoma and Co: disastrous consequences of failed penile augmentation - a single-centre successful surgical management of a challenging entity. *Andrologia* 2018;50:e13109.
4. Schwenke C, Melchior S. Peniscarcinom aus uro-onkologischer Sicht. In: Mirastschijski U, Rimmel E, editors. Intimchirurgie. Berlin: Springer; 2019. pp. 207-13.
5. Harpole BG, Wibbenmeyer LA, Erickson BA. Genital burns in the national burn repository: incidence, etiology, and impact on morbidity and mortality. *Urology* 2014;83:298-302.
6. Balzano FL, Hudak SJ. Military genitourinary injuries: past, present, and future. *Transl Androl Urol* 2018;7:646-52.
7. McAninch JW, Kahn RI, Jeffrey RB, Laing FC, Krieger MJ. Major traumatic and septic genital injuries. *J Trauma* 1984;24:291-8.
8. Mirastschijski U. Buried penis. In: Mirastschijski U, Rimmel E, editors. Intimchirurgie. Berlin: Springer; 2019. pp. 107-14.
9. Mirastschijski U. Classification and treatment of the adult buried penis. *Ann Plast Surg* 2018;80:653-9.
10. Garaffa G, Gentile V, Antonini G, Tsafarakidis P, Raheem AA, et al. Penile reconstruction in the male. *Arab J Urol* 2013;11:267-71.
11. Mirastschijski U, Schwenke C, Schmiedl A. Plastisch-chirurgische rekonstruktion des männlichen genitalen. In: Mirastschijski U, Rimmel E, editors. Intimchirurgie. Berlin: Springer; 2019. pp. 189-205.
12. Hakansson U, Kirrander P, Uvelius B, Baseckas G, Torbrand C. Organ-sparing reconstructive surgery in penile cancer: initial experiences at two Swedish referral centres. *Scand J Urol* 2015;49:149-54.
13. Qin X, Zhang S, Zhang H, Shen Y, Zhu Y, et al. Reconstruction with scrotal skin flaps after wide local resection of penoscrotal extramammary Paget's disease. *BJU Int* 2012;110:E1121-4.
14. Ziyilan O, Acar O, Ozden BC, Tefik T, Donmez MI, et al. A practical approach for the correction of iatrogenic penile skin loss in children: scrotal embedding technique. *Turk J Urol* 2015;41:235-8.
15. Zhao YQ, Zhang J, Yu MS, Long DC. Functional restoration of penis with partial defect by scrotal skin flap. *J Urol* 2009;182:2358-61.
16. Carrera A, Gil-Vernet A, Forcada P, Morro R, Llusa M, et al. Arteries of the scrotum: a microvascular study and its application to urethral reconstruction with scrotal flaps. *BJU Int* 2009;103:820-4.
17. Mirastschijski U. Response to the letter to the editor. *Ann Plast Surg* 2018;81:508.
18. Baroudi R, Ferreira CA. Seroma: how to avoid it and how to treat it. *Aesthet Surg J* 1998;18:439-41.
19. Mirastschijski U. Genital scars. In: Téot L, Thomas M, Midlekoop E, Gauglitz G, editors. Textbook on scar management. Berlin: Springer; 2020.
20. Innocenti A, Tanini S, Mori F, Melita D, Innocenti M. Scrotal dartos-fascio-myo-cutaneous flaps for penis elongation after catastrophic iatrogenic skin shaft sub-amputation: a case of recovery using an extremely adaptable flap. *Int J Surg Case Rep* 2016;28:300-2.
21. Vaca EE, Mundinger GS, Zelken JA, Erdag G, Manahan MA. Surgical excision of multiple penile syringomas with scrotal flap reconstruction. *Eplasty* 2014;14:e21.
22. Westerman ME, Tausch TJ, Zhao LC, Siegel JA, Starke N, et al. Ventral slit scrotal flap: a new outpatient surgical option for reconstruction of adult buried penis syndrome. *Urology* 2015;85:1501-4.
23. Zucchi A, Perovic S, Lazzeri M, Mearini L, Costantini E, et al. Iatrogenic trapped penis in adults: new, simple 2-stage repair. *J Urol* 2010;183:1060-3.
24. Angspatt A, Pungrasmi P, Jindarak S, Tunsatit T. Bilateral scrotal flap: pedicle and dimension of flap in cadaveric dissections. *J Med Assoc Thai* 2009;92:1313-7.
25. Makiyan Z. Systematization of ambiguous genitalia. *Organogenesis* 2016;12:169-82.
26. Kulkarni AA, Bhatia SH, Abhyankar SV, Kulkarni MD, Singh RR. Posterior scrotal artery flap to cover a groin defect: a new technique. *Indian J Surg* 2013;75:52-3.
27. Karim RB, Hage JJ, Ahmed AK, Westerga J. Pedicled scrotal island skin flap in the treatment of anal basal cell carcinoma. *Br J Plast Surg* 2001;54:173-6.
28. Fakin R, Zimmermann S, Jindarak S, Lindenblatt N, Giovanoli P, et al. Reconstruction of penile shaft defects following silicone injection by bipedicled anterior scrotal flap. *J Urol* 2017;197:1166-70.
29. Rosen B, Lundborg G. Sensory re-education after nerve repair: aspects of timing. *Handchir Mikrochir Plast Chir* 2004;36:8-12.

Systematic Review

Open Access



# Effects of various surgical protocols on maxillofacial growth in patients with unilateral cleft lip and palate: a systematic review

Pasquier Corthouts<sup>1,\*</sup>, Fien Boels<sup>1,\*</sup>, Elke Van de Castele<sup>1,2,3</sup>, Nasser Nadjmi<sup>1,2,3,4</sup>

<sup>1</sup>Faculty of Medicine & Health Sciences, University of Antwerp, Campus Drie Eiken, Antwerp 2610, Belgium.

<sup>2</sup>Department of Cranio-Maxillofacial Surgery, Antwerp University Hospital, Edegem 2650, Belgium.

<sup>3</sup>All for Research VZW, Antwerp 2018, Belgium.

<sup>4</sup>Department of Cranio-Maxillofacial Surgery, ZMACK, AZ MONICA Antwerp, Antwerp 2018, Belgium.

\*Co first authors.

**Correspondence to:** Prof. Nasser Nadjmi, Department of Cranio-Maxillofacial Surgery, University of Antwerp, AZ MONICA Antwerp, Harmoniestraat 68, Antwerp 2018, Belgium. E-mail: nasser@nadjmi.com

**How to cite this article:** Corthouts P, Boels F, Van de Castele E, Nadjmi N. Effects of various surgical protocols on maxillofacial growth in patients with unilateral cleft lip and palate: a systematic review. *Plast Aesthet Res* 2020;7:46. <http://dx.doi.org/10.20517/2347-9264.2020.97>

**Received:** 28 Apr 2020 **First Decision:** 6 May 2020 **Revised:** 8 Jun 2020 **Accepted:** 24 Jun 2020 **Published:** 21 Aug 2020

**Academic Editor:** Carroll Ann Trotman **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

**Aim:** The purpose of this study was to ascertain the effect of surgical procedures and their timing on maxillofacial growth in unilateral cleft lip and palate (UCLP) patients through a systematic literature review.

**Methods:** In December 2019, a search was conducted in PubMed and Web of Science on the basis of the keywords: "UCLP", "maxillofacial growth" and "facial growth", complemented by a hand search.

**Results:** Eleven articles were included. An important finding was the wide range of treatment protocols. Eight studies performed a multistage procedure, whereas three studies applied a simultaneous repair of cleft lip, palate, and alveolus in a single surgical session. The findings in these articles were based on cephalometric measures. Comparative tables were constructed regarding method of study and time and technique of closure.

**Conclusion:** The results of the articles were conflicting, and it was clear that more research on this subject is necessary. Overall, most studies agreed on the important factor of palatoplasty in maxillofacial growth. The most common finding was a retrusive maxillary growth in comparison to a noncleft control group. This was illustrated by a negative effect on A-point - nasion - B-point. A lot of discussion remains on the effect of lip closure. However,



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



most studies seemed to agree that lip closure results in retro-inclined upper incisors. In conclusion, it is essential that an agreement be reached on the treatment for UCLP, since this is the most common congenital craniofacial condition.

**Keywords:** Unilateral cleft lip and palate, facial growth, maxillofacial growth

## INTRODUCTION

Cleft lip and/or palate is one of the most common congenital malformations, it occurs in about 1 in 700 children<sup>[1,2]</sup>. This malformation is due to failure in merging the facial processes at the correct time, which normally happens between week 7 and week 12 of gestation<sup>[1,3]</sup>. The etiology of unilateral cleft lip and palate (UCLP) is still not completely clear, it is definitely due to multiple factors and it is assumed to be caused by a combination of genetic and environmental aspects. Treatment of UCLP requires a multidisciplinary approach and a longitudinal follow-up. The team may consist of a maxillofacial surgeon, plastic surgeon, pediatrician, otolaryngologist, geneticist, orthodontist, dentist, psychologist, speech-language pathologist and audiologist. Because of the modern abilities of prenatal screening, UCLP can now already be detected early in gestation.

In patients with repaired UCLP, maxillofacial growth is often disturbed due to iatrogenic scar tissue caused by surgical closure of a cleft<sup>[4]</sup>. A retrusive midfacial region is characteristic of this population and becomes more obvious with age. Sagittal deficiency of the midface resulting in a concave facial profile is the most prominent feature seen in adult UCLP patients with disturbed maxillofacial growth<sup>[3]</sup>. However, in unoperated UCLP patients, midfacial growth is comparable to that in healthy, noncleft children without apparent restriction of growth<sup>[2]</sup>. Therefore an important objective is to restrict the iatrogenic impact of cleft surgery on midfacial growth<sup>[4]</sup>. According to the Eurocleft study, there are 194 different protocols for the treatment of UCLP<sup>[5]</sup>. The most controversial issues in the management of cleft palate are the timing of surgical intervention, speech development after various surgical procedures and the effects of surgery on facial growth<sup>[6]</sup>. UCLP and its treatment can affect the aesthetics, speech, and way of eating and chewing of a patient. A balance has to be found between these aspects to improve the child's quality of life.

The purpose of this review was to summarize the knowledge on the effect of different surgical protocols and surgical timing on maxillofacial growth. At this moment, there is no clear overview of all independent studies. The aim was to determine which timing and surgical approach is associated with the best results in this field.

## METHODS

### Eligibility criteria

This systematic review focused on UCLP patients and their treatment, more precisely the timing of surgical protocols and their effect on maxillofacial growth. For selection of the articles, the following inclusion criteria were applied: non-syndromic UCLP patient population; study population had to be over 6 years old; timing of each surgical procedure had to be known; no orthodontic procedures or orthognathic surgery was performed in the study population after surgical repair and before assessment; a control or comparison group had to be present, the outcome of the article had to be on maxillofacial growth; and evaluation of growth had to be at least based on a cephalogram. From the literature, it was clear that a great variety of landmarks were used in the different studies. The outcome parameters for this review were based on the maxillary position given by the sella-nasion-A-point angle (SNA), the mandibular position given by the sella-nasion-B-point angle (SNB) and the intermaxillary relationship [A-point-nasion-B-point angle (ANB)]. Articles had to provide measurements for at least SNA to be included in this review. Articles were

excluded in which other forms of cleft lip and/or palate (cleft lip without cleft palate, cleft palate without cleft lip, bilateral cleft lip and palate) were analyzed. Furthermore, the following studies were excluded: case reports, literature reviews, systematic reviews and meta-analysis. Studies included in this review had to be written in English or Dutch and published after 2005. The goal of this systematic review was to compare various surgical protocols and to conclude which surgical protocol provides the most benefits regarding maxillofacial growth, functionality and aesthetics in UCLP patients of 6 years and older. Factors that would be assessed included: age of assessment, use of presurgical orthopedics, surgical timing, surgical approach (one-stage surgery, one-stage palatoplasty and two-stage palatoplasty), and ethnicity.

### Information sources

A search of PUBMED and Web of Science was conducted in December 2019. The keywords used were “UCLP” and “Maxillofacial growth” or “Facial growth”. As a search limit, the publication date was set to 2005 or later. The results obtained were stored in a single database (EndNote X9; Thomson Reuters, Philadelphia, USA). Additionally, a hand search of references of included articles in this systematic review was performed.

### Study selection

After removal of duplicates, all articles were screened on title and abstract. This was performed independently by two authors to augment reliability. Any disparity in selected articles was discussed until a consensus was reached. Full-text articles were analyzed regarding our aforementioned inclusion and exclusion criteria. The articles used in the systematic reviews and literature review found through PubMed and Google Scholar were hand searched. The same protocol and eligibility criteria were applied as described above.

### Quality assessment and level of evidence

The included articles were reviewed for good quality based on a checklist adopted from Liao *et al.*<sup>[7]</sup>. This checklist was adjusted for our review on the basis of the theory of Greenhalgh<sup>[8]</sup> and can be found in the left column of Table 1<sup>[6,9-18]</sup>. Studies were considered to be of adequate size if their study population exceeded 100 people; this number was based on the quality assessment applied in the systematic review of Liao *et al.*<sup>[7]</sup>. Using the Oxford Centre for Evidence-Based Medicine 2011 v2.1 the qualified articles were assessed for their level of evidence by two independent reviewers. The levels could be downgraded on the basis of study quality, imprecision, indirectness, or inconsistency between studies, or because the effect size was very small. Disagreements were discussed until the two reviewers gave their consent.

### Data extraction

From the final selection of articles, the following information was retrieved: author(s), publication year, study design, population studied, identification of the study groups, number of patients per group, mean age at time of assessment of facial growth, presurgical orthopedics (yes/no), lip closure technique and timing, soft palate closure technique and timing, hard palate closure technique and timing, alveolar cleft closure technique and timing, and final conclusions. The data were extracted from each article by one author and then checked by the second author before being collected in a database. Disagreements were resolved by discussion of each article to reach a consensus.

## RESULTS

### Study selection

The process of data collection and selection is shown in Figure 1. A total of 314 records were found among the databases investigated and 79 additional records, published after 2005, were identified through hand search of the articles included in this systematic review or provided by specialists on the subjects. After removal of duplicates ( $n = 103$ ), 290 articles remained that had their titles and abstracts assessed in

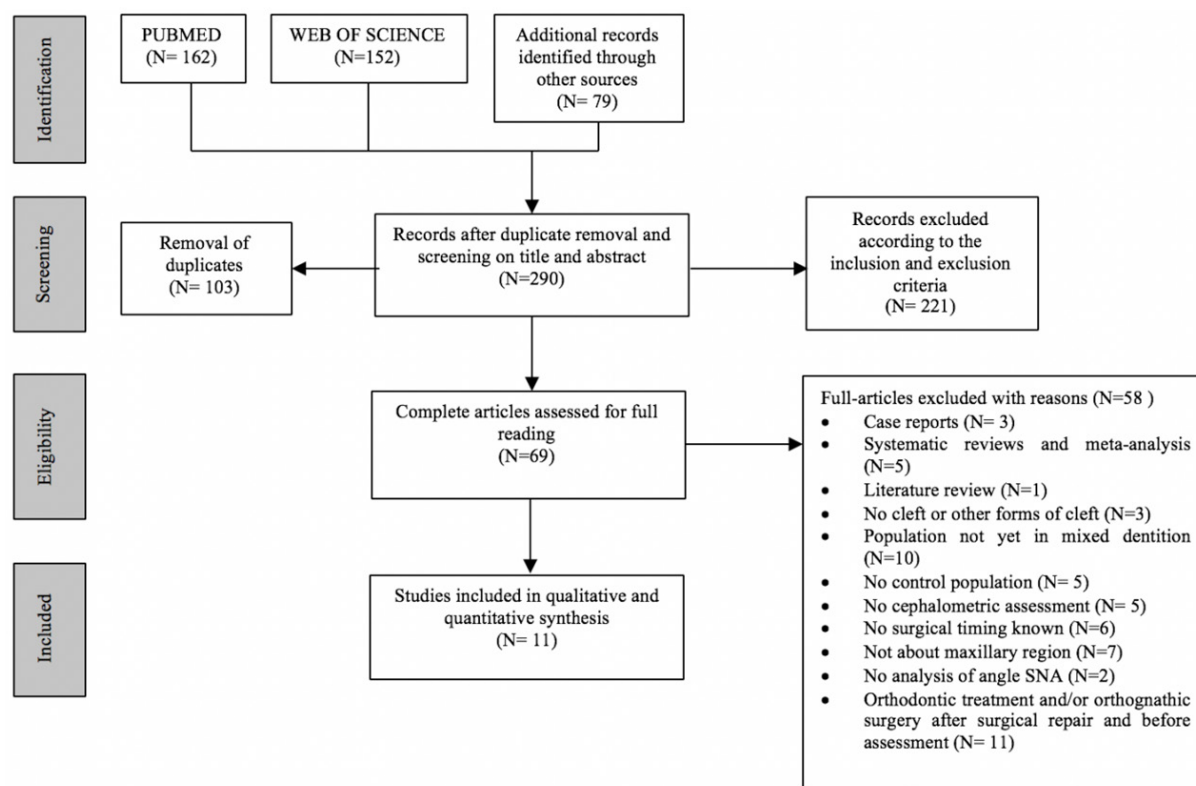




[illegible]

Y: Y; yes; N: no; ?: not specified; D: database; C: consecutively; (a): clinical photographs; (b): alveolar ossification and need for orthognathic surgery; (c): nasalance of speech; (d): one at each center; (e): one surgeon for each protocol performed in the study; (f): two surgeons for the entire population; (g): different surgical approach within a group; (h): some patients received a secondary alveolar bone graft; (i): surgical techniques are not mentioned so there could be an important difference in between patients; (j): healthy control group was selected on the basis of ANB angle; (k): big difference in timing of secondary alveolar bone grafting; (l): conclusion was drawn for the entire unilateral cleft lip and palate population and not just for the population studied in this article

accordance with the inclusion and exclusion criteria. From this initial reading, 221 records were eliminated. After reading the full texts of the remaining 69 records, 58 articles were excluded for the following reasons: case reports ( $n = 3$ ); systematic review and meta-analysis ( $n = 5$ ); literature review ( $n = 1$ ); other forms of cleft (cleft lip without cleft palate, cleft palate without cleft lip, bilateral cleft lip and palate) were studied ( $n = 3$ ); patients were younger than 6 years old and therefore not yet in mixed dentition ( $n = 10$ ); there was no control population ( $n = 5$ ); no cephalometric analysis was performed ( $n = 5$ ); surgical timing of lip and palate repair were not known ( $n = 6$ ); the study did not examine the skeletal maxillary growth pattern ( $n = 7$ ); angle SNA was not measured ( $n = 2$ ); or the patient had undergone orthodontic treatment and/or orthognathic surgery after surgical repair and before assessment ( $n = 11$ ). Therefore, 11 eligible articles were found for this review.



**Figure 1.** Flow chart showing the number of records identified and removed at each stage of the review. SNA: sella-nasion-A-point angle

## Study characteristics

Most studies included in this review (73%) were published after 2010<sup>[6,12-18]</sup> [Table 2]. Six studies were conducted in Asia (55%) and 5 were conducted in Europe (45%). The variety of treatment protocols that were administered in the different studies in the articles, as well as whether or not presurgical orthopedics was performed, is shown in Table 2. Sample sizes consisted of 10 subjects<sup>[12]</sup> to 128 subjects<sup>[18]</sup>. Seven articles included a healthy, noncleft control group in their study<sup>[10,11,13-17]</sup>. The authors used a variety of surgical techniques. Regarding cheiloplasty, the following techniques were used: (modified) Millard rotation-advancement technique<sup>[9-12,15,16]</sup>, triangular technique (by Tennison with modifications)<sup>[9,11,13,18]</sup> and modified Delaire technique<sup>[12]</sup>. Three articles did not specify the technique used for lip repair<sup>[6,14,17]</sup>. Concerning closure of the palate several techniques are listed, which can be categorized as follows: (modified) von Langenbeck<sup>[9,12,13]</sup>, pushback palatoplasty<sup>[10]</sup>, cranial or caudally pedicled vomer flap<sup>[9,12-14,18]</sup>, intravelar veloplasty<sup>[11]</sup>, modified Pigott technique<sup>[12]</sup> and two-flap palatoplasty<sup>[14-16]</sup>. Khanna *et al.*<sup>[6]</sup> provided no description of the technique used for palate repair.

Three different surgical techniques were described for alveolar cleft closure: gingivoperiosteoplasty<sup>[11,12]</sup>, primary bone grafting<sup>[14]</sup> and secondary bone grafting<sup>[11,12,16,18]</sup>. Three studies declared that the patients in the samples had not undergone bone grafting surgery<sup>[9,10,17]</sup>, whereas three studies had no information on whether or not bone grafting surgery had been done<sup>[6,13,15]</sup>.

The mean age at the time of surgical repair in patients with repaired UCLP varied according to the surgical protocol: lip repair before 15 weeks<sup>[6]</sup> until 2 years<sup>[15]</sup>; soft palate closure from 4 months<sup>[12]</sup> to 5 years<sup>[9]</sup>; hard palate closure from 3 months<sup>[12]</sup> to 4 years<sup>[9]</sup> and alveolar cleft repair from 6 months<sup>[14]</sup> to 11 years<sup>[12,16]</sup>.

In 3 studies, the surgical protocol consisted of the primary one-stage surgery of UCLP: simultaneous repair

Table 2. Summary of study

Characteristics				Assessment		Surgical repair UCLP					Conclusion
Author; year; study design	Population studied	Sample	Identification of groups	n	Mean age (year)	PSO	Lip (technique; timing)	Soft palate (technique; timing)	Hard palate (technique; timing)	Alveolar cleft (technique; timing)	
Liao <i>et al.</i> <sup>[9]</sup> , 2005; CS	Sri Lanka	G1: OCLP	58	19		No	Millard or Tennison; 1 y	von Langenbeck; 5 y <sup>a</sup>	Vomerine mucoperiosteal flap or von Langenbeck; 4 y <sup>a</sup>	No bone grafting	Palate repair inhibits the forward displacement of the basal maxilla and anteroposterior development of the maxillary dentoalveolus but has no detrimental effects on the downward displacement of the basal maxilla
	China	G2: OCL	48	23	No	Millard or Tennison; 7 y				No bone grafting	Lip repair is a most important factor in the restraint of maxillary growth in patients with complete UCLP
		G1: OCLP	47	M: 14.2 F: 15.3	No	Modified rotation-advancement; 9 mo		Mucoperiosteal pushback; 38 mo	No bone grafting		
		G2: OCL	35	M: 14.1 F: 13.3	No	Modified rotation-advancement; 9 mo			No bone grafting		
Zemann <i>et al.</i> <sup>[11]</sup> , 2007; CS	Austria and Slovenia	G3: NN	37	M: 13.8 F: 13.9							There were considerable similar sagittal growth of the facial skeleton in both centers which has not been affected by the different surgical protocols so far. A final evaluation should be delayed until the growth of the facial skeleton is complete
		G1: OCLP	20	6.3	Yes	Modified rotation-advancement; 3 mo		Veau; 1 y	Bone graft; 9 y		
		G2: OCLP	20	6.7	Yes	Tennison-Randall; 6 mo	Intravascular veloplasty; 12 mo	Closure with mucoperiosteal closure; 30 mo	Mucoperiosteoplasty; 30 mo		ESGAP seems to have an inhibiting influence on maxillary growth which increases the need for Le Fort 1 osteotomies
		G3: NN	20	7							
		G1: OCLP	15	18.2	Yes	Modified Delaire; 6-9 mo	Modified Pigott; 6-9 mo	Closure with gingivoalveoplasty; 18-24 mo	Gingivoalveoloplasty; 18-24 mo		
Meazzini <i>et al.</i> <sup>[12]</sup> , 2010; C	Italy and Norway	G2: OCLP	10	18.7	Yes	Modified Delaire; 4-6 mo	Modified Delaire; 4-6 mo	Modified Pigott; 4-6 mo	Anterior palate closure by vomer flap; 3 mo	Bone graft; before canine eruption	Significant craniofacial morphological differences were identified between groups 1,2 and 3. This indicates that the technique of hard palate closure has significant influence on craniofacial growth and development
		G3: OCLP	15	18.1	No	Millard; 3 mo	Modified von Langenbeck; 18 mo				
	Kulewicz <i>et al.</i> <sup>[13]</sup> , 2010; C	Poland	G1: OCLP	22	10	Yes	Triangular flap; 7.4 mo	Closure with three layers; 7.4 mo	Bilateral von Langenbeck; 7.4 mo	NR	
			G2: OCLP	22	10	Yes	Triangular flap; 7.2 mo	Closure with three layers; 7.2 mo	Unilateral von Langenbeck; 7.2 mo	NR	
G3: OCLP			22	10	Yes	Triangular flap; 7.3 mo	Closure with three layers; 7.3 mo	Single-layered caudal-pedicled vomer flap; 7.3 mo	NR		
		G4: NN	22	10							

Mueller <i>et al.</i> <sup>[14]</sup> , 2012; C	Switzerland	G1: OCLP	22	MD: 9 PD: 13	NR	6 mo	6 mo	Cranial pedicled vomer flap, two-flap palatoplasty; 6 mo	Bone graft; 6 mo	One-stage procedures led to significant disturbance in growth, but the degree of this was similar to mean values of multistage procedures in the Eurocleft study. Primary alveolar bone grafting led to inconsistent alveolar ossification and was suspected to interfere with anterior maxillary growth
Khanna <i>et al.</i> <sup>[6]</sup> , 2012; CS	India	G1: OCLP G2: NOCLP	25 47	17.5 17.7	NR	< 15 wk		< 14 mo	NR	Surgical intervention has a restraining effect on growth in the facial region due to the scar tissue in lip and palate region
Chen <i>et al.</i> <sup>[15]</sup> , 2012; CS	China	G1: OCLP	33	MD: 8.5 PD: 20.4	NR	Millard; < 2 y		2-flap; < 3 y	NR	It appears that there may be the potential normal maxillary growth in UCLP patients, and early surgical repair of the cleft palate may affect sagittal maxillary growth pattern in patients with cleft
		G2: OCL	30	MD: 8.6 PD: 20.2	NR	Millard; < 2 y				
		G3: NN	30	MD: 8.6 PD: 20.2						
Zheng <i>et al.</i> <sup>[16]</sup> , 2016; CS	China	G1: OCLP	20	9.3	Yes	Modified rotation- advancement; 4-8 mo		2-flap; 12-14 mo	Bone graft; 9-11 y	Differences in the facial morphology can be ascribed to the difference in the primary anomaly in the UCLP groups, but isolated surgery has minor effects on growth disturbances
		G2: NOCLP	20	9.4						
		G3: NN	20	9.3						
		G1: OCLP	37	15.2 <sup>C</sup>	No	< 6 mo		12-18 mo	No bone grafting	Patients with lip operated, whether cleft palate operated or not, tend to have a smaller length of maxilla sagittally and this deformity progresses with age
		G2: OCL	37	14.6 <sup>C</sup>	No	< 6 mo			No bone grafting	
		G3: NN	37	14.7 <sup>C</sup>						
Brudnicki <i>et al.</i> <sup>[18]</sup> , 2019; CS	Poland	G1: OCLP	128	10.1	No	Modified Tension- Randall; 8 mo	8 mo	Vomer flap; 8 mo	Bone graft; 3.5 y	SABG performed before 8 years of age can have limited negative effect on craniofacial morphology
		G2: OCLP	39	9.9	No	Modified Tension- Randall; 10 mo	10 mo	Vomer flap; 10 mo	No bone grafting	

<sup>a</sup>37 of 58 patients received one-stage palate repair with palatal mucoperiosteal flaps, the von Langenbeck procedure; <sup>b</sup>mean value from all centers that completed the Eurocleft study; <sup>c</sup>mean age for both the mixed and permanent dentition. C: cohort; CS: cross-sectional; ESGAP: early secondary gingivovulvoplasty; F: female; G: group; LOE: level of evidence; M: male; mo: months; MD: mixed dentition; N: number sample size; NN: noncleft normal control group; NOCLP: surgically untreated unilateral cleft lip and palate; NR: not reported; OCL: unilateral cleft lip and palate with operated cleft lip only; OCLP: unilateral cleft lip and palate with operated cleft lip and palate; PD: permanent dentition; PSO: presurgical orthopedic treatment; SABG: secondary alveolar bone graft; UCLP: unilateral complete cleft lip, alveolus and palate; wk: weeks; y: years

of cleft lip, palate, and alveolus in a single surgical session<sup>[13,14,18]</sup>. Five studies applied one-stage palatoplasty<sup>[6,10,15-17]</sup> and 2 studies applied both one-stage and two-stage palatoplasty<sup>[9,11]</sup>. Meazzini *et al.*<sup>[12]</sup> compared early anterior palate closure by vomer flap during lip repair at 3 months and a two-stage palatoplasty.

The mean age at assessment of maxillary growth through cephalometric analysis varied from 6 to 20 years. The cephalometric values described for each study are summarized in Table 3 and Table 4, classified according to the study population. Cephalometric values of UCLP patients with operated cleft lip and

**Table 3. Comparison of cephalometric values of each study with UCLP patients in mixed dentition (6-12 y), classified according to study population. Specification of groups can be found in Table 2**

	First author	Identification of groups	n	SNA (°)		SNB (°)		ANB (°)	
				Mean	SD	Mean	SD	Mean	SD
Asian	Chen <i>et al.</i> <sup>[15]</sup> , 2012	G1: OCLP	18	74.7	3.7	76.8	4.8	-2	4.5
		G2: OCL	15	77.5	3.6	76.1	4.1	1.5	4.1
		G3: NN	15	77.7	3.7	75.2	3.1	2.5	1.5
		P value*		NS		NS		S <sup>ab</sup>	
	Zheng <i>et al.</i> <sup>[16]</sup> , 2016	G1: OCLP	20	79	NR	77.4	NR	1.6	NR
		G2: NOCLP	20	78.4	NR	77	NR	1.4	NR
		G3: NN	20	80.4	NR	76	NR	4.4	NR
		P value*		NS		NS		S <sup>bc</sup>	
	Liu <i>et al.</i> <sup>[17]</sup> , 2018	G1: OCLP	37	75.1	3.9	NR	NR	NR	NR
		G2: OCL	37	79.3	3.3	NR	NR	NR	NR
		G3: NN	37	80.2	3.9	NR	NR	NR	NR
		P value*		S <sup>ab</sup>		NR		NR	
European	Kulewicz <i>et al.</i> <sup>[13]</sup> , 2010	G1: OCLP	22	76.5	3.6	75.0	3.8	1.6	3.5
		G2: OCLP	22	78.2	3.7	75.1	3.5	3.2	4.2
		G3: OCLP	22	79.4	4.1	75.8	4	3.4	2
		G4: NN	22	79.8	3.7	76.5	3.6	3.3	2.2
		P value*		S <sup>bd</sup>		NR		S <sup>bd</sup>	
	Zemann <i>et al.</i> <sup>[11]</sup> , 2007	G1: OCLP	20	80.1	2.8	75.4	2.7	4.7	1
		G2: OCLP	20	80.5	2.3	75.1	1.9	5.4	1.8
		G3: NN	20	80.5	3.4	77.0	3.1	3.4	2
		P value*		NR		NR		NR	
	Mueller <i>et al.</i> <sup>[14]</sup> , 2012	G1: OCLP	15	76	4	NR	NR	3	3
		G2: Eurocleft†	25	77	4	NR	NR	3	3
		G3: NN	62	81	3	NR	NR	5	2
		P value*		S <sup>bc</sup>		NR		S	
	Brudnicki <i>et al.</i> <sup>[18]</sup> , 2019	G1: OCLP	128	75.7	4.8	75.6	4.1	0.2	3.9
		G2: OCLP	39	78.2	5.1	76.5	5.1	1.7	3.9
		P value*		S <sup>a</sup>		NS		S <sup>a</sup>	

†Mean value from all centers that completed the Eurocleft study; <sup>a</sup>G1-G2: *P*-value < 0.05; <sup>b</sup>G1-G3: *P*-value < 0.05; <sup>c</sup>G2-G3: *P*-value < 0.05; <sup>d</sup>G1-G4: *P*-value < 0.05. \**P*-value < 0.05 was regarded as significant. Comparisons between groups are mentioned only when undertaken in the study and regarded as significant. G: group; F: female; M: male; n: number of sample size; NN: noncleft normal control group; NOCLP: surgically untreated unilateral cleft lip and palate; NR: not reported; NS: not significant; OCL: unilateral cleft lip and palate with operated cleft lip only; OCLP: unilateral cleft lip and palate with operated cleft lip and palate; UCLP: unilateral cleft lip and palate; S: significant; SD: standard deviation; y: years

palate (OCLP) in each study were compared with the following groups: UCLP patients treated according to a different protocol<sup>[11-13,18]</sup>; UCLP patients with operated cleft lip and unoperated cleft palate<sup>[9,10,15,17]</sup>; and non-treated UCLP patients<sup>[6,16]</sup>, mean value from all centers that completed the Eurocleft study<sup>[14]</sup>. In seven studies<sup>[10,11,13-17]</sup> noncleft children served as normal controls. Among the abovementioned groups, 10 of the 11 included articles<sup>[6,9-11,13-18]</sup> reported a *P*-value less than 0.05 for one or more of the cephalometric values SNA, SNB and ANB, whereas one study<sup>[12]</sup> did not report a corresponding *P*-value. A *P*-value less than 0.05 was regarded as significant.

### Quality assessment and level of evidence

The methodological quality of the 11 articles was evaluated using the aforementioned checklist, which can be seen in Table 1. None of the included articles were of perfect methodological quality, they showed different deficiencies, but overall, they were deemed of good quality. Only 4 studies<sup>[9,10,17,18]</sup> were deemed large enough, this showing that there is a need for more research with a substantial study population. All included studies were retrospective (level 3 evidence).

### Surgical repair and maxillofacial growth

Six out of 11 included articles evaluated the effect that surgery itself has on maxillofacial growth in children with UCLP<sup>[6,9,10,15-17]</sup>. Khanna *et al.*<sup>[6]</sup> compared a group of treated UCLP patients with a group of non-



**Table 4. Comparison of cephalometric values of each study with UCLP patients in permanent dentition (12-23 y), classified according to study population. Specification of groups can be found in Table 2**

Authors		Identification of groups	n	SNA (°)		SNB (°)		ANB (°)	
				Mean	SD	Mean	SD	Mean	SD
Asian	Liao <i>et al.</i> <sup>[9]</sup> , 2005	G1: OCLP	58	79.6	NR	78.3	NR	1.4	NR
		G2: OCL	48	83.0	NR	78.6	NR	4.4	NR
		P value*		S <sup>a</sup>		NS		S <sup>a</sup>	
	Li <i>et al.</i> <sup>[10]</sup> , 2006	G1: OCLP (M/F)	47	73.5/73.2	4.7/6.8	74.8/75.8	4.7/4.9	-1.3/-2.6	2.8/3.9
		G2: OCL (M/F)	35	72.6/75.1	5.3/3.4	73.3/75.2	4.3/6.0	-0.6/-0.0	4.0/4.2
		G3: NN (M/F)	37	82.1/80.3	2.6/3.2	78.5/77.4	2.5/3.0	3.6/2.9	2.2/1.3
		P value (M/F)*		S <sup>bc</sup> /S <sup>bc</sup>		S <sup>bc</sup> /NS		S <sup>bc</sup> /S <sup>abc</sup>	
	Khanna <i>et al.</i> <sup>[6]</sup> , 2012	G1: OCLP	25	73.2	13.9	NR	NR	NR	NR
		G2: NOCLP	47	83.6	4.3	NR	NR	NR	NR
		P value*		S <sup>a</sup>		NR		NR	
	Chen <i>et al.</i> <sup>[15]</sup> , 2012	G1: OCLP	15	75.5	6.6	79.7	6.4	-4.2	5.1
		G2: OCL	15	79.3	4.9	79	3.3	0.3	4.4
		G3: NN	15	80.6	3.0	77.2	2.9	3.4	1.9
		P value*		S <sup>b</sup>		NS		S <sup>abc</sup>	
	Liu <i>et al.</i> <sup>[17]</sup> , 2018	G1: OCLP	37	75.8	5.1	NR	NR	NR	NR
		G2: OCL	37	77.3	4.8	NR	NR	NR	NR
		G3: NN	37	81.7	2.9	NR	NR	NR	NR
		P value*		S <sup>bc</sup>		NR		NR	
European	Meazzini <i>et al.</i> <sup>[12]</sup> , 2010	G1: OCLP	15	74.9	3.5	76.9	3.0	-1.9	2.7
		G2: OCLP	10	76.7	3.3	77.4	2.6	-0.8	3.3
		G3: OCLP	15	75.8	3.5	77.1	4.3	-1.3	1.9
		P value*		NR		NR		NR	
	Mueller <i>et al.</i> <sup>[14]</sup> , 2012	G1: OCLP	7	76	4	NR	NR	-0.2	3
		G2: Eurocleft†	25	75	4	NR	NR	0.9	3
		G3: NN	71	81	4	NR	NR	4	2
		P value*		S <sup>bc</sup>		NR		S <sup>bc</sup>	

†Mean value from all centers that completed the Eurocleft study; <sup>a</sup>G1-G2: *P*-value < 0.05; <sup>b</sup>G1-G3: *P*-value < 0.05; <sup>c</sup>G2-G3: *P*-value < 0.05. \**P*-value < 0.05 was regarded as significant. Comparisons between groups are mentioned only when undertaken in the study and regarded as significant. G: group; F: female; M: male; n: number of sample size; NN: noncleft normal control group; NOCLP: surgically untreated unilateral cleft lip and palate; NR: not reported; NS: not significant; OCL: unilateral cleft lip and palate with operated cleft lip only; OCLP: unilateral cleft lip and palate with operated cleft lip and palate; UCLP: unilateral cleft lip and palate; S: significant; SD: standard deviation; y: years

treated UCLP patients between the age of 12 and 20 years old. They found different values by comparing the cephalometric measurements of the two groups, and they concluded that surgical intervention does interfere with growth in the facial region due to scar tissue in the lip and palate.

Four articles identified the effects of palate repair on maxillary morphology<sup>[9,10,15,17]</sup>. These studies recruited patients with non-syndromic UCLP who had lip repair only (OCL) and patients with non-syndromic UCLP who had lip and palate repairs (OCLP). Palate repair at an early stage in patients with UCLP seems to result, in the long run, in a larger retrusion of the maxilla (SNA) and smaller anteroposterior jaw relation (ANB) than in the OCL group, who demonstrated an almost normal maxillary growth<sup>[9,15,17]</sup>. Opposed to this view, Li *et al.*<sup>[10]</sup> reported a smaller SNA angle in both OCL and OCLP groups than the normal control group and concluded that lip repair is primarily responsible for the midfacial hypodevelopment in cleft patients.

However, Zheng *et al.*<sup>[16]</sup> attributes the difference in cephalometric results to the intrinsic effect of UCLP on the maxilla resulting in a developmental deficiency and claims that surgery has minor effects on growth disturbances. They discovered that the tendency in patients with UCLP (with or without surgical repair) toward a less protruded alveolar maxilla (SNA) and a more protruded alveolar mandible (SNB) gave rise to the low anteroposterior jaw relation at the alveolar level (ANB).

### Surgical technique and maxillofacial growth

Five of the 11 included articles evaluated the effect of different surgical techniques and protocols on maxillofacial growth in children with UCLP<sup>[11-14,18]</sup>. Three of the 11 studies looked into the implementation of a one-stage surgery and compared their results with a healthy control population<sup>[11,13,14]</sup>. Considering the age at the time of assessment in these 3 studies, the results concerning sagittal growth were very diversified when comparing the outcomes. The study of Zemmann *et al.*<sup>[11]</sup> showed no significant difference regarding the angles SNA, SNB and ANB at the age of 6 years old when comparing patients treated according to various one-stage protocols. Furthermore, the acquired values were equivalent to those in a healthy control group. However, Mueller *et al.*<sup>[14]</sup> concluded that maxillary protrusion (SNA) and anteroposterior jaw relation (ANB) in the one-stage groups differed significantly from those of the noncleft, healthy control group, but the degree of disturbance in growth was similar to mean values of multistage approaches in the Eurocleft study. Kulewicz *et al.*<sup>[13]</sup> conducted comparative research into 3 different techniques of palate repair applied to a one-stage surgical approach and checked this against healthy controls. Cephalometric parameter comparison analysis demonstrated significant differences between the 4 groups regarding maxillary prominence (SNA) and maxillo-mandibular relationship (ANB). This indicates that the technique of hard palate closure has a substantial influence on maxillofacial growth and development.

Meazzini *et al.*<sup>[12]</sup> did a comparison between UCLP patients treated with 3 different protocols to evaluate the long-term results between closure of the hard palate at 18-36 months together with early secondary gingivopalatoplasty (ESGAP) and alveolar cleft repair at 9-11 years of age. Using a longitudinal cephalometric evaluation, they found that patients who underwent ESGAP had a decreased maxillary prominence (SNA) and showed an inhibition of maxillary growth compared with the 2 secondary bone graft groups, while mandibular prominence (SNB) increased in the 3 groups. Nonetheless, performing alveolar bone grafting before 8 years of age is suspected to interfere with anterior maxillary growth, and the timing of bone grafting can be essential to maxillofacial growth. Studies suggested that performing the surgery at a later age would prove the most beneficial<sup>[14,18]</sup>.

### DISCUSSION

Maxillary growth in UCLP patients has already been widely addressed in the literature; nevertheless, a wide variation in results was found. More often than not, no consensus was reached relating to the vertical and anteroposterior growth pattern in UCLP patients. On the one hand, they propose that there may be a potentially normal maxillary growth in untreated UCLP patients<sup>[15]</sup>, and on the other hand, they propose that regardless of the treatment, UCLP patients show retrusion of the maxilla and decreased maxillary length, where there are many causes to be considered. Some reports attribute this retrusion to the intrinsic defect of the cleft<sup>[16,17]</sup>, while others claim it is from surgical intervention<sup>[6,13-15,19]</sup> and even dependent on the skill of the surgeon. The disturbing effect on the growth of the maxillary skeleton after surgical repair is due to devascularization, disturbance of the periosteum or the restrictive effect of the scar<sup>[16]</sup>. Therefore, surgery leads to maxillary hypoplasia: the maxillary angle (SNA) and the maxillomandibular angle (ANB) were smaller and negative when matched to the normal population<sup>[19]</sup>. Unoperated cleft patients had a more favorable morphology of craniofacial structures when compared with surgically treated patients, indicating that due to alteration of the peri-oral functional matrix, surgical intervention interferes with the growth process in UCLP patients. They point to the scar tissue in lip and palate region being the factor due to its restraining effect on maxillofacial growth. The alterations in these functional matrices are important in determining the growth of facial structures. Moreover, maxilla length was found to be significantly reduced in surgically treated UCLP patients, and they showed a significant reduction in cranial base angle<sup>[6]</sup>.

Without doubt, palate closure is the most documented part of the treatment protocol for UCLP. Many surgical protocols exist, using different techniques and surgical timings and have been evaluated in terms of benefits to maxillary growth, speech development, velopharyngeal function and quality of life. An

important objective is to reduce the number of operations as they are considered to be stressful for the family and to make it more difficult to cope successfully. Likewise, the number of surgeries has an impact on the psychological well-being of the patient. There does not seem to be any consensus on the best time to perform palate closure, where every timing has its own advantages and disadvantages<sup>[7,20]</sup>. Some studies<sup>[15,19,21,22]</sup> assumed that the early surgical repair of the cleft palate is responsible for the impaired maxillary growth and concluded that it was better to delay surgical palate repair. During the maxillary growth spurt an important proportion of the final length of the maxilla is gained. It is possible that the benefit of delayed hard palate on maxillofacial growth closure can only be achieved by closing when the greatest proportion of the final maxillary length is already achieved<sup>[19]</sup>. However, Zheng *et al.*<sup>[16]</sup> claim that isolated surgery has minor effects on growth disturbances and conclude that early palatal closure should therefore be performed because it will not negatively affect maxillofacial growth. Furthermore, early primary repair operations facilitate ease of feeding and good speech development, and there is a strong desire from the patient's parents themselves to have the cleft closed as early as possible<sup>[7,22,23]</sup>. Nevertheless, the growth spurt of these children should be awaited before conclusive results are formed concerning the measured cephalometric values regarding sagittal growth of the skeleton, since the results in patients in mixed dentition show a lot of variability. Regarding this concept, researchers should be aware of the fact that the end of growth in cleft children is later than in healthy noncleft children<sup>[24]</sup>.

Whereas most studies agree that palatal closure is the most detrimental factor for the evolution of maxillary growth, other studies are convinced that lip repair is the most important factor in the restraint of maxillary growth in patients with UCLP<sup>[10,25]</sup>. There is however agreement that pressure from a tense upper lip causes retro inclined upper incisors, a retruded maxilla and obtuse nasolabial angle<sup>[26]</sup>. This usually results in an anterior cross bite<sup>[2]</sup>. It is crucial to stretch the importance of an optimal result of lip closure. Lip, nose and chin are the key regions in a patient's face and they have the most significant impact on facial aesthetics, self-esteem and self-image. Thus lip, nose and columella<sup>[27]</sup> are most frequently surgically revised in UCLP patients.

There is still a lot of discussion about which technique and timing is most beneficial for alveolar closure. Alveoloplasty is performed to stabilize the maxillary arch, facilitate the eruption of the canine (and the lateral incisor), raise the alar base of the nose and to reconstruct the residual nasoalveolar fistula<sup>[28]</sup>. Overall, 3 used techniques can be distinguished<sup>[2]</sup>: gingivoperiosteoplasty, primary bone grafting and secondary bone grafting. Although gingivoperiosteoplasty has the big advantage that it requires fewer surgeries, it seemed to have an inhibitory effect on maxillary growth<sup>[29]</sup>. Primary bone grafting led to inconsistent alveolar ossification and was suspected to interfere with anterior maxillary growth<sup>[14]</sup>. Patients treated with secondary bone grafting seemed to have better maxillary growth and appeared to be needing less orthognathic surgery<sup>[29]</sup>. Brudnicki *et al.*<sup>[18]</sup> discovered that maxillary length increased when alveolar bone grafting was performed at a later age, specifically when performed beyond the age of eight years old. This would suggest that the timing of bone grafting is critical to maxillofacial growth.

Unanimity with regard to a superior treatment protocol in terms of closure of the lip, closure of the palate and closure of the alveolar cleft, was not reached in this systematic review. The reasons for conflicting results from the selected studies include the great variance in treatment protocols, as shown by the varied timing of surgical repair and different surgical techniques [Table 2]. This systematic review also had some methodological deficiencies [Table 1] and limitations. First, 4 studies<sup>[6,9,12,18]</sup> did not compare operated UCLP patients with a noncleft control group. Consequently, it is not clear how the measured cephalometric outcomes are related to a healthy, normal population. Second, some studies were well designed and well-executed but had small sample sizes. Seven<sup>[6,11-16]</sup> of the 11 included articles had samples less than 100 patients. This could imply that the statistical power of these studies was too low to detect differences. Third, one study<sup>[10]</sup> examined the cephalometric values for males and females separately and this might have

resulted in an analysis bias, whereas another study<sup>[12]</sup> did not provide a corresponding p-value for their cephalometric outcomes. Fourth, the study population used in the different articles included in this review had a lot of ethnic diversity. Therefore, it is unsure if all findings apply to all different ethnical groups. It is important to take this into account when using the results of this systematic review. Fifth, none of the included studies had a level of evidence higher than 3. This means that there was a shortage of high-quality randomized controlled trials on the effects of surgical timing and techniques on maxillofacial growth. To get more high-quality studies, follow-up of patients should be over a longer period of time. Preferably, patients should be followed starting from mixed dentition until after their growth spurt, ending at adult age. Sixth, detailed documentation of the study population, the technique for surgical cleft closure, number of surgeons, grade of surgeon and information whether orthodontic or orthognathic treatment was performed, were insufficiently described or lacking, making the studies unsuitable for meta-analysis. Hence, no attempt was made to perform pooled analysis, and the evidence was summarized qualitatively.

Future treatment research should be established with special attention towards methodology, well described study population, number of surgeons, grade of surgeon, technique of surgical closure and information on the undergoing of orthodontic or orthognathic treatments since early intervention may result in a better outcome. Kappen *et al.*<sup>[4]</sup> proposed that a multidisciplinary and multicenter database of cleft children should be set up. If this would be the case, a prospective study could be conducted on these patients. This might help in the further determination of the best time of closure of both lip and palate. Consequently, on the basis of a study like this, a universal protocol might be possible for the treatment of cleft children to guarantee them the best results. Furthermore, they stressed the importance of calculating the burden on the caregivers as well as the costs of the procedures into the determination of the best protocol.

In conclusion, most studies agree that palatoplasty is the main factor attributing to disturbance of maxillofacial growth; in addition, it is crucial to limit the amount of postoperative scar tissue. In palatoplasty performed after the growth spurt, the maxillofacial growth is least affected. But studies also agree that it is important to find a balance between aesthetics, functionality and quality of life. Therefore, it is not recommended to perform palatoplasty only after the growth spurt despite of the better effect on maxillofacial growth because this impedes speech development too much.

There is however a consensus about the timing of lip closure. It has to be performed between three and six months of age. It is also widely accepted that lip closure could have a negative influence on maxillofacial growth.

From the studies on alveoloplasty, it can be concluded that secondary bone grafting has the most beneficial outcome on maxillofacial growth; however, when using gingivoperiosteoplasty, there is less need of a third surgery.

In the articles studied in this review the functional result of UCLP repair is considered to be more important than the aesthetic result. More studies still need to be conducted to ascertain the best timing of surgery and to design a technique that creates both optimal functional and aesthetic results to guarantee the well-being of the patient.

It is important to properly understand the causal factors that result in an impeded maxillary growth. This will help in enabling proper planning of treatment, minimizing orthodontic treatment time and in reducing major secondary corrective surgeries. All of this combined illustrates that in the treatment of UCLP, a longitudinal follow-up and a multidisciplinary approach are crucial. More studies still need to be conducted to make sure the best outcome can be acquired.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Corthouts P, Boels F, Van de Castele E, Nadjmi N

Performed data acquisition and provided administrative, technical, and material support as well: Corthouts P, Boels F, Van de Castele E, Nadjmi N

Reviewed the manuscript for content and grammar/spelling mistakes: Corthouts P, Boels F, Van de Castele E, Nadjmi N

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Mossey PA, Little J, Munger RG, Dixon MJ, Shaw WC. Cleft lip and palate. *Lancet* 2009;374:1773-85.
2. Farronato G, Kairyte L, Giannini L, Galbiati G, Maspero C. How various surgical protocols of the unilateral cleft lip and palate influence the facial growth and possible orthodontic problems? Which is the best timing of lip, palate and alveolus repair? literature review. *Stomatologija* 2014;16:53-60.
3. Goyenc YB, Gurel HG, Memili B. Craniofacial morphology in children with operated complete unilateral cleft lip and palate. *J Craniofac Surg* 2008;19:1396-401.
4. Kappen IFPM, Yoder WR, Mink van der Molen ABM, Breugem CC. Long-term craniofacial morphology in young adults treated for a non-syndromal UCLP: a systematic review. *J Plast Reconstr Aesthet Surg* 2018;71:504-17.
5. Shaw WC, Semb G, Nelson P, Brattstrom V, Molsted K, et al. The eurocleft project 1996-2000: overview. *J Craniomaxillofac Surg* 2001;29:131-40; discussion 41-2.
6. Khanna R, Tikku T, Wadhwa J. Nasomaxillary complex in size, position and orientation in surgically treated and untreated individuals with cleft lip and palate: a cephalometric overview. *Indian J Plast Surg* 2012;45:68-75.
7. Liao YF, Mars M. Hard palate repair timing and facial growth in cleft lip and palate: a systematic review. *Cleft Palate Craniofac J* 2006;43:563-70.
8. Greenhalgh T. Assessing the methodological quality of published papers. *BMJ* 1997;315:305-8.
9. Liao YF, Mars M. Long-term effects of palate repair on craniofacial morphology in patients with unilateral cleft lip and palate. *Cleft Palate Craniofac J* 2005;42:594-600.
10. Li Y, Shi B, Song QG, Zuo H, Zheng Q. Effects of lip repair on maxillary growth and facial soft tissue development in patients with a complete unilateral cleft of lip, alveolus and palate. *J Craniomaxillofac Surg* 2006;34:355-61.
11. Zemmann W, Mossbock R, Karcher H, Kozelj V. Sagittal growth of the facial skeleton of 6-year-old children with a complete unilateral cleft of lip, alveolus and palate treated with two different protocols. *J Craniomaxillofac Surg* 2007;35:343-9.
12. Meazzini MC, Rossetti G, Garattini G, Semb G, Brusati R. Early secondary gingivo-alveolo-plasty in the treatment of unilateral cleft lip and palate patients: 20 years experience. *J Craniomaxillofac Surg* 2010;38:185-91.
13. Kuliewicz M, Dudkiewicz Z. Craniofacial morphological outcome following treatment with three different surgical protocols for complete unilateral cleft lip and palate: a preliminary study. *Int J Oral Maxillofac Surg* 2010;39:122-8.



14. Mueller AA, Zschokke I, Brand S, Hockenjos C, Zeilhofer HF, et al. One-stage cleft repair outcome at age 6- to 18-years - a comparison to the Eurocleft study data. *Br J Oral Maxillofac Surg* 2012;50:762-8.
15. Chen ZQ, Wu J, Chen RJ. Sagittal maxillary growth pattern in unilateral cleft lip and palate patients with unrepaired cleft palate. *J Craniofac Surg* 2012;23:491-3.
16. Zheng ZW, Fang YM, Lin CX. Isolated influences of surgery repair on maxillofacial growth in complete unilateral cleft lip and palate. *J Oral Maxillofac Surg* 2016;74:1649-57.
17. Liu X, Chen Z. Effects of palate repair on cranial base and maxillary morphology in patients with unilateral complete cleft lip and palate. *Cleft Palate Craniofac J* 2018;55:1367-74.
18. Brudnicki A, Sawicka E, Brudnicka R, Fudalej PS. Effects of different timing of alveolar bone graft on craniofacial morphology in unilateral cleft lip and palate. *Cleft Palate Craniofac J* 2020;57:105-13.
19. Kappen IFPM, Bittermann GKP, Schouten RM, Bittermann D, Etty E, et al. Long-term mid-facial growth of patients with a unilateral complete cleft of lip, alveolus and palate treated by two-stage palatoplasty: cephalometric analysis. *Clin Oral Investig* 2017;21:1801-10.
20. Salgado KR, Wendt AR, Fagundes NCF, Maia LC, Normando D, et al. Early or delayed palatoplasty in complete unilateral cleft lip and palate patients? A systematic review of the effects on maxillary growth. *J Craniomaxillofac Surg* 2019;47:1690-8.
21. Liao YF, Cole TJ, Mars M. Hard palate repair timing and facial growth in unilateral cleft lip and palate: a longitudinal study. *Cleft Palate Craniofac J* 2006;43:547-56.
22. Gopinath VK, Samsudin AR, Noor SNFM, Sharab HYM. Facial profile and maxillary arch dimensions in unilateral cleft lip and palate children in the mixed dentition stage. *Eur J Dent* 2017;11:76-82.
23. Holland S, Gabbay JS, Heller JB, O'Hara C, Hurwitz D, et al. Delayed closure of the hard palate leads to speech problems and deleterious maxillary growth. *Plast Reconstr Surg* 2007;119:1302-10.
24. Batwa W, Almoammar K, Aljohar A, Alhussein A, Almujeel S, et al. The difference in cervical vertebral skeletal maturation between cleft lip/palate and non-cleft lip/palate orthodontic patients. *Biomed Res Int* 2018;2018:5405376.
25. Bichara LM, Araujo RC, Flores-Mir C, Normando D. Impact of primary palatoplasty on the maxillomandibular sagittal relationship in patients with unilateral cleft lip and palate: a systematic review and meta-analysis. *Int J Oral Maxillofac Surg* 2015;44:50-6.
26. Ebin LE, Zam NMZ, Othman SA. Cephalometric analysis of Malay children with and without unilateral cleft lip and palate. *Aust Orthod J* 2010;26:165-70.
27. Moreira I, Suri S, Ross B, Tompson B, Fisher D, et al. Soft-tissue profile growth in patients with repaired complete unilateral cleft lip and palate: a cephalometric comparison with normal controls at ages 7, 11, and 18 years. *Am J Orthod Dentofacial Orthop* 2014;145:341-58.
28. Lilja J. Alveolar bone grafting. *Indian J Plast Surg* 2009;42:S110-5.
29. Meazzini MC, Capasso E, Morabito A, Garattini G, Brusati R. Comparison of growth results in patients with unilateral cleft lip and palate after early secondary gingivopalveoplasty and secondary bone grafting: 20 years follow up. *Scand J Plast Reconstr Surg Hand Surg* 2008;42:290-5.

Review

Open Access



# Conventional surgical techniques and emerging transplantation in complex penile reconstruction

Nima Khavanin, Richard J. Redett

Department of Plastic and Reconstructive Surgery, Johns Hopkins University School of Medicine, Baltimore, MD 21287, USA.

**Correspondence to:** Dr. Richard J. Redett, Department of Plastic and Reconstructive Surgery, Johns Hopkins University School of Medicine, 601 N Caroline St, Baltimore, MD 21287, USA. E-mail: rjr@jhmi.edu

**How to cite this article:** Khavanin N, Redett RJ. Conventional surgical techniques and emerging transplantation in complex penile reconstruction. *Plast Aesthet Res* 2020;7:47. <http://dx.doi.org/10.20517/2347-9264.2020.63>

**Received:** 7 Apr 2020 **First Decision:** 19 Jun 2020 **Revised:** 20 Jun 2020 **Accepted:** 30 Jul 2020 **Published:** 1 Sep 2020

**Academic Editor:** Marlon E. Buncamper **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Complex penile reconstruction continues to pose a significant challenge to surgeons and patients alike. The ideal phalloplasty is one that can be reproducibly performed in a single stage, creates a neourethra that allows for voiding while standing, produces a phallus with tactile and erogenous sensation, allows for penetrative sexual intercourse, and offers satisfactory aesthetic results. With recent advances in microsurgery and perforator flap dissection, several techniques and modifications thereof have been described that aim to achieve these reconstructive goals. All of these now conventional techniques, however, fall short in one way or another - often with regards to urinary transport, the ability to achieve an erection, and the need for multiple surgical stages and revision operations. These limitations of conventional reconstruction have led some surgeons to explore new avenues for complex penis reconstruction, giving birth to the novel field of penile transplantation. In this article, we discuss the complexities of male genitourinary reconstruction in the context of conventional methods for reconstruction as well as the burgeoning field of penile transplantation.

**Keywords:** Phalloplasty, total penile reconstruction, free flap, vascularized composite allotransplantation, penis transplantation, reconstructive surgery, urologic reconstruction

## INTRODUCTION

Despite nearly a century's worth of cumulative experience in complex penile reconstruction, the ideal neophallus continues to elude us, presenting a significant challenge to reconstructive surgeons. The first



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



attempted total penile reconstruction was reported in 1936 by Russian surgeon Nikolaj Bogoraz<sup>[1]</sup>. His use of a bipedicle abdominal flap with rib cartilage aimed to provide stiffness for potential sexual intercourse, but fell short of attempting urethral reconstruction or taking measures to ensure adequate protective and/or erogenous sensation. In 1948, Gilles and Harrison<sup>[2]</sup> expanded upon this technique to introduce the contemporary “tube within a tube” design for creation of the neourethra; however, reconstruction required three or more stages and yielded highly variable results.

Since then, the advent of microsurgical techniques has vastly expanded our toolkit in complex genitourinary reconstruction, enabling substantial improvements in function and aesthetic outcomes. Free tissue transfer using the radial forearm free flap (RFFF), anterolateral thigh flap (ALT), fibular osteocutaneous flap (FOF), and latissimus dorsi myocutaneous flap (LDMF) allow for the transfer of a large amount of tissue in a single surgical procedure with relatively minimal donor site morbidity<sup>[3,4]</sup>. Our improved understanding of nerve regeneration and coaptations has allowed for the preservation of erogenous sensation and the ability to achieve orgasm in most cases<sup>[5,6]</sup>. Most recently, building upon the global experience in hand and face allotransplantation, penile transplantation has emerged as a viable alternative in carefully selected patient populations, promising to even further advance the potential of penile reconstruction<sup>[7-11]</sup>.

Despite these advances, there is no consensus on the ideal reconstructive approach, and, in many cases, complications are both commonplace and potentially serious. In this article, we aim to review the goals of complex penile reconstruction and to discuss the various surgical options within this context. We discuss both “conventional” microsurgical techniques and the emerging field of penile transplantation, including its various technical and ethical considerations<sup>[7-11]</sup>.

## EPIDEMIOLOGY

There are several distinct patient populations who undergo phalloplasty including those who seek oncologic reconstruction, traumatic reconstruction, gender affirming surgery, and correction of congenital abnormalities. Although males with anxiety regarding small penis size may occasionally seek consultation for phalloplasty, surgery is rarely indicated in this patient population, as many benefit from expert psychosexual therapy alone.

Oncologic penectomy, although relatively uncommon, may be increasing in its prevalence with considerable variations in its incidence geographically<sup>[3]</sup>. Squamous cell carcinoma of the penis makes up 0.4%-0.6% of all malignancies within Europe and the United States<sup>[3]</sup>. In 2019 alone, the estimated number of new cases of penile cancers in the United States was 2080<sup>[12]</sup>. The incidence is increased by as much as 10% in other parts of the world including South America, Africa, and Asia, with reports that it accounts for as many as 17% of all malignancies in certain areas of Brazil<sup>[13]</sup>. The most common age of presentation is between 50 and 70 years<sup>[14]</sup>.

Trauma is a well-established etiology for genitourinary injury; nonetheless, the incidence of penile trauma has not been well described. Recent conflicts in Iraq and Afghanistan have brought to light the potentially devastating consequences of male genitourinary trauma. Although these injuries are not new, with historical rates of injury between 0.5% and 8% in World War II and Vietnam, the increased use of improvised explosive devices and improvements in body armor that result in more soldiers surviving previously fatal injuries increased the rate to 14% of all servicemen in 2011<sup>[15,16]</sup>. It is important to note that a large percentage of service members who sustain penile trauma present with concomitant multi-extremity trauma and/or amputation that minimizes the availability of donor tissue for reconstruction. Of the 1367 male servicemen who sustained genitourinary trauma in Operation Iraqi Freedom and Operation Enduring freedom, 28.7% sustained at least one extremity amputation as well<sup>[17]</sup>.

The true incidence of transgender or gender nonconforming patients has been difficult to identify, and the proportion of those patients who are interested in pursuing phalloplasty is even less clear. Recent estimates suggest that 0.6% of the United States' population identifies as transgender or gender nonconforming<sup>[18]</sup>. The rate of phalloplasty within this population is further confounded by challenges related to health insurance availability and coverage of hormonal and surgical therapy. Nonetheless, gender affirming phalloplasty remains a major area of unmet demand, with many patients experiencing wait times of several years or more<sup>[3]</sup>.

Among children the most common indications for phalloplasty include ambiguous genitalia, micropenis/severe penile inadequacy, aphallia, and phallic inadequacy associated with epispadias/bladder exstrophy<sup>[19]</sup>. Bladder and cloacal exstrophy have reported incidences of 1:10,000 to 1:50,000 and 1:200,000 to 1:400,000 live births, respectively<sup>[20]</sup>. Male children with bladder or cloacal exstrophy may have ambiguous genitalia, and, historically, some of these patients have been gender-converted to female during infancy and later decide to pursue phalloplasty as an adolescent or adult.

## ANATOMY

The intricate anatomy of the penis allows for its several complex functions but also makes it particularly difficult to reconstruct in the setting of phalloplasty. Its general arrangement is that of a cylinder with two central corpora cavernosa bound together tightly by the tunica albuginea. Ventrally lies the corpus spongiosum, which encases the urethra. Overlying these structures is the deep penile fascia, or Buck's fascia, which tightly binds the corpus spongiosum and the corpora cavernosa into a single functioning entity. Buck's fascia also carries several neurovascular structures, including the deep dorsal veins, arteries, and nerves of the penis, the circumflex arteries and veins, and the penile lymphatics.

The glans is a vascular spongiosa which contains unique erogenous and tactile sensory nerve endings. The glans epithelium is distinct from that of the shaft and includes sensory cells, particularly around the corona. In an uncircumcised penis, the glans is protected by a bilaminar prepuce with an inner lamina consisting of uroepithelium similar to that of the glans and an outer lamina with glabrous skin similar to that of the shaft. Deep in the skin lies the superficial fascial system of the penis, or the Dartos fascia. This fascial layer is in continuation with Scarpa's fascia superiorly and Colles' fascia inferiorly.

## Vascular anatomy

There are two distinct arterial systems that perfuse the penis - both of which are necessary to adequately perfuse the penis and all of its overlying skin<sup>[21]</sup>. The deep system originates from the internal pudendal arteries (branches of the internal iliac artery) that gives off perineal and scrotal branches before continuing as the common penile artery. Each common penile artery gives off three branches, the bulbar, urethral, and cavernosal, before terminating in the tortuous dorsal artery of the penis.

The superficial system originates from the external pudendal arteries, which are branches of the femoral artery. There are typically separate superficial and deep external pudendal arteries. The superficial supplies vascularity to the dartos fascia and genital skin, while the deep travels separately to further perfuse the dorsolateral and ventral shaft skin.

The venous drainage is similarly composed of two systems. A superficial system runs within the Dartos to drain the penile shaft skin, whereas the deep system drains the circumflex veins and deep dorsal veins into the prosthetic plexus and the crural and cavernosal veins into the internal pudendal veins.

## Sensation

The nerves to the penis also arise from a dual source that run with the arteries. The dorsal penile nerves provide erogenous sensation but do not provide sensation to the penile shaft skin. The pudendal nerve

is mixed motor, sensory, and autonomic, originating from the sacral roots S2 through S3 and is the main penile sensory nerve. The shaft is also innervated by ancillary erogenous nerves including the ilioinguinal nerves.

## GOALS OF RECONSTRUCTION

In their 1987 article, Gilbert and Winslow<sup>[22]</sup> described these five necessary criteria to achieve the ideal phallic reconstruction:

1. A reproducible procedure that takes place in one stage
2. Creation of a neourethra that facilitates voiding while standing
3. A phallus with erogenous and tactile sensibility
4. Sufficient bulk to permit the placement of a penile prosthesis, allowing for penetrative sexual intercourse
5. A satisfactory aesthetic result

These five goals must of course be weighed against donor site morbidity, as all techniques require the transfer of tissues from elsewhere in order to restore the missing skin, urethra, and soft tissue bulk. Meeting these criteria with conventional reconstructive options continues to challenge surgeons over thirty years later. In the sections that follow, we review each of the individual donor sites commonly employed in modern, conventional phalloplasty, assessing their ability to achieve these reconstructive goals.

## CONVENTIONAL SURGICAL TECHNIQUES

At least at the surface, each of the conventional techniques for phalloplasty has the potential to achieve the above listed goals set forth by Gilbert and Winslow<sup>[22]</sup> Ultimately, there are advantages and disadvantages to each flap, and therefore the choice of donor site should be a combination of both the individual patient's preference as well as the surgeon's ability to produce a consistent result.

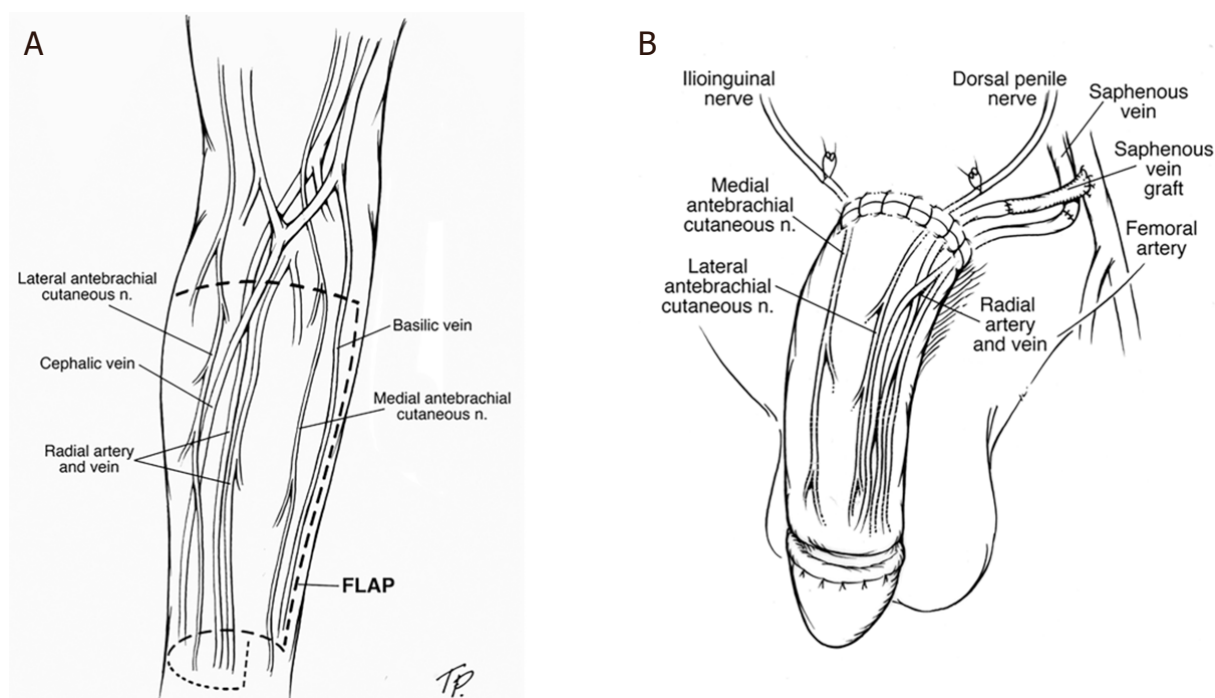
### RFFF

RFFF [Figure 1] is among the most common techniques for phalloplasty and considered by some to be the modern "gold standard" technique. The donor site is thin, pliable, and relatively hairless allowing for a flap that can be easily tubed and shaped with excellent aesthetic outcomes. The medial and lateral antebrachial cutaneous nerves can be coapted to the ilioinguinal and dorsal penile or clitoral nerves in order to provide excellent protective and erogenous sensation in most patients [Figure 2]. In transgender men, the clitoris can remain at the base of the phallus for stimulation as well. Technically, the radial artery of the flap can be anastomosed to several donor vessels including the profunda femoris, lateral circumflex femoral, circumflex iliac, or the inferior epigastric artery and their respective venae comitans. In some cases, the cephalic vein of the flap will also be included and anastomosed to a branch of the greater saphenous vein.

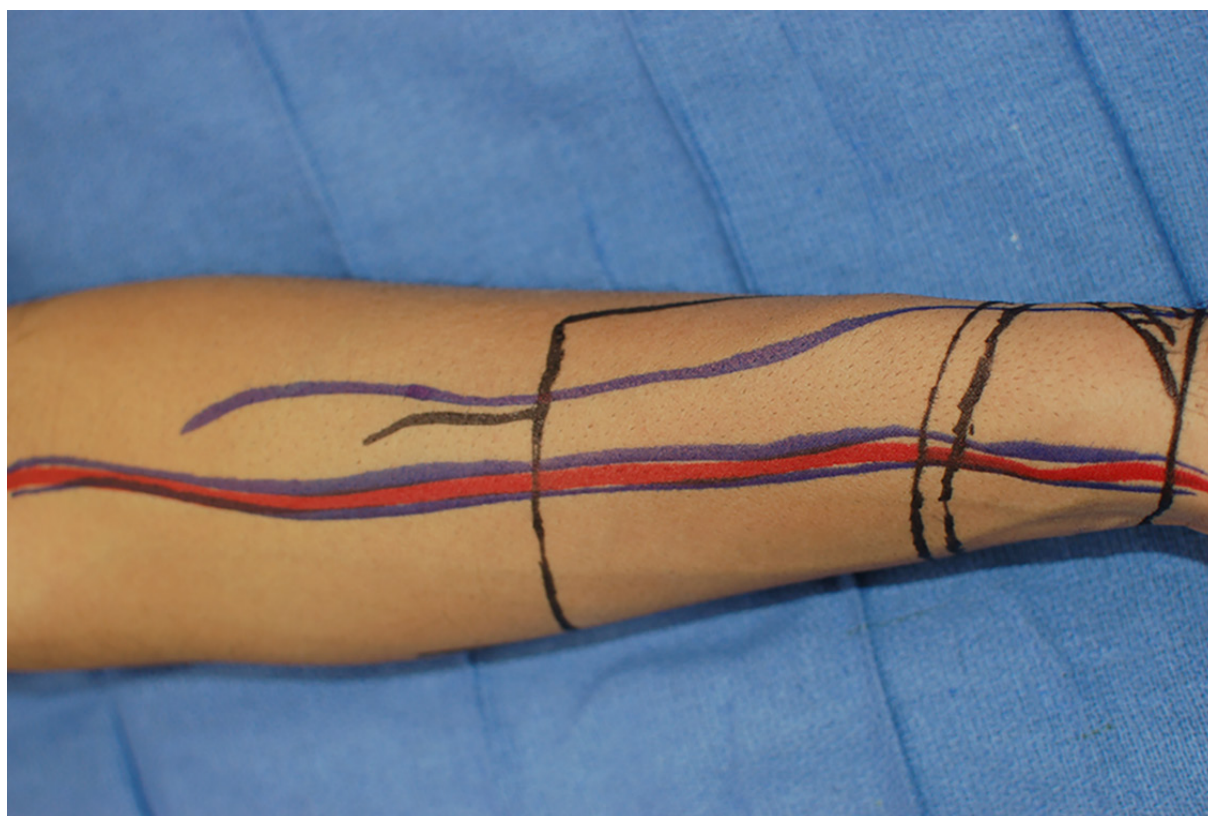
The technique for RFFF phalloplasty can be carried out in as many as 1-4 or more stages and depends on the needs of the patient and surgeon preference<sup>[3]</sup>. The first stage almost always involves the harvest and inset of the flap in the perineum. The flap is typically harvested from the non-dominant arm, with a portion of it used to form a narrow skin tube stented using a 16-French Foley catheter that is to function as the neourethra [Figure 3]. The donor site can often be very conspicuous due to the large amount of tissues required to create the neophallus. Donor site coverage is achieved with either a thick split or a full thickness skin graft at the time flap harvest [Figure 4], or in two stages with the initial application of an Integra Dermal Regeneration Template (Integra LifeSciences Corp., Princeton, NJ).

The aesthetics of the phallus can be refined by the creation of a corona using a local flap and a full thickness skin graft as described by Monstrey *et al.*<sup>[23]</sup> Although some surgeons perform this "glansplasty" at the

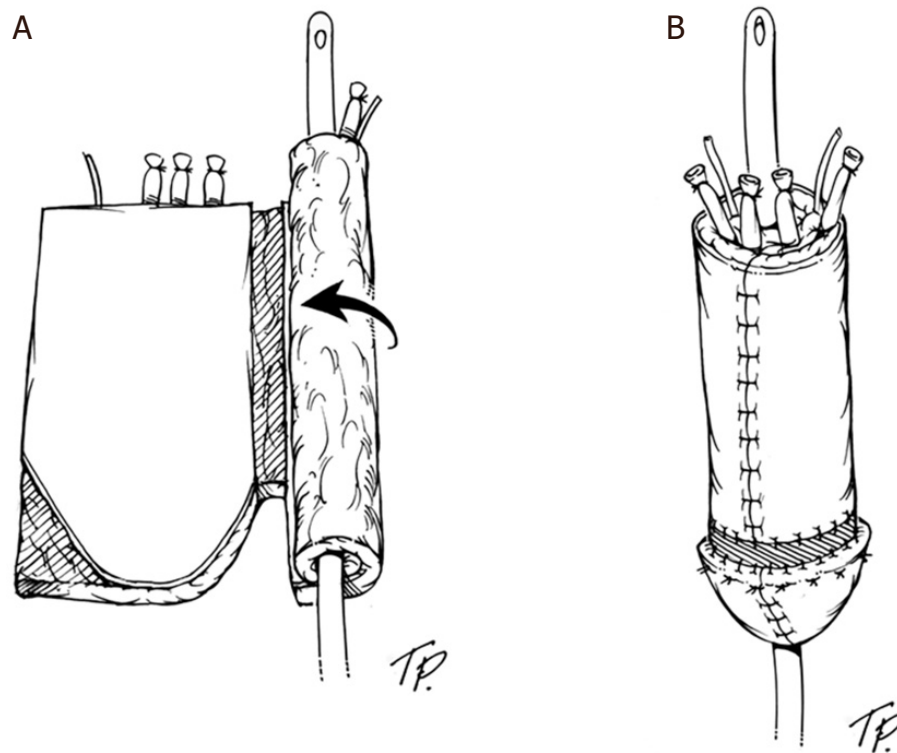




**Figure 1.** Outline of the radial forearm free flap phalloplasty on the arm. The flap is designed to include the lateral and medial antebrachial cutaneous nerves as well as the radial artery and veins and the cephalic vein (A); representation of the flap following inset (B)



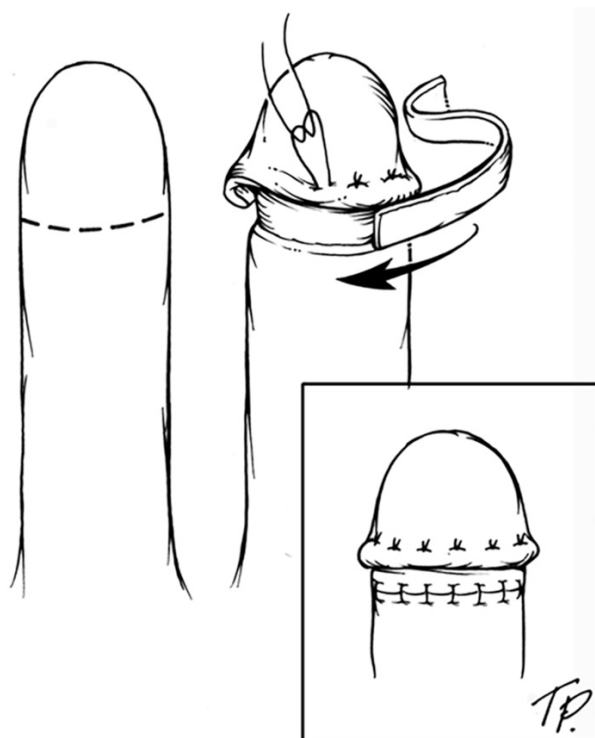
**Figure 2.** Markings of the radial forearm free flap *in situ* within the forearm. The outline of the flap, including the markings for glansplasty, is made in black. The courses of the radial artery (red) along with its two venae comitans (paired blue) and the cephalic vein (single blue) are marked out as well



**Figure 3.** The flap is divided into three section prior to tubularization: the outer skin envelope of the neophallus; the de-epithelialized portion, which separates the skin and urethra; and the ulnar-sided skin paddle, which serves as the neourethra (A); the flap is tubularized over a 16-French Foley catheter (B)



**Figure 4.** Well healed radial forearm donor site resurfaced with a thick split thickness skin graft at the time of flap harvest



**Figure 5.** During the glansplasty, the distal flap is de-epithelialized and curled onto itself to recapitulate the corona. A full thickness skin graft is harvested from the groin and placed below. Before the return of sensation to the neophallus, this can be further refined with tattooing if the patient so wishes once the wounds have healed

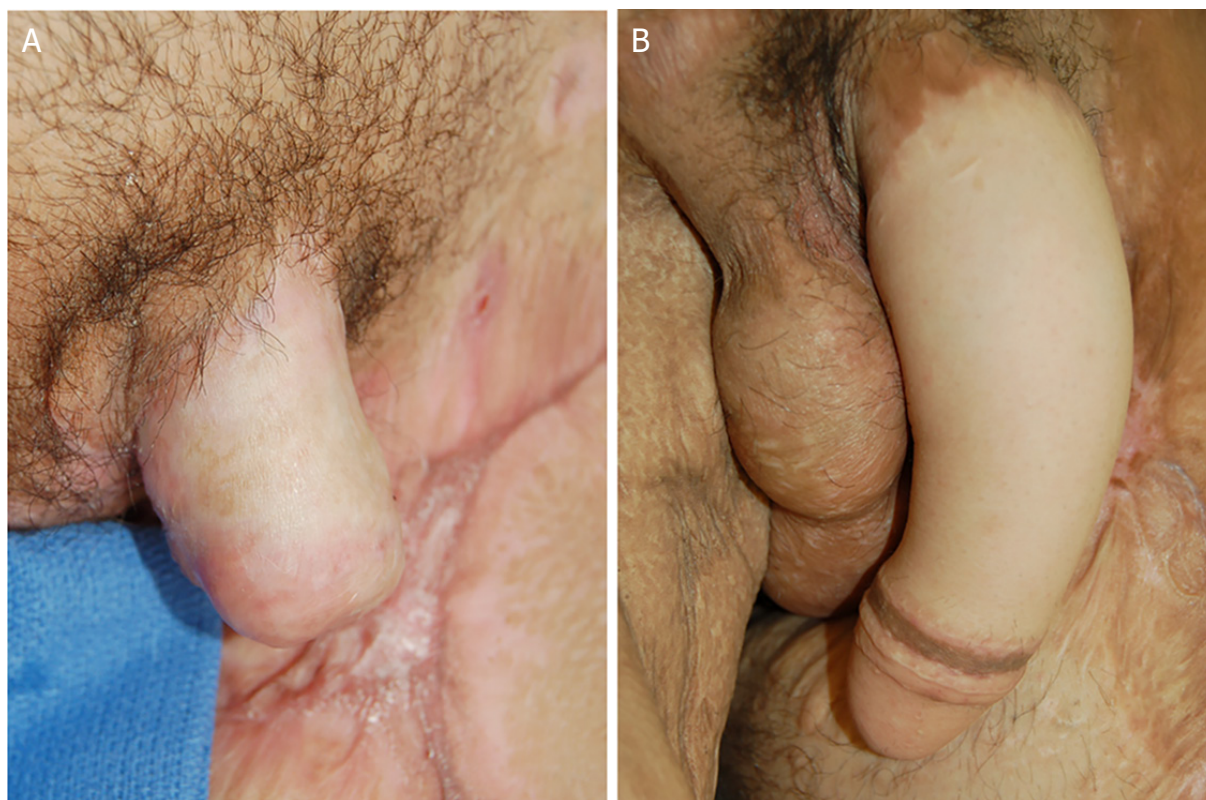
time of flap transfer, other will stage it for at least three months to allow the tissues to heal from the initial operation [Figure 5]. The corona can be further refined before the return of sensation by tattooing in order to match the color of the areola.

The anastomosis of the neourethra and native urethra may also vary in timing, from the time of flap transfer to a separate stage several months later. In many cases, the need to perform a urethral anastomosis is determined by the indication. For example, children with exstrophy may have a continent umbilical bladder stoma and not need urethral reconstruction. In these children, the native glans can be de-epithelialized and brought out the ventral and proximal surface of the neophallus to allow for ejaculation from the native urethra. Without the normal peristalsis of the corpora bodies, the ejaculate may not reach the tip of the neophallus if a full-length urethra is constructed. Furthermore, the ability to urinate standing up may not be a priority for some transgender men, who may wish to forego urethral lengthening and anastomosis in order to avoid the relatively high complication rates.

The final stage of RFFF phalloplasty is typically the insertion of a penile prosthesis. This is normally performed up to one year after the index procedure in order to allow for adequate regeneration of protective sensation. Although some surgeons have described osteocutaneous modifications to RFFF in order to provide stiffness of the flap and avoid the need for a penile prosthesis, this procedure is associated with an increased risk of donor site fractures and not commonly employed<sup>[24,25]</sup>. Penile prostheses are discussed in further detail in a separate section below.

Overall, despite being the “gold standard” reconstructive technique [Figure 6], the outcomes of RFFF fall short in many ways. A recent meta-analysis by Yao *et al.*<sup>[3]</sup> included 925 RFFF phalloplasties that included nearly 90% female-to-male gender-affirming surgeries. Although complications varied greatly based on the





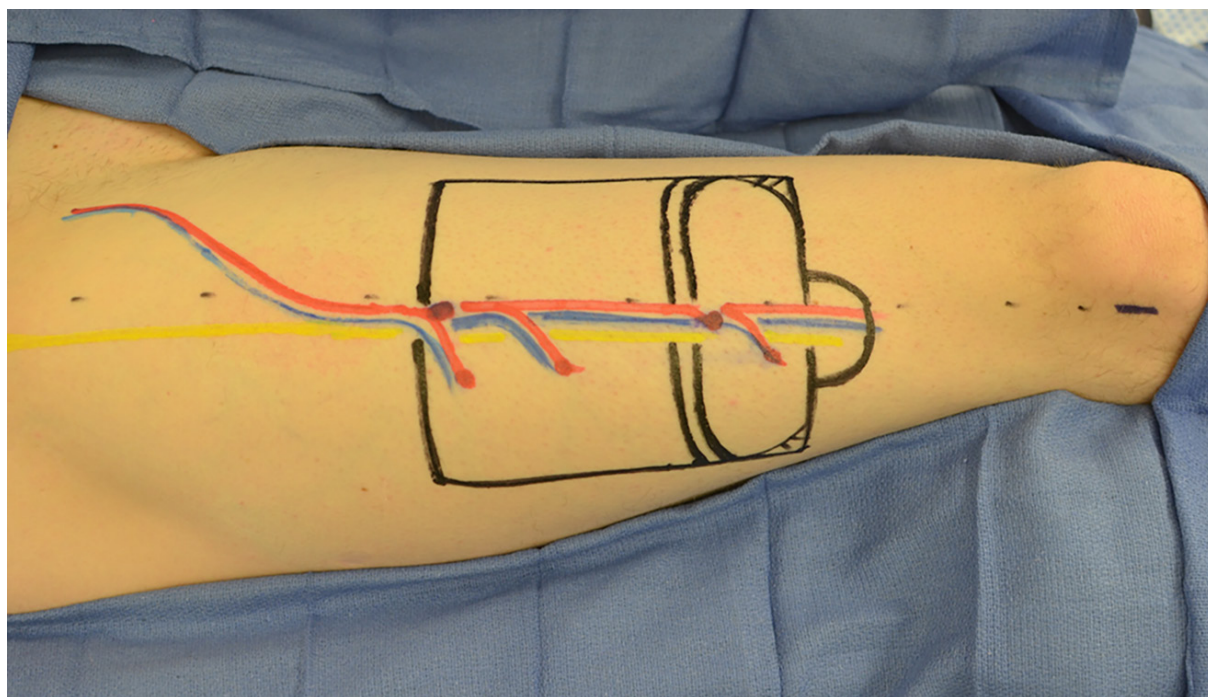
**Figure 6.** Before (A) and after (B) images of a patient undergoing radial forearm free flap phalloplasty following partial amputation of the penis due to an improvised explosive device. The grafted skin just proximal to the corona can be tattooed to further refine the aesthetics of the neophallus

various technical modifications performed, total flap loss was rare with an incidence of 1.5%, with another 7.4% experiencing partial/distal flap loss. Urethral fistulas were much more common with an incidence of nearly 30% - of which 41% were able to be managed conservatively. Urethral strictures were less common and occurred in 8.2% of phalloplasties<sup>[3]</sup>. In one of the largest single center experiences with RFFF phalloplasties, Monstrey *et al.*<sup>[23]</sup> reported similar outcomes with a 41% incidence of urologic complications and a 44% rate of penile prosthesis removal.

Nonetheless, patients tend to be happy with their RFFF reconstruction. As many as 75%-100% report the ability to void while standing, 97% are satisfied with cosmesis, and 87% of patients reported sensation in the neophallus<sup>[26]</sup>. With appropriate innervation, 80% of patients are able to achieve orgasm<sup>[27]</sup>.

## ALT

The ALT flap [Figure 7], based on perforators of the descending branch of the lateral circumflex femoral vessels, can be used as both a pedicled and free flap for phalloplasty. Sensation to the flap is provided by the lateral circumflex femoral nerve. Some authors described harvesting an additional cuff of fascia with the flap that can be used to create a neo-tunica that will cover the eventual penile prosthesis<sup>[28]</sup>. Because of the bulk of the thigh subcutaneous tissues relative to the forearm, some surgeons do not arrange the flap in the “tube within a tube” configuration; instead, a separate skin graft is often harvested and wrapped around a catheter and sewn to the native urethra proximally. In thinner patients, a 1.5 cm strip can be de-epithelialized and tabularized as in RFFF at the cost of a bulkier construct at the time of initial flap transfer. Other surgeons, including Mutfak and colleagues, have described a chimeric flap in which the skin perfused by the sartorius perforators is harvested and used to create the neourethra within the tubed ALT<sup>[29,30]</sup>.



**Figure 7.** Markings of the ALT flap including glansplasty are made in black. The descending branch of the lateral femoral circumflex artery and its paired veins are marked in red and blue, respectively, along with the location of three perforators identified using Doppler ultrasonography. The course of the lateral femoral cutaneous nerve is depicted in yellow

Although technically more complex, this technique benefits from the use of vascularized tissue to form the urethral passage.

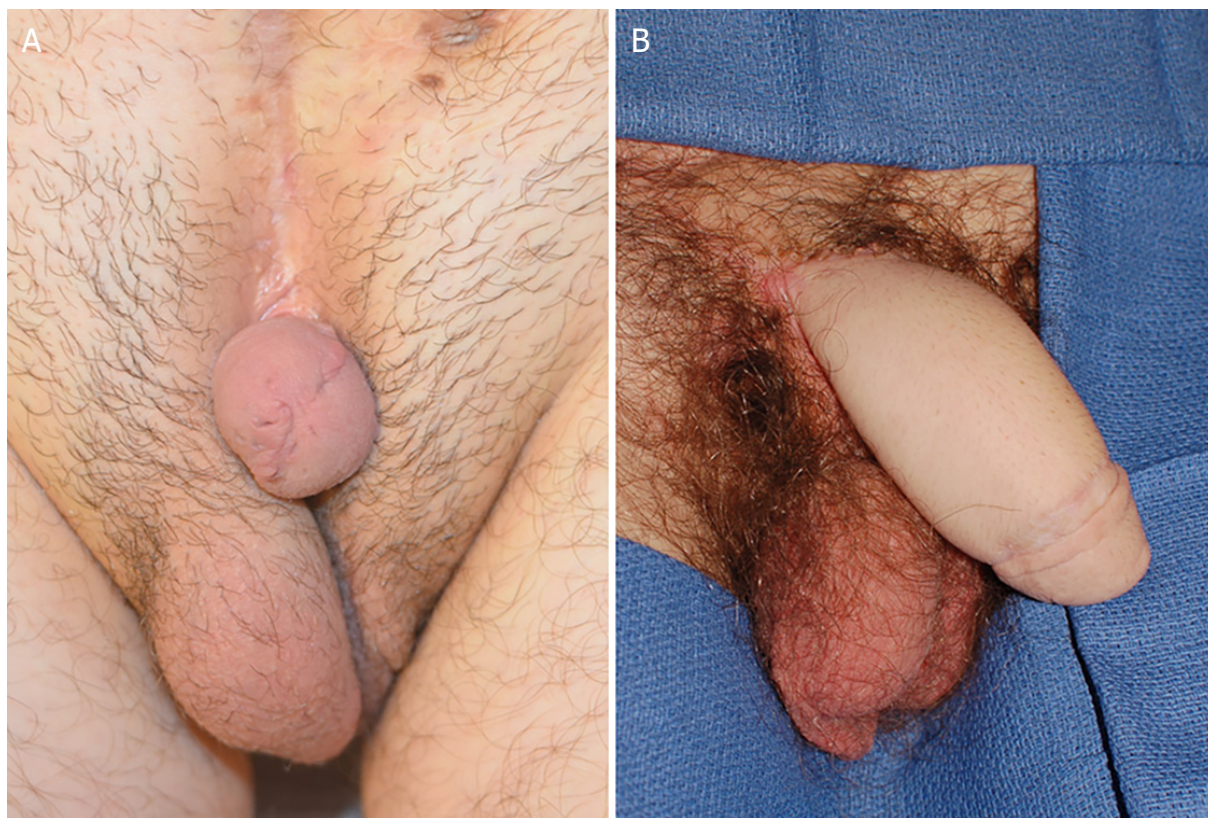
The pedicled ALT is a particularly powerful option in patients who have undergone several previous operations, such as in children with bladder exstrophy, patients who are very thin and RFFF would not provide enough bulk, or those in whom a free flap is relatively contraindicated. One of the major benefits of ALT is the relatively inconspicuous donor site location on the thigh that is more easily hidden than the forearm. Similar to RFFF, the number and timing of stages is typically dictated by patient needs and surgeon preference, and everything short of the penile prosthesis can theoretically be preformed in as few as one stage<sup>[30]</sup>. In all but the thinnest patients, however, debulking of the flap via serial excision and/or liposuction may be necessary in order to achieve an appropriately sized neophallus, and the vast majority of patients should be prepared for several stages before achieving an acceptable result.

Published functional and aesthetic results are relatively scarce and subject to a great deal of heterogeneity in surgical technique and outcomes [Figure 8]. Although complete flap loss is rare, particularly with the pedicled ALT, partial flap loss can occur in the periphery of the flap and in areas where the tissue has been folded. Limited patient reported outcomes studies have demonstrated that the vast majority of patients report satisfaction with the phallus and nearly two-thirds report the ability to urinate while standing and to undergo penetrative intercourse<sup>[31]</sup>. The most common complications are associated with the formation of urethral fistulas (~22%) and stricture/stenosis (~7%)<sup>[31]</sup>. Sensation in the ALT flap is generally considered to be good with coaptation of a dorsal penile/clitoral nerve to the lateral circumflex femoral nerve, with 100% of patients reporting at least sensation in the proximal half of the neourethra<sup>[3]</sup>.

## FOF

FOF remains the most commonly used option that includes a vascularized boney component in order to allow for long-term rigidity without the need for a penile prosthesis. It also benefits from a relatively





**Figure 8.** Before (A) and after (B) images of a patient undergoing anterolateral thigh flap phalloplasty

hidden donor site, while the primary disadvantages of FOF relate to potentially unpredictable bone resorption, risk for fracture, and the inability to modulate the stiffness of the neophallus, as with a penile prosthesis<sup>[3]</sup>. Dabernig *et al.*<sup>[32]</sup> described the use of a fasciocutaneous fibula flap without any bone in phalloplasty; although not typically employed in this fashion, this may represent one possible option in patients who wish to avoid the forearm donor site and in whom ALT is relatively contraindicated due to excessive subcutaneous tissue bulk. When the bone is included, it can be fixated proximally to either the penile corpora cavernosa or the pubic symphysis. Hage *et al.*<sup>[33]</sup> suggested including a segment of bone ~2 cm longer than the skin paddle so that the phallus is not floppy after fixation.

Because the tissues of the lower leg are relatively stiff and less amenable to rolling on itself, many authors report prelamination the neourethra with a skin graft in a separate stage before flap transfer. Urethral lengthening and/or anastomosis may occur in a separate third stage or at the time of flap transfer. As with most other flaps used in conventional penile reconstruction, FOF allows for phalloplasty in as few as one or as many as three or more stages. There is some evidence to suggest staging the procedure with prelamination of the neourethra reduces the incidence of urethral fistulas<sup>[33-35]</sup>.

Sensation to the flap is provided by coaptation of the donor nerves to the lateral sural cutaneous nerve, which lies posterior to the posterior crural intermuscular septum in nearly 75% of cases with an anterior branch in 26%<sup>[36]</sup>. The nerve has been shown to be within 4 cm of the septum in 86% of cases; as such, we prefer to orient the skin paddle such that it is located posterior to the septum and in the proximal two-thirds of the lower leg<sup>[35,36]</sup>. Although studies of sensation following FOF phalloplasty are very limited, Schaff and Papadopoulos<sup>[37]</sup> compared their patient reported outcomes to RFFF, demonstrating potentially worse sensibility with FOF.

Overall, the published outcomes of the fibula are not dissimilar from the other flaps discussed and largely suffer from the same limitations mentioned above. Flap loss, either complete or partial, range in the 1%-2% and 10%-15% ranges, respectively<sup>[3]</sup>. Urethral fistula formation remains the most commonly reported complication, followed by urethral strictures<sup>[3]</sup>. Nonetheless, the majority of patients report that they are able to urinate standing up, partake in sexual intercourse, and that they are satisfied with their overall result<sup>[3,37,38]</sup>.

## LDMF

Perovic and colleagues have reported extensively on the use of LDMF in phalloplasty with excellent results<sup>[39,40]</sup>. The advantages of this donor site include its well concealed location, ability to be closed primarily in most situations, relatively hairless donor site, and a large amount of tissues allowing for an aesthetic reconstruction. Owing to the less sensitive nature of the back skin, however, there are concerns that erogenous sensation may be relatively difficult to achieve with LDMF. The flap is based on the thoracodorsal vessels and nerve. Typically, only a thin strip of muscle around the pedicle is harvested in order to minimize donor site morbidity. As described by Perovic, reconstruction with LDMF takes place in several stages including flap harvest and creation of the neophallus, two stages of urethroplasty using a buccal mucosa inlay, and finally the insertion of a penile prosthesis. Muscle sparing thoracodorsal artery perforator flaps have also been described in phalloplasty<sup>[41]</sup>, as well as other perforator flaps based on the subscapular vessel system including the scapular and parascapular flaps<sup>[3,42,43]</sup>.

Limited outcomes studies have demonstrated largely similar complication rates with LDMF as with other options for phalloplasty. Although urethral fistula and stricture rates have not been rigorously studied, they are believed to be largely on the same order of magnitude as other reconstructive options<sup>[3]</sup>. Two unique complications to consider with the use of the back as a donor site are a potentially increased risk of hematoma and/or seroma at the donor site. Although the majority of the muscle is open spared, minimizing the amount of dead space at the back, the need to widely undermine the skin in order to achieve primary closure can may result in blood or fluid collections in as many as 10% of patients<sup>[3]</sup>. Patients who received LDMF may also experience the phenomenon of “paradox erection,” in which muscle contraction results in stiffening of the neophallus, potentially allowing for penetrative intercourse (82% of patients in one study)<sup>[44]</sup>. Otherwise, protective sensation has been noted in the proximal neophallus for up to two years after surgery, and patients generally report the ability to void while standing and satisfaction with flap aesthetics<sup>[44]</sup>.

## Penile prosthesis

The rigidity required for penetrative intercourse is an important component of a successful phalloplasty, however it is to date impossible to adequately reconstruct the erectile tissues of the penis using autologous tissue. As discussed above, the osteocutaneous fibula flap attempts to address this need by incorporating a length of bone to provide rigidity to the neophallus. This is far from the ideal solution, however, as the bone remains permanently erect and the osseous component is susceptible to warping, fracture, and unpredictable resorption.

As such, the hydraulic erectile implant has become the standard at many centers across the world<sup>[3,19,45]</sup>. These implants are typically placed no less than 6-12 months following the completion of reconstruction once the wounds have completely healed, protective sensation is fully restored, and the phallus is at its final size. The prosthesis is often left semi-inflated for one or more weeks to enable capsule formation around the cylinder. After the incisions have healed the implant may be inflated to create an erection and deflated at other times as needed.

Although a key component of a successful phalloplasty, the penile prosthesis is unfortunately also the portion of reconstruction with one of the highest rates of complication. The rate of explanation is over 40% in most large series<sup>[22,45]</sup>, and most often secondary to infection, erosion of the overlying soft tissues, or device dysfunction. The absence of a tunica albuginea is believed to predispose the device to trauma and erosion when compared to the use of these devices to treat impotence, leading some authors to fabricate a neo-tunica using vascularized fascia lata to protect the device<sup>[28,46]</sup>. Despite these drawbacks, over 80% of patients with prosthesis report satisfactory sexual intercourse<sup>[47]</sup>. This is undoubtedly an important area of ongoing research that requires additional long-term follow-up and innovation moving forward.

## PENILE TRANSPLANTATION

### History

Some of the earliest animal models for penile transplantation were developed in the early 2000s. The feasibility of allogeneic penile transplantation through nonvascular anastomosis and later arterial anastomosis to the distal corpus spongiosum were first demonstrated in a rat model<sup>[48,49]</sup>. Auto-transplantation rat models have also been developed to evaluate the viability and functionality of re-planted phalluses<sup>[50]</sup>. Since then, several advancements including a canine model<sup>[51]</sup>, deceased donor anatomic studies<sup>[21]</sup>, and an *ex vivo* model to assess graft rejection and its effect on erectile function have helped to bridge the gap between research and practice in preparation for the first few cases of penile transplantation across the globe<sup>[52]</sup>.

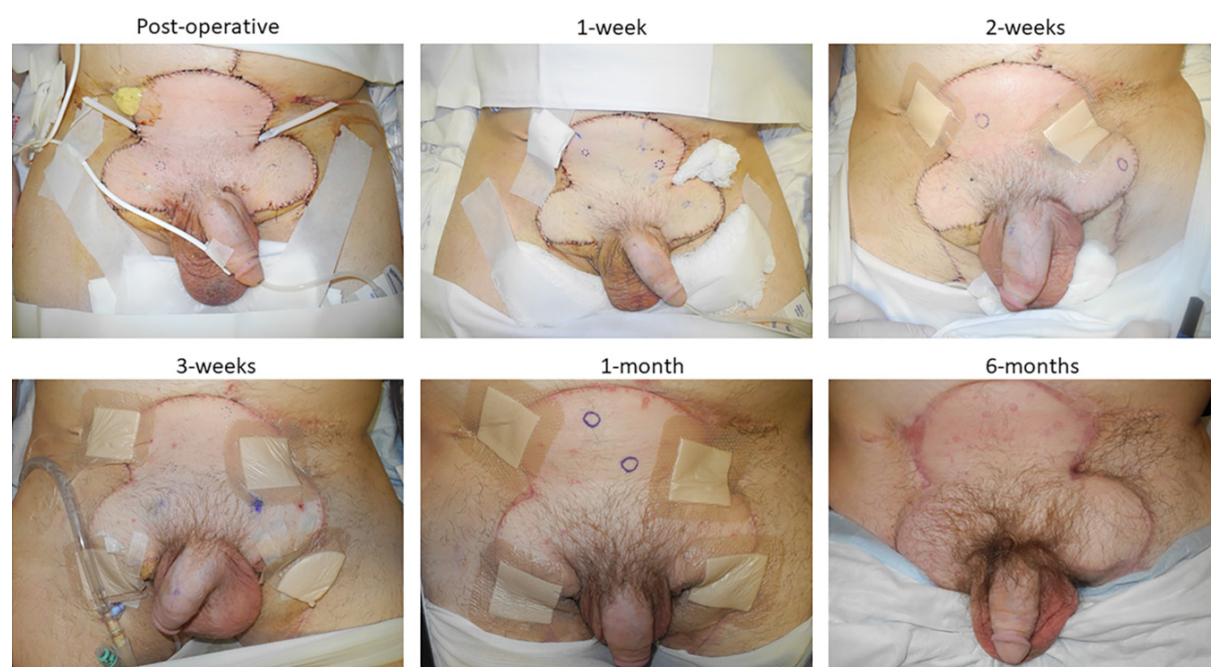
The first in human penile transplantation was attempted in 2006 in China. A 44-year-old man who had sustained a traumatic injury underwent a technically successful procedure, however the graft was explanted after two weeks due to psychological rejection<sup>[10]</sup>. In 2015, the first successful penis transplant was performed in South Africa on a 21-year-old man with a penile injury sustained during cultural circumcision<sup>[9]</sup>. Two years following this procedure, the recipient reported the ability to urinate and to achieve erections, orgasms, and ejaculation<sup>[53]</sup>. In 2017, the same group in South Africa performed its second penis transplantation<sup>[54]</sup>. In the United States, a partial penis transplant from a deceased donor to a 64-year-old man following oncologic amputation was performed in 2016<sup>[8]</sup>. Six months following the operation, the patient had recovered sensation, was voiding successfully, and reported partial erectile function. Finally, in 2018, the entire penis, scrotum, and part of the abdominal wall were transplanted from a deceased donor to a patient who had sustained a blast injury to the abdominal wall and perineum. One year postoperatively, the patient is voiding successfully and has return of erogenous sensation and the ability to obtain a full erection [Figure 9]. Most importantly, he reports that his transplanted penis feels “normal”<sup>[7]</sup>.

### Indications

Most discussions on the use of penis transplantation have focused on traumatic etiologies. In this context, extremity amputation or injuries may compromise reconstructive donor sites, precluding conventional options for phalloplasty. That said, the lack of conventional reconstructive options is not alone an indication for penis transplantation. Traditionally, penile transplantation has been considered a last resort after several failed attempts at phalloplasty<sup>[16]</sup>. This thought process has been recently questioned within the literature, as there are considerable downsides to undergoing several failed reconstructive attempts before transplantation<sup>[55]</sup>.

We believe that surgeons should preserve conventional reconstructive options as a contingency plan in the event of allograft failure. There are several benefits to pursuing transplantation before exhausting options for phalloplasty. First, the superior functional and cosmetic outcomes of transplantation may outweigh its associated risks for certain patients. Furthermore, there may be no appropriate salvage options available for





**Figure 9.** Clinical images following total penis, scrotum, and lower abdominal wall transplantation over the course of six months postoperatively. Circular scars at biopsy sites can be appreciated on the skin of the abdomen and groin. The graft healed unevenly and has incorporated well without evidence of rejection

penile reconstruction if the transplant were to fail. Finally, without long-term data on graft longevity, we should make sure to preserve several “back-up” reconstructive options.

### Patient selection

The allocation of life-enhancing grafts, including penile transplants, should follow the standard practices used for life-saving transplantation: the resource is allocated based on equity, priority, and net benefit<sup>[56]</sup>.

The ethical and practical challenges of penis transplantation in a child have thus far limited the procedure to adults<sup>[57,58]</sup>. The cumulative risks of a transplant are greater in children than adults due to the extended exposure to immunosuppression over their lifetime. Additionally, adherence to immunosuppressive regimens may also prove challenging in children, particularly during the adolescent period. Furthermore, it is not clear when a child can provide adequate consent to such a life altering procedure, and parental permission may not be sufficient ethically given the circumstances. Third, pediatric penile transplant is complicated by issues related to donor matching. It would not be appropriate to transplant an adult phallus onto a child, but the alternative of using an age-congruent phallus risks issues later in adulthood because of an age-incongruent phallus. Finally, we do not currently know what a reasonable lifespan for a penis transplant is, and childhood recipients would likely require another transplant in adulthood. As such, conventional reconstruction should remain the mainstay for these children until an appropriate decision regarding transplantation can be made in adulthood.

The majority of transplants to date were performed for traumatic indications, however oncologic extirpation remains another important avenue for potential transplantation. We have expanded our eligibility criteria to include patients with a five-year history of remission following oncologic penectomy. The largest study to date on recurrence in penile cancer reported that all local and distant recurrences occur within a five-year window following initial resection<sup>[59]</sup>. Furthermore, most local recurrences occurred after penile preserving treatment. Given that all remaining native penile skin can be resected before transplantation, the risk of

locoregional recurrence should be negligible. We also believe that criteria for transplantation should be expanded to include men with congenitally ambiguous genitalia or true micropenis. To date, even the most sophisticated techniques for conventional penile reconstruction are fraught with urinary complications and issues related to penile prosthesis<sup>[3,19]</sup>. Although there are greater risks with penis transplantation, this may be outweighed by the improved function and aesthetics of transplanted phallus. At this time, further deliberation on the use of penile transplantation in transgender men is needed as the lack of proximal corpora would preclude the ability to achieve an erection with the transplanted penis.

## Technical considerations

### *Donor selection*

Limited donor availability represents a major hurdle for any kind of transplantation<sup>[60]</sup>. In addition to HLA matching and screening for a healthy donor phallus that is without vascular disease, sexually transmitted infection, and/or complications of diabetes, attention should be paid to recipient aesthetic preferences. The appearance of donor phallus must be inline with the recipient's desired appearance and discussed frankly before listing, in an attempt to limit psychological stresses.

### *Surgical planning*

Given the anatomical complexity, penile transplantation presents unique challenges including how proximally the graft will need to be harvested. Anastomosing a distal portion of the penis requires fewer structural anastomoses than transplantation of the entire penis with portions of the pelvic floor. Larger grafts may be required to address wartime injuries when extensive damage to the genitalia, pelvic floor, and abdominal tissues can occur from the upward blast of an improvised explosive device. In congenital anomalies, there may be insufficient tissue development to provide adequate proximal corpora to be anastomosed.

The penis has three main vascular perfusion territories that have been previously described in detail<sup>[21]</sup>. The first includes the shaft skin, which is perfused by the external pudendal arteries bilaterally. The second territory includes the glans and corpus spongiosum, which are supplied by the dorsal arteries. Finally, the corpora cavernosa are perfused by the cavernous arteries. The dorsal and cavernous arteries both originate from the internal pudendal artery. However, depending on the level of the penis transplantation, each may require its own vascular anastomosis<sup>[29]</sup>. Venous outflow similarly depends upon the extent of graft required. In a mid-shaft transplantation, this could be limited to as little as the deep and superficial dorsal veins, whereas, in the most extensive penis, scrotum, and abdominal wall transplantation performed to date, the dorsal veins were anastomosed in addition to the bilateral saphenous veins<sup>[7]</sup>. Similar to replantation, the donor dorsal penile nerves can be coapted with the recipient dorsal penile nerves, and the donor urethra anastomosed with the recipient urethra in a spatulated fashion. The tunica albuginea, Buck's fascia, and dartos fascia are also connected in addition to the skin between donor and recipient tissues.

## Postoperative care

Postoperatively, transplant recipients must have access to the appropriate monitoring to minimize both medical and psychological risks. Psychological counselling should begin during the pre-transplant work-up and be continued afterward to ensure that the patient integrates the graft with their sense of identity. Certain sexually transmitted infections may be particularly devastating to the graft within the context of systemic immunosuppression, and safe sex counselling is essential to the patient's long-term safety. Given the intimate nature of penile transplants, sexual partners should also be involved in care if the recipient so wishes. Relationship counselling provides an opportunity to ensure that the recipient has a stable support network to assist with the necessary emotional adjustments and can also be an essential component of these patients' care.



Our preference for perioperative antibacterial prophylaxis is intravenous piperacillin/tazobactam 3.375 g prior to and during the operation. Prophylaxis is continued with cefazolin 2 g IV every 8 h for additional three days. For antiviral prophylaxis, we use valacyclovir 500 mg twice daily for 12 months. Testing is performed for herpes simplex virus I/II, varicella zoster virus, Epstein-Barr virus, and cytomegalovirus, and prophylaxis can be adjusted as needed based on these results. Pneumocystis prophylaxis is with daily trimethoprim-sulfamethoxazole 400 mg/80 mg for a total of 12 months. Anti-candida prophylaxis is with fluconazole 800 mg loading dose followed by 400 mg daily for one month.

Immunosuppression regimens vary from one center to the other. At our center, a recipient is treated with an immunomodulatory regimen consisting of monoclonal antibody induction, calcineurin inhibitor (tacrolimus) monotherapy maintenance, and a donor bone marrow cell infusion.

## Outcomes

Successful urinary transport is generally achieved in penis transplantation. This is not surprising given the high success rates with replantation and primary anastomotic urethroplasties where two segments of healthy urethra are reapproximated. This is unlike phalloplasty, where tubularized skin is used to create a neourethra, allowing for the high complication rates discussed above. Several case series have demonstrated that it is possible to obtain natural erections after penile replantation<sup>[61]</sup>. Thus far, the experience has been similar with the limited series of penile transplantations<sup>[7]</sup>. Furthermore, at one year postoperatively, the most recent patient who received the most extensive penile transplantation to date reports the ability to achieve orgasm, as well as substantial improvements in pleasure scores on patient-reported outcome measures<sup>[7]</sup>.

## CONCLUSION

Over 30 years after Gilbert and Winslow<sup>[22]</sup> outlined the five criteria to achieve ideal phallic reconstruction, the surgical community has finally realized this objective. Although conventional techniques including RFFF, ALT, FOF, and LDMF have gained popularity as common flaps used in penile reconstruction, these procedures are often fraught with complications and require multiple stages and revisions before achieving an acceptable result. Penile transplantation represents a single-stage procedure that allows the recipient to void while standing, provides protective and erogenous sensation as well as the ability to achieve an erection, and results in an unmatched aesthetic outcome [Figures 2, 8, and 9].

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception, design, and execution of the review: Khavanin N, Redett RJ

### Availability of data and materials

All data and materials used in the current article are available from the corresponding author upon request.

### Financial support and sponsorship

None.

### Conflicts of interest

Both authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

The Johns Hopkins School of Medicine Penile transplantation protocol has been reviewed and approved by the Institutional Review Board. All participants of the program undergo extensive medical and psychosocial

screening and provide explicit written informed consent for study participation. Study procedures are performed in accordance to the Declaration of Helsinki.

### Consent for publication

All individuals whose personal data were included within this review provided explicit written consent for publication of these data.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Bogoraz N. On complete plastic reconstruction of a penis sufficient for coitus. *Sov Surg* 1936;8:303-9.
2. Gillies SH, Harrison R. Congenital absence of the penis. *Br J Plast Surg* 1948;1:8-28.
3. Yao A, Ingargiola MJ, Lopez CD, Sanati-Mehrziy P, Burish NM, et al. Total penile reconstruction: a systematic review. *J Plast Reconstr Aesthet Surg* 2018;71:788-806.
4. Kropp B, Cohn JE, Wang W, Sokoya M, Ducic Y. Free tissue transfer penile reconstruction. *Semin Plast Surg* 2019;33:24-9.
5. Salmerón-González E, Simón-Sanz E, García-Vilarino E, Sánchez-García A, Pérez-García A, et al. Technical detail on nerve coaptation in phalloplasty: use of fibrin glue instead of sutures. *Sex Med Rev* 2019;7:376.
6. Massanyi EZ, Gupta A, Goel S, Gearhart JP, Burnett AL, et al. Radial forearm free flap phalloplasty for penile inadequacy in patients with exstrophy. *J Urol* 2013;190:1577-82.
7. Redett RJ 3rd, Etra JW, Brandacher G, Burnett AL, Tuffaha SH, et al. Total penis, scrotum, and lower abdominal wall transplantation. *N Engl J Med* 2019;381:1876-8.
8. Cetrulo CL Jr, Li K, Salinas HM, Treiser MD, Schol I, et al. Penis transplantation: first US experience. *Ann Surg* 2018;267:983-8.
9. Bateman C. World's first successful penis transplant at Tygerberg Hospital. *S Afr Med J* 2015;105:251-2.
10. Hu W, Lu J, Zhang L, Wu W, Nie H, et al. A preliminary report of penile transplantation: part 2. *Eur Urol* 2006;50:1115-6; discussion 1116.
11. Hu W, Lu J, Zhang L, Wu W, Nie H, et al. A preliminary report of penile transplantation. *Eur Urol* 2006;50:851-3.
12. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin* 2018;68:7-30.
13. Ornellas AA. Management of penile cancer. *J Surg Oncol* 2008;97:199-200.
14. Pow-Sang MR, Ferreira U, Pow-Sang JM, Nardi AC, Destefano V. Epidemiology and natural history of penile cancer. *Urology* 2010;76:S2-6.
15. Hudak SJ, Morey AF, Rozanski TA, Fox CW Jr. Battlefield urogenital injuries: changing patterns during the past century. *Urology* 2005;65:1041-6.
16. Tuffaha SH, Cooney DS, Sopko NA, Bivalacqua TJ, Lough DM, et al. Penile transplantation: an emerging option for genitourinary reconstruction. *Transpl Int* 2017;30:441-50.
17. Janak JC, Orman JA, Soderdahl DW, Hudak SJ. Epidemiology of genitourinary injuries among male U.S. service members deployed to Iraq and Afghanistan: early findings from the trauma outcomes and urogenital health (TOUGH) project. *J Urol* 2017;197:414-9.
18. The Williams Institute. How many adults identify as transgender in the United States? Available from: <https://williamsinstitute.law.ucla.edu/wp-content/uploads/How-Many-Adults-Identify-as-Transgender-in-the-United-States>. [Last accessed on 5 Aug 2020]
19. Bluebond-Langner R, Redett RJ. Phalloplasty in complete aphallia and ambiguous genitalia. *Semin Plast Surg* 2011;25:196-205.
20. Gearhart JP, Mathews R. Exstrophy-epispadias complex. In: Wein AJ, Kavoussi LR, Novick AC, Partin AW, Peters CA, editors. *Campbell-Walsh Urology*. 9th ed. Philadelphia: Saunders; 2007. pp. 3497-555.
21. Tuffaha SH, Sacks JM, Shores JT, Brandacher G, Lee WP, et al. Using the dorsal, cavernosal, and external pudendal arteries for penile transplantation: technical considerations and perfusion territories. *Plast Reconstr Surg* 2014;134:111e-9.
22. Gilbert DA, Winslow BH. Penis construction. *Semin Urol* 1987;5:262-9.
23. Monstrey S, Hoebeke P, Selvaggi G, Ceulemans P, Van Landuyt K, et al. Penile reconstruction: is the radial forearm flap really the standard technique? *Plast Reconstr Surg* 2009;124:510-8.
24. Satteson ES, Satteson AC, Waltonen JD, Li Z, Wiesler ER, et al. Donor-site outcomes for the osteocutaneous radial forearm free flap. *J Reconstr Microsurg* 2017;33:544-8.
25. Biemer E. Penile construction by the radial arm flap. *Clin Plast Surg* 1988;15:425-30.
26. Garaffa G, Raheem AA, Christopher NA, Ralph DJ. Total phallic reconstruction after penile amputation for carcinoma. *BJU Int* 2009;104:852-6.
27. Ma S, Cheng K, Liu Y. Sensibility following innervated free radial forearm flap for penile reconstruction. *Plast Reconstr Surg* 2011;127:235-41.
28. Rubino C, Figus A, Dessy LA, Alei G, Mazzocchi M, et al. Innervated island pedicled anterolateral thigh flap for neo-phallic reconstruction in female-to-male transsexuals. *J Plast Reconstr Aesthet Surg* 2009;62:e45-9.
29. Mutaf M. A new surgical procedure for phallic reconstruction: Istanbul flap. *Plast Reconstr Surg* 2000;105:1361-70.

30. Mutaf M, Isik D, Bulut O, Büyükgöral B. A true one-stage nonmicrosurgical technique for total phallic reconstruction. *Ann Plast Surg* 2006;57:100-6.
31. Morrison SD, Shakir A, Vyas KS, Kirby J, Crane CN, et al. Phalloplasty: a review of techniques and outcomes. *Plast Reconstr Surg* 2016;138:594-615.
32. Dabernig J, Chan LK, Schaff J. Phalloplasty with free (septocutaneous) fibular flap sine fibula. *J Urol* 2006;176:2085-8.
33. Hage JJ, Winters HAH, Van Lieshout J. Fibula free flap phalloplasty: modifications and recommendations. *Microsurgery* 1996;17:358-65.
34. Capelouto CC, Orgill DP, Loughlin KR. Complete phalloplasty with a prelaminated osteocutaneous fibula flap. *J Urol* 1997;158:2238-9.
35. Christiano JG, Dorafshar AH, Rodriguez ED, Redett RJ. Repair of recurrent cleft palate with free vastus lateralis muscle flap. *Cleft Palate Craniofac J* 2012;49:245-8.
36. Woerdeman LAE, Chaplin BJ, Griffioen FMM, Bos KE. Sensate osteocutaneous fibula flap: anatomic study of the innervation pattern of the skin flap. *Head Neck* 1998;20:310-4.
37. Schaff J, Papadopoulos NA. A new protocol for complete phalloplasty with free sensate and prelaminated osteofasciocutaneous flaps: experience in 37 patients. *Microsurgery* 2009;29:413-9.
38. van Rooij JC, Plomp R. The effect of linguistic entropy on speech perception in noise in young and elderly listeners. *J Acoust Soc Am* 1991;90:2985-91.
39. Djordjevic ML, Bumbasirevic MZ, Vukovic PM, Sansalone S, Perovic SV. Musculocutaneous latissimus dorsi free transfer flap for total phalloplasty in children. *J Pediatr Urol* 2006;2:333-9.
40. Perovic SV, Djinic R, Bumbasirevic M, Djordjevic M, Vukovic P. Total phalloplasty using a musculocutaneous latissimus dorsi flap. *BJU Int* 2007;100:899-905; discussion 905.
41. Lugo-Fagundo C, Ahn H, O'Brien-Coon D, Fishman EK. The role of cinematic rendering in pre-operative planning of a thoracodorsal artery perforator flap (TDAP) phalloplasty: a case study. *BJR Case Rep* 2019;5:20180084.
42. Djordjevic ML. Novel surgical techniques in female to male gender confirming surgery. *Transl Androl Urol* 2018;7:628-38.
43. Dong L, Dong Y, He L, Liu C, Zhang Z, et al. Penile reconstruction by preexpanded free scapular flap in severely burned patient. *Ann Plast Surg* 2014;73 Suppl 1:S27-30.
44. Ranno R, Vesely J, Hyza P, Stupka I, Justan I, et al. Neo-phalloplasty with re-innervated latissimus dorsi free flap: a functional study of a novel technique. *Acta Chir Plast* 2007;49:3-7.
45. Hoebeke PB, Decaestecker K, Beysens M, Opendakker Y, Lumen N, et al. Erectile implants in female-to-male transsexuals: our experience in 129 patients. *Eur Urol* 2010;57:334-40.
46. Garaffa G, Raheem AA, Ralph DJ. An update on penile reconstruction. *Asian J Androl* 2011;13:391-4.
47. Monstrey SJ, Ceulemans P, Hoebeke P. Sex reassignment surgery in the female-to-male transsexual. *Semin Plast Surg* 2011;25:229-44.
48. Sonmez E, Nasir S, Siemionow M. Penis allotransplantation model in the rat. *Ann Plast Surg* 2009;62:304-10.
49. Koga H, Yamataka A, Wang K, Kato Y, Lane GJ, et al. Experimental allogenic penile transplantation. *J Pediatr Surg* 2003;38:1802-5.
50. Akyurek M, Ozkan O, Safak T, Ozgentas HE, Dunn RM. The penile flap in the rat: description and autotransplantation. *Ann Plast Surg* 2005;55:94-100; discussion 101.
51. Zhao Y, Hu W, Zhang L, Guo F, Wang W, et al. Penis allotransplantation in beagle dog. *Biomed Res Int* 2016;2016:1489204.
52. Sopko NA, Matsui H, Lough DM, Miller D, Harris K, et al. Ex vivo model of human penile transplantation and rejection: implications for erectile tissue physiology. *Eur Urol* 2017;71:584-93.
53. van der Merwe A, Graewe F, Zühlke A, Barsdorf NW, Zarrabi AD, et al. Penile allotransplantation for penis amputation following ritual circumcision: a case report with 24 months of follow-up. *Lancet* 2017;390:1038-47.
54. Ngaage LM, Elegbede A, Sugarman J, Nam AJ, Cooney CM, et al. The baltimore criteria for an ethical approach to penile transplantation: a clinical guideline. *Transpl Int* 2020;33:471-82.
55. Diaz-Siso JR, Borab ZM, Plana NM, Parent B, Stranix JT, et al. Vascularized composite allotransplantation: alternatives and catch-22s. *Plast Reconstr Surg* 2018;142:1320-6.
56. Sixty-Third World Health Assembly, World Health Organization. WHO Guiding Principles on Human Cell, Tissue and Organ Transplantation. *Cell Tissue Bank* 2010;11:413-9.
57. Marchac A, Kushner T, Paris J, Picard A, Vazquez MP, et al. Ethical issues in pediatric face transplantation: should we perform face transplantation in children? *Plast Reconstr Surg* 2016;138:449-54.
58. Amaral S, Levin LS. Pediatric and congenital hand transplantation. *Curr Opin Organ Transplant* 2017;22:477-83.
59. Leijte JA, Kirrander P, Antonini N, Windahl T, Horenblas S. Recurrence patterns of squamous cell carcinoma of the penis: recommendations for follow-up based on a two-centre analysis of 700 patients. *Eur Urol* 2008;54:161-8.
60. Pomahac B, Alhefzi M, Bueno EM, McDiarmid SV, Levin LS. Living donation of vascularized composite allografts. *Plast Reconstr Surg* 2018;142:405e-11.
61. Sopko NA, Tuffaha SH, Lough D, Brandacher G, Lee WPA, et al. Penile allotransplantation for complex genitourinary reconstruction. *J Urol* 2017;198:274-80.

Original Article

Open Access



# Facial morphospace: a clinical quantitative analysis of the three-dimensional face in patients with cleft lip and palate

Chihiro Tanikawa

Department of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, Osaka University, Osaka 565-0871, Japan.

**Correspondence to:** Assoc Prof./Dr. Chihiro Tanikawa, Department of Orthodontics and Dentofacial Orthopedics, Graduate School of Dentistry, Osaka University, Osaka 565-0871, Japan. E-mail: ctanika@dent.osaka-u.ac.jp

**How to cite this article:** Tanikawa C. Facial morphospace: a clinical quantitative analysis of the three-dimensional face in patients with cleft lip and palate. *Plast Aesthet Res* 2020;7:48. <http://dx.doi.org/10.20517/2347-9264.2020.136>

**Received:** 15 Jun 2020 **First Decision:** 27 Jul 2020 **Revised:** 5 Aug 2020 **Accepted:** 20 Aug 2020 **Published:** 12 Sep 2020

**Academic Editor:** Carroll Ann Trotman **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

**Aim:** The aims were: (1) to examine the clinical application of a geometric morphometric method (GMM) that quantifies the three-dimensional (3D) configuration of the facial soft tissues in patients with a repaired unilateral cleft lip and palate (UCLP); and (2) to determine the morphological characteristics that distinguish between non-cleft participants and patients with UCLP.

**Methods:** 3D facial images at rest were recorded from Japanese patients with a repaired UCLP (Cleft group;  $n = 60$ ) and healthy adults featuring a straight type facial profile with normal occlusion (Control group;  $n = 200$ ) using 3D photogrammetric cameras. For each participant, wire mesh fitting was conducted based on the assignment of landmarks to each 3D facial image. This method generated landmark-based GMM models consisting of 6017 nodes on the fitted wire mesh. For each node, the mean and standard deviation were determined in the Control group and were used as the normative range of the faces. With this normative range, the Z-scores before and after surgery were evaluated for patients with UCLP who underwent orthognathic bimaxillary surgery. Further, the morphological characteristics of the Cleft group were evaluated using a principal component (PC) regression analysis that distinguished between two subject groups. In addition, K-means clustering analysis and MANOVA were used to examine the morphological variation of the Cleft group.

**Results:** A patient with UCLP was evaluated with the system. After surgery, the normal area increased by 8%-20% on all axes, which means that the surgery was effective for normalizing the patient's face. However, even after



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



surgery, the protrusion of the lower lip and asymmetry remained. Nine PCs were extracted, and seven PCs were selected for the regression model to discriminate two subject groups, e.g., midfacial retrusion, nasal bump, and chin protrusion. The MANOVA also revealed significant differences between both the Cleft and Control groups and the sex subgroups, and the effects of cleft on the facial morphology was found to be related to sex (all,  $P < 0.01$ ).

**Conclusion:** The clinical application of GMM was confirmed to be effective. GMM detected variations of the Cleft group and morphological characteristics. GMM is considered to be a powerful tool to quantitatively evaluate faces in clinics.

**Keywords:** Cleft lip and palate, face, geometric morphometric analysis, three-dimensional analysis

## INTRODUCTION

The extent of recognition of an individual's own face by others exerts a great sociopsychological influence on that individual's sense of acceptance by his or her community. It is known that there are several facial deformities associated with congenital cleft lip after primary lip revision surgery. It is thus crucial for patients with cleft lip and/or palate to normalize their facial morphology with primary repair and any subsequent revision surgery. The extent and type of these facial deformities varies among patients due to the extent of the original abnormality and any prior surgeries performed<sup>[1-3]</sup>. Several experts have recommended using subjective checklists to provide assessments of the extent and type of facial abnormalities<sup>[4,5]</sup>; however, this could result in bias. The development of a systematic method for the evaluation and classification of the morphological traits of the cleft facial shape could greatly facilitate the surgical/orthodontic diagnosis and the design of treatment plans for the optimization of treatment outcomes.

The analysis of the form of the nose and lip is important for revision surgery and many attempts have been made to establish systematic analytical methods. Conventional photographs have been employed in some studies<sup>[3,6,7]</sup>. Several three-dimensional (3D) analysis techniques, including direct anthropometry of the human face<sup>[8,9]</sup>, stereophotogrammetry<sup>[10,11]</sup>, laser scanning, gypsum cast<sup>[12-14]</sup>, and computer tomography<sup>[15]</sup>, have been employed to assess the facial morphology of patients with cleft lip. Because these analyses are primarily conducted with the use of linear and/or angular measurements between/among landmarks, the detailed morphological characteristics of the entire facial surface forms are not considered. There have also been a few attempts to extract detailed morphological characteristics of the entire facial surface forms using the slice lines. In these studies, traced nasal lines in an axial view were assessed using a curve fitting method that provides the extent of asymmetry<sup>[12]</sup>, and an automatically extracted nostril shape was assessed<sup>[16]</sup>. These studies succeeded in describing the morphological characteristics of the nasal surface; they mainly attempted to describe the nasal forms in an axial view and the nostril shapes before and after surgery. A 3D quantitative analysis of the lip was also reported, in which the morphological traits of the lip surfaces were measured from the frontal and lateral views<sup>[17]</sup>. However, thus far, few studies have reported the 3D holistic analysis of the entire facial form from all three views (i.e., frontal, lateral, and axial views).

With recent computation advances, landmark-based geometric morphometric methods (GMMs) have recently emerged that-together with conventional measurements in medicine-have revealed some statistical variation in the shape and size of target objects (phenotypic variation). In developmental biology, GMMs use homologous landmarks, which can be defined as precise locations on biological specimens that hold some functional, structural, developmental, or evolutionary significance, and which are directly comparable between specimens<sup>[18]</sup>. GMMs have four types of landmarks: Type I landmarks can be clearly defined on a



structure (e.g., the corner of the mouth); Type II landmarks are more ambiguous and usually describe the maxima of curvature (e.g., nasal tip); Type III landmarks are geometric constructions generated from lines or Type I and/or II landmarks (e.g., midpoint of the right and left eyes); and semi-landmarks represent surfaces or curves between landmarks<sup>[18]</sup>. A key concept of GMM is based on the fact that morphology can be mapped systematically, often within a “morphospace”, with the use of these landmarks. Morphospaces are maps that show how shapes are defined by quantitative traits. GMMs rely on the superimposition of landmark coordinate data to place individuals in a common morphospace.

Recently, the clinical application of a GMM—a novel 3D quantitative analysis method—in the quantification and visualization of the 3D configuration of the facial soft tissues was reported<sup>[19]</sup>. 3D faces were fitted with mathematical wire meshes based on 26 landmarks, and the nodes of the fitted meshes were used as semi-landmarks. All faces were superimposed based on the landmarks and were statistically analyzed in the facial morphospace. The analysis included the average range of faces with regard to sex, age, and race, and the method compared a patient's face with this normal range. This enabled us to understand the patient's static facial form characteristics quantitatively and instantaneously. In their report, the applicability of the system to three cases, namely one case each of Class II malocclusion, Class III malocclusion, and jaw deviation, was reported; however, the applicability of this system to the cleft facial shape remains unclear.

Thus, the objectives of this study were as follows: (1) to examine whether the previously published soft-tissue evaluation methods developed based on GMMs<sup>[19]</sup> could be applied to patients with a cleft lip before or after treatment; (2) to determine soft-tissue morphological characteristics that distinguish non-cleft participants from patients with a cleft lip; and (3) to examine the variations of cleft facial shapes. Furthermore, we discuss the clinical application of the GMM based on the results of Objectives (1)–(3).

## METHODS

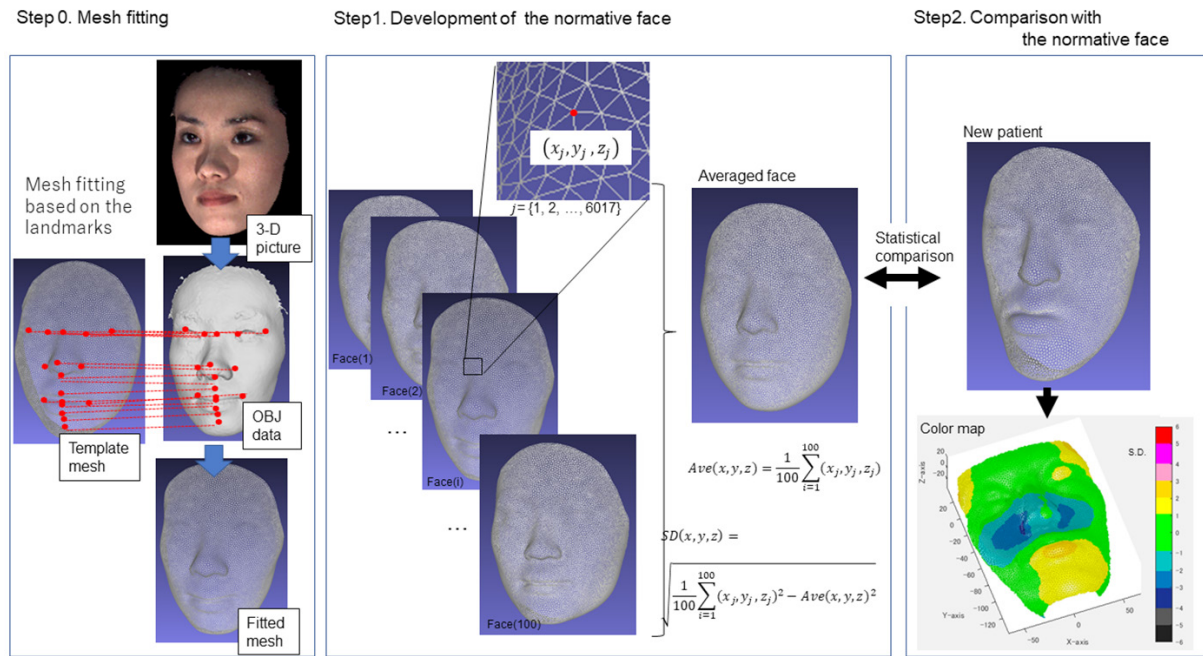
The study was approved by the ethics committee for medical research at Osaka University Dental Hospital (ID: H25-E37-1). An informed consent to participate in the study was obtained from all participants.

### Systems

A system for evaluating facial morphology that was developed in a previous study<sup>[19]</sup> was employed in the present study. The overview of the system is shown in Figure 1. The system was divided into three parts: (1) mesh fitting on the 3D facial picture; (2) development of the normative ranges of faces; and (3) evaluation of the new face with the normative face. In the present study, the normative range of the face was defined as the average and  $\pm 1$  standard deviation ( $\pm 1$  SD), and the patient data were compared to the normative range of faces. To develop the normative ranges, 200 participants (female,  $n = 100$ ; male,  $n = 100$ ) between 18 and 35 years of age were recruited from the students and faculty of Osaka University in Japan. For the detailed inclusion criteria, please see the previous study<sup>[19]</sup>. Then, the normative faces, including the mean coordinate values and the standard deviation for each sex group, were used to evaluate new patients. The equations were as follows:

$$\begin{aligned} Z\text{-score}_{(x)} &= |p_{(x)} - m_{(x)}|/s_{(x)} \\ Z\text{-score}_{(y)} &= -(p_{(y)} - m_{(y)})/s_{(y)} \\ Z\text{-score}_{(z)} &= (p_{(z)} - m_{(z)})/s_{(z)} \end{aligned}$$

where  $p_{(x)}$ ,  $p_{(y)}$ , and  $p_{(z)}$  indicate the coordinate values of each image of the sample patients;  $m_{(x)}$ ,  $m_{(y)}$ , and  $m_{(z)}$  indicate the average coordinate values of the control group; and  $s_{(x)}$ ,  $s_{(y)}$ , and  $s_{(z)}$  indicate the standard deviation of the coordinate values of the control group in the x-, y-, and z-directions, respectively. To visualize the results, the Z-scores were visualized as color values. For a detailed description, please see the previous study<sup>[19]</sup>.



**Figure 1.** A schematic illustration of the wire mesh fitting (Step 0), the generation of averaged faces (Step 1), and their statistical analysis (Step 2)

## Samples

### *Application of the system to patients with unilateral cleft lip and palate*

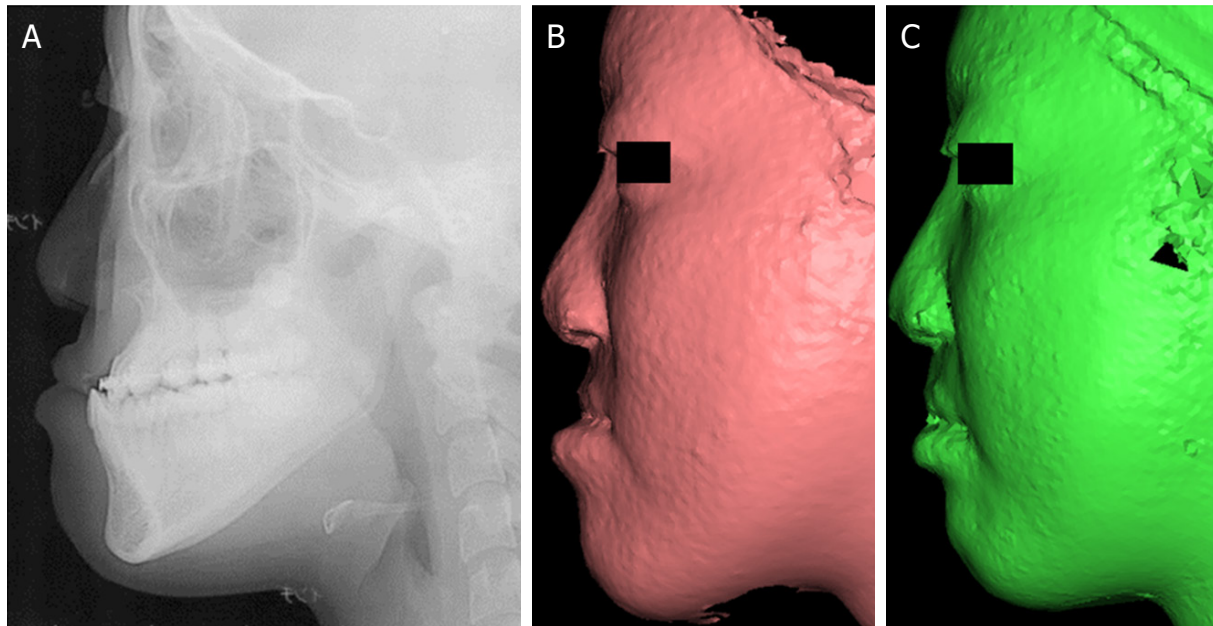
To demonstrate the applicability of the system, one Japanese patient with unilateral cleft lip and palate (UCLP) (example case) was selected from the patients who visited our department to undergo orthognathic bimaxillary surgery (Le Fort I maxillary surgery and bilateral sagittal split osteotomy, [Figure 2](#)). The details of the treatment have been described elsewhere<sup>[20]</sup>.

### *Morphological characteristics of the faces of patients with UCLP*

Japanese patients with a repaired UCLP (Cleft group,  $n = 60$ ; mean age,  $22.0 \pm 5.0$  years; male,  $n = 36$ ; female,  $n = 24$ ) and healthy adults with a straight-type facial profile with normal occlusion (Control group:  $n = 200$ ; mean age,  $25.4 \pm 5.3$  years; male,  $n = 100$ ; female,  $n = 100$ ) were enrolled in the present study. The Control group samples were the same as those used in the previous study<sup>[21]</sup>. The inclusion criteria for the Cleft group were as follows: UCLP, patients who visited the orthodontic department, Osaka University Dental Hospital, during 2011-2015; age 15-37 years; positive overjet (i.e., most patients were in the post-treatment “retention” period); no facial paralysis; body mass index, 18.50-24.99; and no maxillofacial plastic surgery in the past six months. A written informed consent form was distributed to and signed by all participants.

## Data acquisition

The subjects were asked to sit on a fixed chair with a natural head position without head support. Facial images at rest were recorded once with a 3D image capturing device (3-DMDcranial System, 3-DMD, Atlanta, GA, USA). This data acquisition was conducted once for each patient. For an example case to show the pre- and post-treatment changes, 3D images were acquired three months before and six months after treatment.



**Figure 2.** Cephalogram and 3D facial images of a patient with cleft lip and palate: cephalogram (A); 3D image pre-treatment (B); and 3D image post-treatment (C). These images were used for applicability of the system proposed in the previous study<sup>[19]</sup>

## Data processing

### Landmark identification

Each 3D facial image was displayed on a monitor, and the positions of 18 landmarks [nasion, pronasale, subnasale, labiale superius, stomion, labiale inferius, submentale, pogonion, porion (right and left), exocanthion (right and left), endocanthion (right and left), alar curvature (right and left), and cheilion (right and left)<sup>[8]</sup>] were identified and digitized using a commercial software program (Face Rugle, Medic Engineering Co., Kyoto, Japan). Facial images were standardized with a common coordinate system [Figure 3]<sup>[21]</sup>.

### Wire mesh fitting

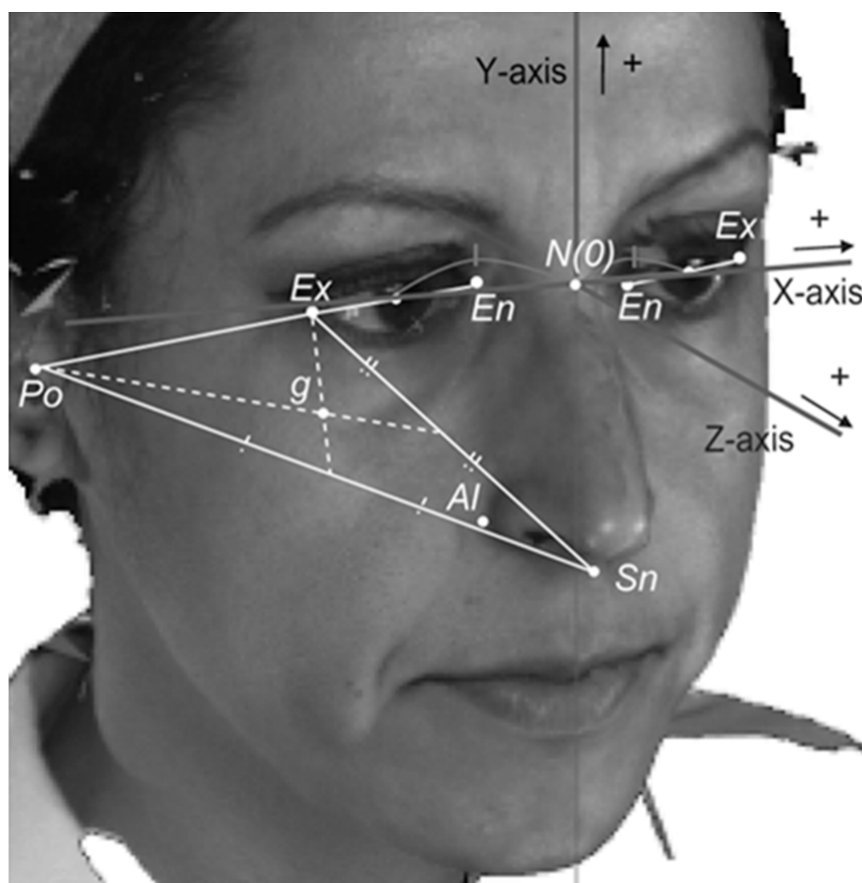
For each participant, landmark-based GMM analysis<sup>[22]</sup> was performed using HBM software (National Institute of Advanced Industrial Science and Technology, Japan). This analysis generated a fitted mesh consisting of a set of 6017 points as nodes. The arithmetic means  $m_{(x)}$ ,  $m_{(y)}$ , and  $m_{(z)}$  and standard deviations  $s_{(x)}$ ,  $s_{(y)}$ , and  $s_{(z)}$  of the points were computed, and  $m_{(x)} \pm s_{(x)}$ ,  $m_{(y)} \pm s_{(y)}$ , and  $m_{(z)} \pm s_{(z)}$  were used as the normative range for each point in the X-, Y-, and Z-directions, respectively.

### Quantitative facial evaluation before and after treatment

For the example case, the system was applied to exemplify the system results. Surface areas showing  $-1$  SD  $<$  Z-score  $< 1$  SD were defined as the normal area. The percentages of the surface before and after treatment were evaluated in each axis and presented as radar charts.

### Morphological differences between Cleft and Control groups

For the Cleft and Control groups, to determine morphological characteristics of the facial soft tissue surface, the coordinate values of each node of the wire mesh on the facial surface on the x, y, and z axes were statistically analyzed. A two-sample *t*-test was performed to compare each axis in the two subject groups. To visualize the differences between the two subject groups, the results were represented as a color map showing the *P*-values for the comparison between the two subject groups (hereafter referred to as the significance probability map) and a color map representing the differences between two subject groups (hereafter referred to as the distance map)<sup>[21,22]</sup>. *P* values of  $< 0.05$  were considered to indicate statistical significance.



**Figure 3.** The coordinate system<sup>[19]</sup>. The 3D coordinate system. The nasion (N) was defined as the origin (O). The sagittal plane was defined as the plane passing through the origin and perpendicular to the line through the midpoint of the right exocanthion (Ex) and endocanthion (En) and the midpoint of the left Ex and En. The axial plane was defined as the plane passing through the origin and parallel to the line connecting the porion and the geometric center (g) of the porion (Po), subnasale (Sn), and Ex on the image projected onto the sagittal reference plane. The coronal plane was defined as a plane passing through the origin and perpendicular to both the axial and sagittal planes (cited from Tanikawa *et al.*<sup>[21]</sup>). Images of the participants with a right unilateral cleft lip were “mirrored” to left side to simulate a left unilateral cleft lip

### *Morphological variation of the Cleft group*

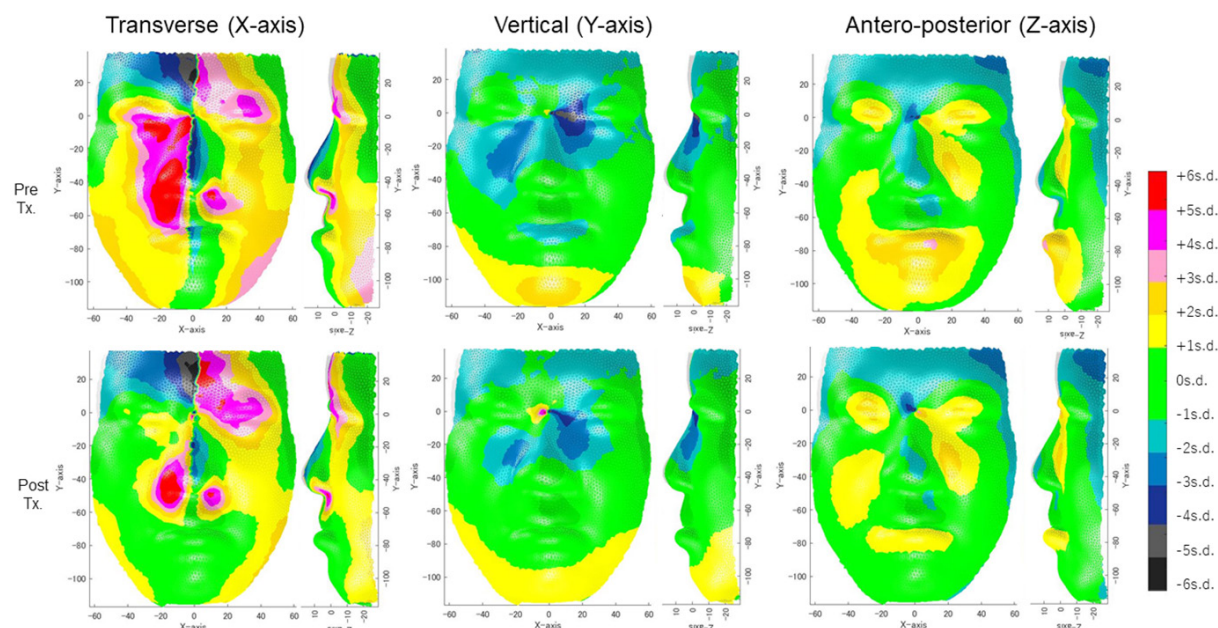
To examine the variance in each group, each sex, and their interactions, the following two analyses were also conducted. We first performed a principal component analysis for the 6017 coordinates of each point on the facial surface in the x, y, and z axes to reduce dimensionality. Significant principal components (PCs) were determined by a scree plot analysis (90% was used as the cut off value). Significant PCs were entered into a regression model that decimated the Control and Cleft groups ( $P < 0.01$ ). PCs were also entered into a MANOVA to test for significance of factors (i.e., cleft/non-cleft and sex). Then, to examine the patterns of the face and variation of the Cleft group, the patients in the Cleft group were categorized based on the similarities in the morphospace constructed above PCs using a clustering method (k-means and Elbow method).

## **RESULTS**

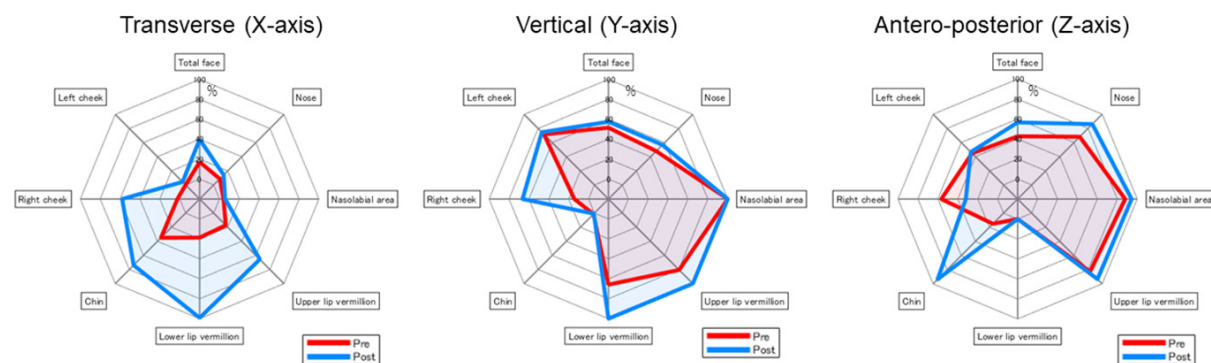
### **Quantitative facial evaluation before and after treatment**

Figure 4 shows the results of the application of the system to a patient with UCLP before and after treatment. The percentage of the normal area ( $-1 \text{ SD} < Z\text{-score} < 1 \text{ SD}$ ) on each axis is shown in Figure 5 for each facial region before and after treatment. In total, the percentage of the normal area was increased from 20% to 40% on the x-axis (transverse direction); from 52% to 59% on the y-axis (vertical direction);





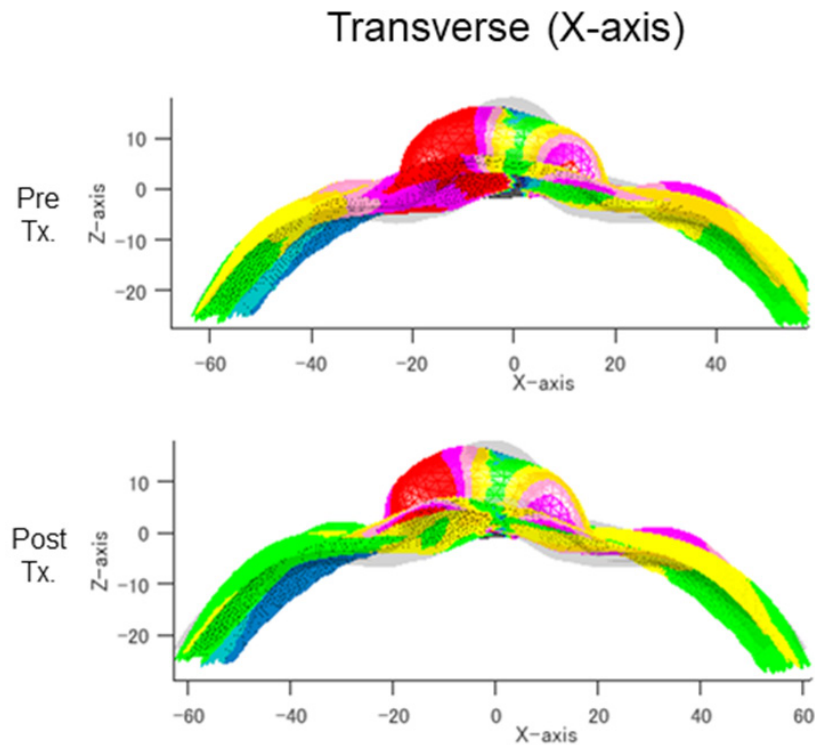
**Figure 4.** Verification of the system using a cleft case. Grey profile indicates an average face: (top) pre-surgery; and (bottom) post-surgery. (left) The standardized X-values of the 3D face when compared with the normative mean (transverse direction). Red, pink, light yellow, and deep yellow represent the point clouds located outwards when compared with the normative mean; light blue, deep blue, grey, and black indicate the points located inwards (close to the medial line). Green represents the range within  $\pm 1$  SD. The left column indicates the standardized Z-value (transverse direction). Before treatment, the right nasal alar and nasal tip were deviated to the right side by more than 6 SD. After treatment, the area showing more than 1 SD was decreased; however, the right nasal alar was still deviated by 6 SD (for the axial view, please see Figure 6). (middle) The standardized Y-value (vertical direction). Red, pink, light yellow, and deep yellow indicate the corresponding point clouds located inferiorly, whereas light blue, deep blue, grey, and black indicate the points located superiorly when compared with the normative mean. In this patient, before treatment, the lower lip was located superiorly by 1 SD, and the chin was located inferiorly by more than 2 SD and showed greater facial height. After treatment, the lower lip was located within the normal range, indicating that the lower lip had moved downwards after maxillary advancement and mandibular set-back movement due to the surgery. (right) The standardized Z-value (antero-posterior direction). Red, pink, light yellow, and deep yellow indicate the corresponding point clouds located posteriorly when they were compared to the normative mean. In this patient, before treatment, the nasal dorsum and left upper lip (repaired cleft site) retruded by more than 1 SD, while the chin protruded by more than 3 SD. After treatment, the chin showed normal antero-posterior position while the lower lip showed slight retrusion by more than 1 SD. The area of the retrusion in the nasal dorsum and upper lip was also decreased



**Figure 5.** Radar chart of the normal area for the example case with unilateral cleft lip and palate [Figure 2]. Surface areas showing  $-1 \text{ SD} < \text{Z-score} < 1 \text{ SD}$  were defined as the normal area. The percentage of the surface before (red) and after treatment (blue) was evaluated on: x-axis (left); y-axis (middle); and z-axis (right)

and from 41% to 59% on the z-axis (antero-posterior direction). In detail, the figures for the x-axis show that before surgery there was greater asymmetry in the cheeks, the nose, nasolabial region, upper lip,





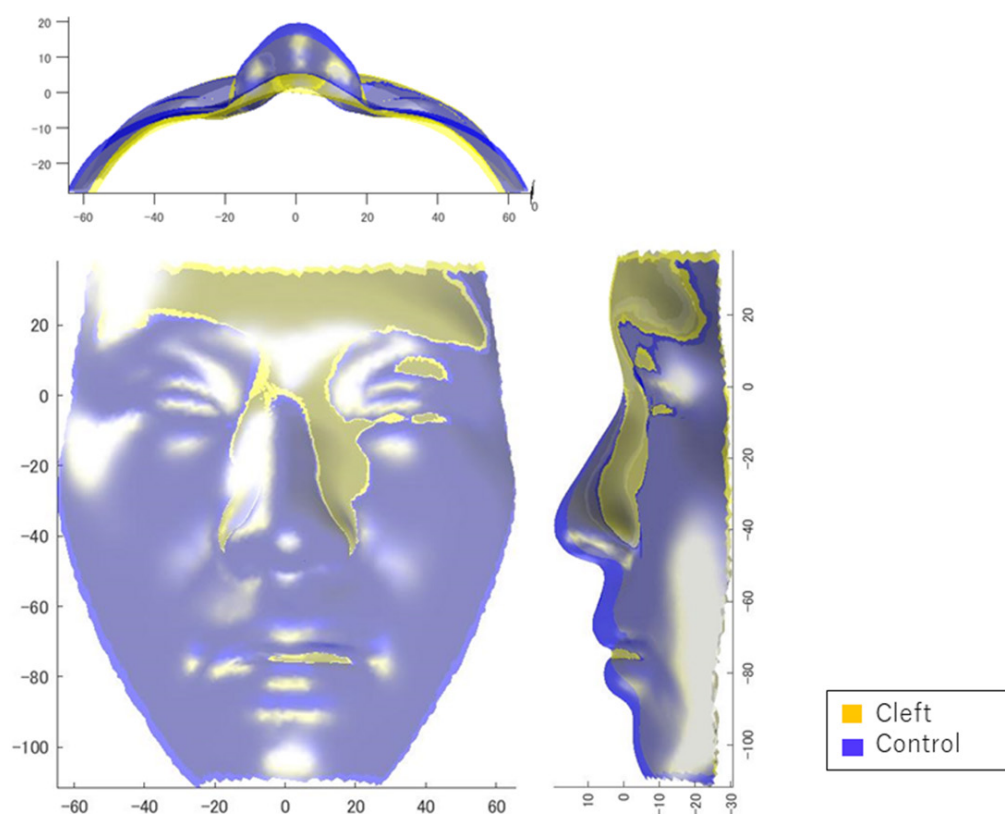
**Figure 6.** Axial view of the standardized X-values of the 3D nasal dorsum in comparison to the normative mean (transverse direction): (top) pre-surgery; and (bottom) post-surgery. Grey indicates the averaged face of the Control group (female;  $n = 100$ ). Red, pink, light yellow, and deep yellow represent the point clouds located outwards when compared with the normative mean; light blue, deep blue, grey, and black indicate the points located inwards (close to the medial line). Green represents the range within  $\pm 1$  SD. Before treatment, the right nasal base was deviated to the right side by more than 6 SD before and after treatment. The left nasal base was also deviated to the left side by more than 5 SD before and after treatment. These results indicate that the width of the nose was greater in comparison to the normative range, which was not corrected, even after bimaxillary surgery

lower lip, and chin. After surgery, the asymmetry in the upper and lower lip vermillion (the normal area changed from 19% to 84% and from 20% to 100%, respectively; [Figure 5](#), left) and the right cheek improved remarkably (from 20% to 60%); however, the nose and nasolabial area and left cheek showed deviation to the right (x-axis), which is clearly observed in [Figure 6](#) showing the axial view. In the z-axis, the figures also show that, before surgery, the patient had a retruded area corresponding the cleft scar (-1 SD) and a protruded chin (+1 to +2 SD). After surgery, the retrusion of the chin (normal area was changed from 18% to 98%; [Figure 5](#), right) and lower lip improved remarkably, yet maintained a mean of -1 SD of the normative face for the lower lip (z-axis). As for the vertical direction, the upper lip vermillion was displaced downward (y-axis) into the normative range after surgery (from 90% to 100%; [Figure 5](#), middle), while the greater facial height remained after surgery (from 20% to 20%; [Figure 5](#), middle).

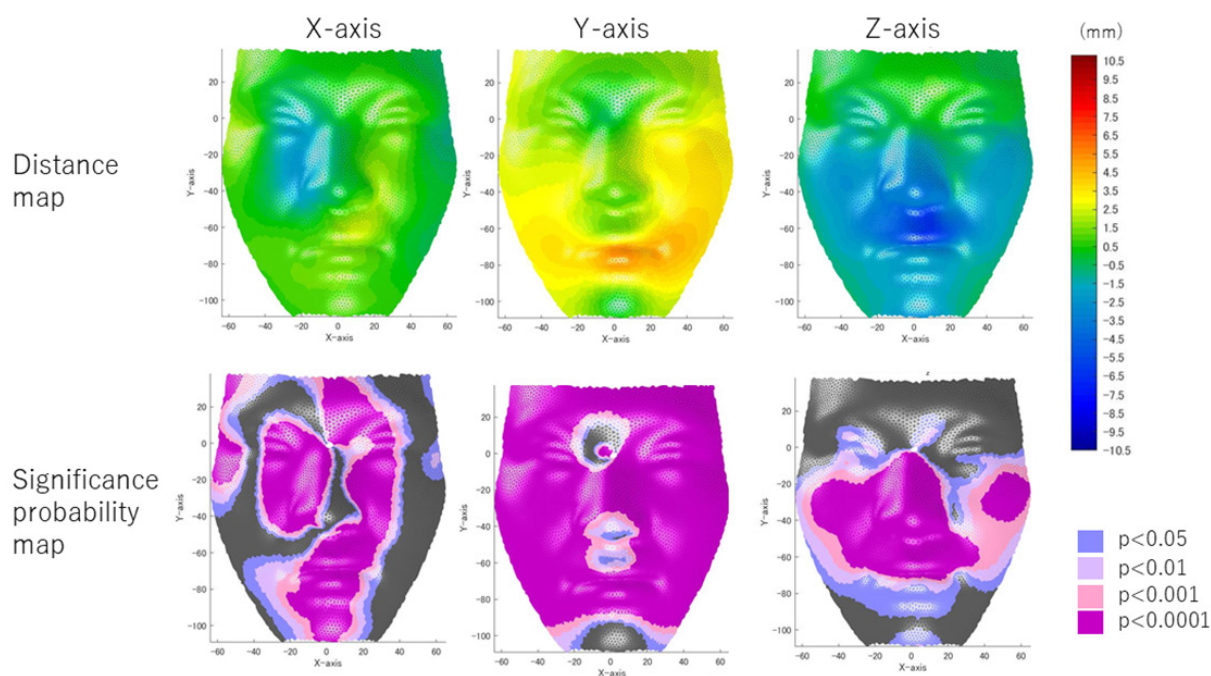
#### Morphological differences between the Cleft and Control groups

[Figures 7 and 8](#) show the average differences between the Control and Cleft groups and the significance probability map of the X-, Y-, and Z-values, respectively.

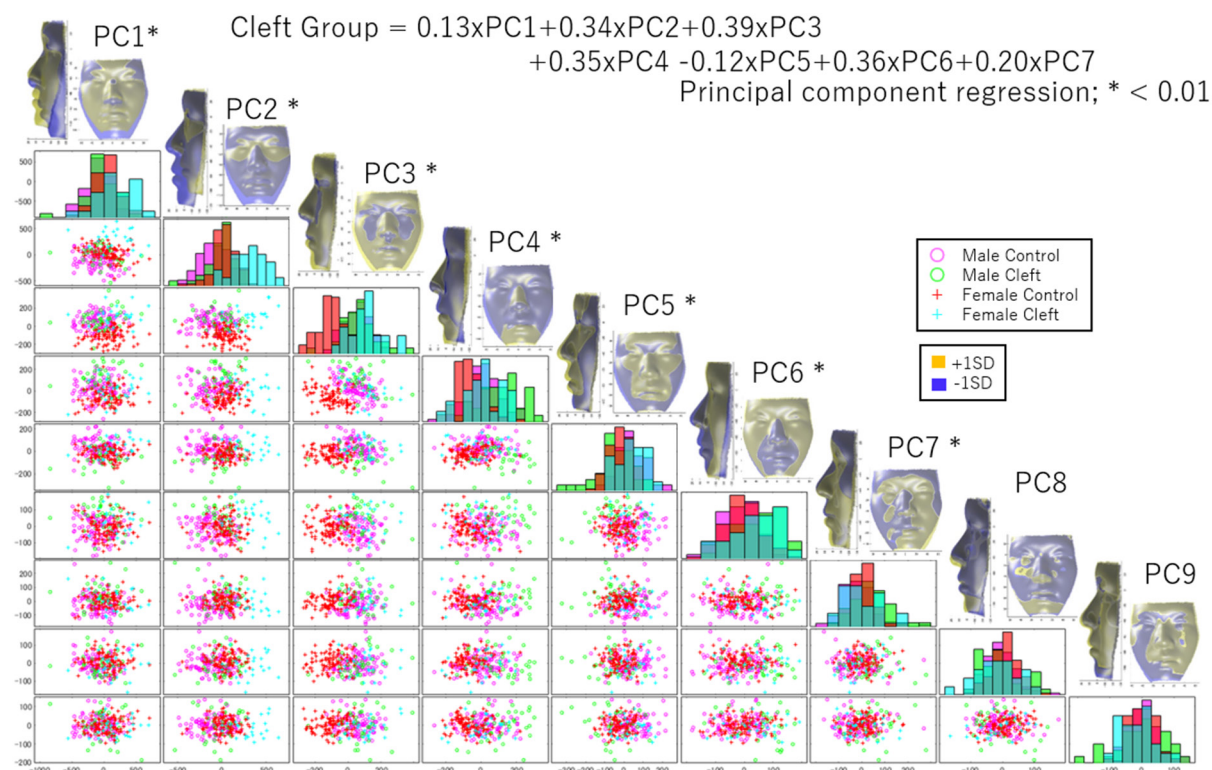
In the transverse direction (x-axis), the Cleft group showed that significant widening of the nasal wall at the non-cleft side, and the nasal dorsum and nasal alar of the non-cleft side was deviated to the non-cleft side. The upper lip, lower lip, and chin were significantly deviated to the cleft side ( $P \leq 0.001$ ). This indicates that the nasal tip was more rounded on the non-cleft side.



**Figure 7.** Average shape of the Cleft group (yellow) and Control group (blue). The statistical significance of the differences is shown in Figure 8



**Figure 8.** Distance maps (top) and significance probability maps (bottom) of the difference in the coordinate values of nodes of the fitted mesh between the Control and Cleft groups in three directions [horizontal (left); vertical (middle); and anteroposterior (right) directions]. For the significance probability maps, the colors designate: blue,  $P \leq 0.05$ ; pale pink,  $P \leq 0.01$ ; dark pink,  $P \leq 0.001$ ; and purple,  $P \leq 0.0001$ . For the distance maps, red indicates that the difference in the coordinate values between two subject groups (Cleft group - Control group) is a positive value, whereas blue indicates that the difference in the coordinate values between the two subject groups (Cleft group - Control group) was a negative value



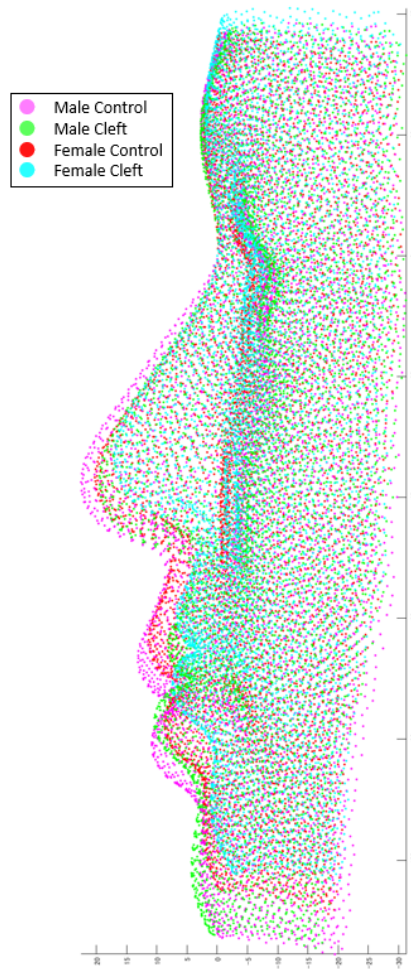
**Figure 9.** A scatter plot matrix of the principal component (PC) scores in the Cleft and Control groups with a histogram in the diagonal cells. PCs 1-9 explain 90.5% of the shape variation across samples. Pink denotes facial configurations in the male Control group (male); green denotes those in the male Cleft group; red denotes those in the female Control group; and cyan denotes those in the female Cleft group. The PCs are defined as +1 SD (yellow) and -1 SD (blue) in the top column. For example, the top column indicates that a +1 SD value of PC2 represents a smaller facial height, which was represented as yellow (1 SD). The histogram shows that the bimodal distribution and female patients in the Cleft group (cyan) showed a greater PC2 value, suggesting that the female Cleft group had a smaller facial height

In the vertical direction (y-axis), the position of the lower lip was significantly higher by approximately 4.5 mm in the Cleft group; however, the upper lip showed vertically normal position ( $P \leq 0.001$ ).

In the antero-posterior direction (z-axis), the Cleft group showed significant retrusion of the nose, cheek, upper lip, and lower lip in comparison to the Control group at rest, by approximately 2.5-4.5 mm ( $P \leq 0.001$ ). On the other hand, the nasal alar at the cleft side showed no differences between two groups, indicating nasal alar at the cleft side was flattened.

### Morphological variation of the Cleft group

The first nine significant PCs, which explained 90% of the sample's variance, were determined to be significant by a scree plot analysis [Figures 9 and 10]. Based on the principal component regression analysis, PC3 (weight = 0.39), PC6 (0.36), PC4 (0.35), and PC2 (0.34) were extracted as important features to discriminate the Cleft and Control groups. Visualization of the between-group structure of the surface data revealed that PC3 was characterized by midfacial retrusion, well-developed nasal bone, and chin protrusion, and the Cleft group showed greater PC3 values ( $P < 0.001$ ). PC6 was characterized by retrusion of the nose, upper lip, and lower lip, and the Cleft group showed a greater PC6 value ( $P < 0.000$ ). PC4 was characterized by the retrusion of the cheek, eyes, and upper lip, and the Cleft group showed a greater PC4 value than the Control group. PC2 was characterized by a vertically short face. The Cleft group showed greater PC2 values, indicating that the patients in the Cleft group showed vertically shorter faces.



**Figure 10.** Superimposition of the averaged coordinate values of a male Control group patient (pink), a male Cleft group patient (green), a female Control group patient (red), and a female Cleft group patient (cyan)

MONOVA revealed that there were significant differences in the facial morphology in the Cleft and Control groups and sex the subgroups; and that the effects of cleft on the facial morphology were related to sex [ $P < 0.01$ , Table 1].

With these nine PCs, the optimal number of clusters was mathematically created. The Elbow method showed that the samples could be classified into four typical patterns of the Cleft group (i.e., codes) [Figures 11 and 12]. Using color maps, the differences among the codes were described in detail: midface retrusion with short mandibular height (Code 1), midface retrusion combined with mandibular protrusion with well-developed nasal bone (Code 2), smaller nasal height and retruded cheeks (Code 3), and severe mandibular protrusion (Code 4).

Table 2 shows the demographic data of patients in each code. Code 1 consists of female patients, while Codes 2-4 consist of male patients. It was difficult to compare the surgical techniques or orthodontic treatment, because the sample size in each attribution was small.

## DISCUSSION

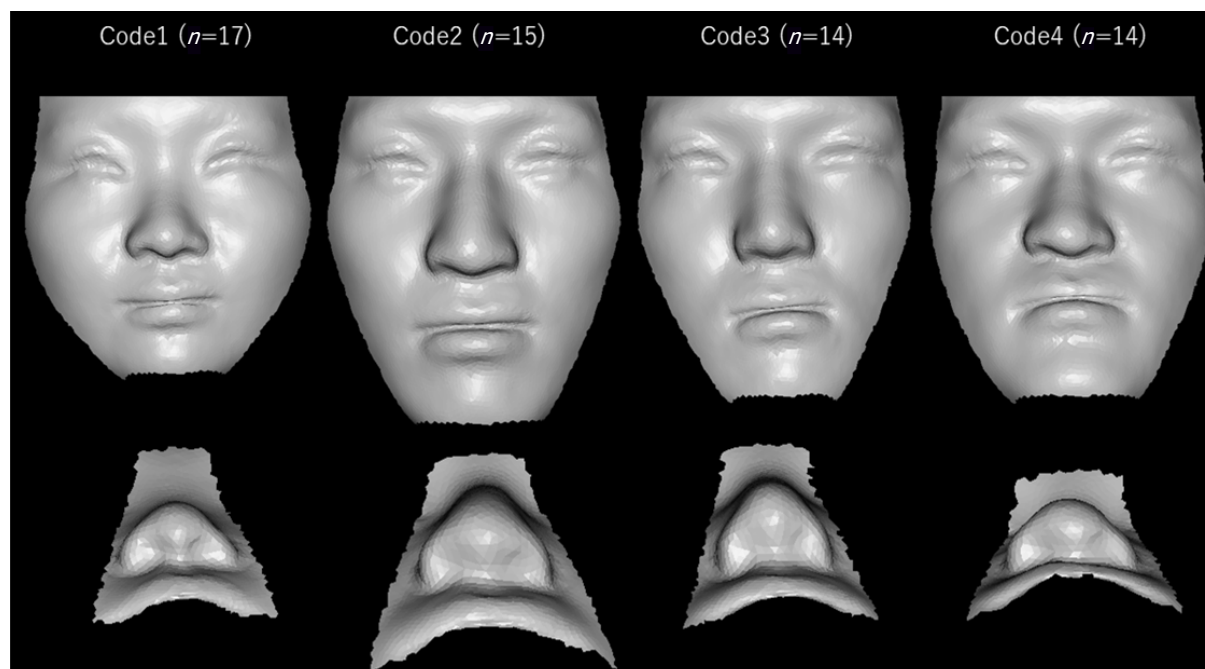
In the present research, we took advantage of GMM to propose a 3D quantitative analysis method for quantifying and visualizing the 3D configuration of the soft tissues of the face at rest. This method was



**Table 1. The multifactor analysis of variance of the surface-based model**

	Df	Pillai	Approx F	Num Df	Den Df	Pr(> F)
Cleft	1	0.8149	108.756	10	247	< 2.2e-16*
Sex	1	0.6727	50.761	10	247	< 2.2e-16*
Cleft: Sex	1	0.1969	6.056	10	247	< 3.13e-8*
Residuals	256					

\* $P < 0.01$ . Df: degrees of freedom; Pillai: Pillai's trace, which is a test statistic in the multifactor analysis of variance. This is a positive valued statistic ranging from 0 to 1. Increasing values means that effects are contributing more to the model; Approx F: the F statistic for the given predictor and test statistic; Num DF: the number of degrees of freedom in the model; Den Df: the number of degrees of freedom associated with the model errors; Pr(> F): the  $P$ -value associated with the F statistic of a given effect and test statistic. The null hypothesis that a given predictor has no effect on either of the outcomes is evaluated with regard to this  $P$ -value



**Figure 11.** The results of the clustering method. Sixty samples were classified into four categories (codes). Figures 11 and 12 show that Code 1 represents patients having midface retrusion with short mandibular height; Code 2 represents those with midface retrusion combined with mandibular protrusion with well-developed nasal bone; Code 3 represents those with smaller nasal height, retruded cheeks, and asymmetric face; and Code 4 represents those with severe mandibular protrusion with asymmetric nose

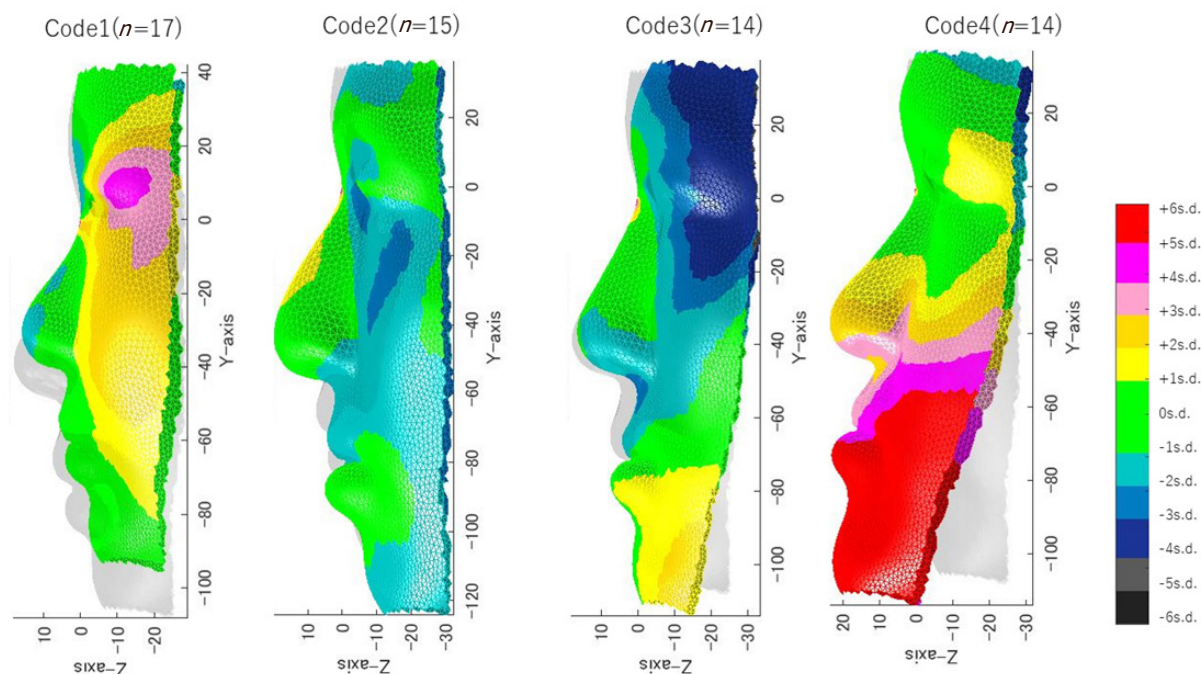
applied to the face of one example patient with cleft lip and palate. GMM is considered to be effective for describing the changes from before to after surgery.

A key concept of GMM is based on the fact that morphology can be mapped in the same dimensions as space, i.e., “the morphospace”, using these landmarks. Once 3D face images are converted to the morphospace, we can apply several statistical analyses to these face images. Examples of applications of GMM include the detection of facial sexual dimorphism<sup>[21]</sup>, the examination of relationships between faces and genetics<sup>[23,24]</sup>, the clinical diagnosis of dysmorphology<sup>[25]</sup>, computer vision<sup>[26]</sup>, and computer graphics<sup>[27]</sup>. In the present study, we used GMM for the quantitative evaluation of the treatment effects in patients with CLP and for the examination of the variation of the faces of patients with CLP based on a combination of principal component regression, MANOVA, and the clustering method (described below).

#### *Quantitative evaluation of the treatment effect in patients with UCLP*

GMM could be applied to detect the normal area before and after surgery. For the selected case, the percentage of the normal area was increased for all axes; however, several portions showing deformities





**Figure 12.** Z-scores for the four categories (Code) of Cleft patients. The average shape was evaluated with the normative range<sup>[19]</sup>. Only the z-axis (anterior-posterior direction) results were exemplified. A grey profile indicates the average face. Z-score was calculated by the equation:  $Z\text{-score}_{(z)} = (p_{(z)} - m_{(z)})/s_{(z)}$  where  $p_{(z)}$  indicates the averaged coordinate values of each code,  $m_{(z)}$  indicates the average coordinate values of the control group, and  $s_{(z)}$  indicates the standard deviation of the coordinate values of the control group in the z-direction. Please note that Code 1 shows that the anterior-posterior position of the upper and lower lips was within 1 SD. In Codes 2 and 3, the cheeks (midface) were remarkably retruded, while the anterior-posterior position of the upper lip was within 1 SD. Codes 4 shows greater protrusion of upper and lower lips

**Table 2. Demographic data in each code**

	Code 1	Code 2	Code 3	Code 4
Patient number	17	15	14	14
Sex				
Male	1	13	11	11
Female	16	2	3	3
Cleft side				
Left	11	13	11	11
Right	6	2	3	3
Orthodontic treatment				
Camouflage	4	4	2	3
Surgery	13	11	12	11
Surgical technique for the palatoplasty				
Wardill-Kilner push-back	15	15	12	12
Early two-stage with modified Furlow veloplasty	2	0	2	2
Age (years old)				
Mean	20.6	21.9	22.2	23.5
Standard deviation	3.2	2.3	7.5	5.6

remained, even after surgery. This was considered to be due to treatment limitations. Another possibility is that there were no measures to analyze the 3D face when we had performed surgery. The method introduced in the present article helps both patients and orthodontists share a mutual understanding of the soft tissue problems of patients with CLP. The patient's facial soft tissues can be quantified and visualized three-dimensionally. This is useful for developing optimum treatment plans based on the evaluation of soft tissue.

### *Examination of the variation of the faces of patients with CLP*

In the present study, we also clarified the morphological variations and characteristics of the face in patients with CLP using several statistical methods. This became possible because the faces could be converted to vectors of the same dimension (i.e., morphospace). Because the present study only included patients with positive overjet (i.e., most patients were in the post-treatment “retention” period), our study mainly detected antero-posterior facial deformities. If we examined patients before or during treatment, our method would be able to detect transverse facial deformities as well.

Decreased nasal height and its related obscure nasal tip were identified as characteristics of the Cleft group in the present study. These results were compatible with those of previous reports<sup>[6,28-30]</sup>. The Cleft group was also characterized by downward rotation of the dorsum and a well-developed nasal bone (PC3 and Code 2). This can be explained by the assumption that the retruded midface and deficient maxilla of the nose in patients resulted in a significant nasal hump and downward rotation of the columella. This observation was well-matched with a previous analysis from the lateral view<sup>[15]</sup>, which stated that this “beaky type nose” was often observed in adult patients with a cleft lip<sup>[15,29]</sup>.

A MANOVA also showed that the facial morphology was significantly different between the Cleft and Control groups and sex subgroups, and that the effects of a cleft on the facial morphology were related to sex. [Figure 10](#) shows that patients in the Cleft group tended to have greater lower lip protrusion, and this was exaggerated in males. As our samples were from patients who underwent treatment in our hospital, this fact might be related to the treatment goal or treatment demands in males in our hospital.

### *The 3D analysis of patients with CLP*

The reason 3D technology has not shown any further clinical progress thus far can be due to technical challenges in the analysis of 3D structures<sup>[19]</sup>. To solve the problem that 3D images containing an enormous quantity of information cannot be fully utilized in GMMs, this study introduced a new method to statistically analyze the entire 3D morphology and to simply visualize the results in patients with CLP. For the treatment of patients with CLP, a 3D quantitative analysis of the craniofacial structures is important for determining the treatment objectives and results.

### *Limitations*

There are two major limitations in this study that could be addressed in future research. First, the sample size for the exemplification of the system application was limited. Secondly, the specific features and categories identified in this study might be different from those observed in other centers.

### *Conclusion*

GMM was applied for quantifying and visualizing the 3D configuration of the soft tissues of the face. This method was effective in describing the changes from before to after surgery. GMM was also used to detect morphological variations of the face in patients with CLP. GMM will be a powerful tool to instantaneously and comprehensively evaluate faces in clinics.

## **DECLARATIONS**

### **Authors' contributions**

The author contributed solely to the article.

### **Availability of data and materials**

Not applicable.

### Financial support and sponsorship

This work was supported by JSPS KAKENHI (No. JP 19K10403). The funding body has no roles in the experiment design, collection, analysis and interpretation of data, and writing of the manuscript.

### Conflicts of interest

The author declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Research involving human subjects, human material or human data was performed in accordance with the Declaration of Helsinki and approved by the ethics committee for medical research at Osaka University Dental Hospital (project ID: H25-E37-1). An informed consent to participate in the study was obtained from participants.

### Consent for publication

A statement that a written informed consent for publication was obtained.

### Copyright

© The Author(s) 2020.

### REFERENCES

1. Sykes JM, Jang YJ. Cleft lip rhinoplasty. *Facial Plast Surg Clin North Am* 2009;17:133-44.
2. Yamada T, Mori Y, Minami K, Mishima K, Sugahara T. Three-dimensional facial morphology, following primary cleft lip repair using the triangular flap with or without rotation advancement. *J Craniomaxillofac Surg* 2002;30:337-42.
3. Fujimoto T, Imai K, Hatano T, Takahashi M, Tamai M. Follow-up of unilateral cleft-lip nose deformity after secondary repair with a modified reverse-U method. *J Plast Reconstr Aesthet Surg* 2011;64:747-53.
4. Mori A, Nakajima T, Kaneko T, Sakuma H, Aoki Y. Analysis of 109 Japanese children's lip and nose shapes using 3-dimensional digitizer. *Br J Plast Surg* 2005;58:318-29.
5. Anastassov Y, Chipkov C. Analysis of nasal and labial deformities in cleft lip, alveolus and palate patients by a new rating scale: preliminary report. *J Craniomaxillofac Surg* 2003;31:299-303.
6. Kim DW, Kim JT, Hong HK, Nam KC, Park JH. Statistical evaluation of the cleft lip nose deformity image. *Conf Proc IEEE Eng Med Biol Soc* 2006;1:3840-2.
7. Nagy K, Mommaerts MY. Analysis of the cleft-lip nose in submental-vertical view, Part I--reliability of a new measurement instrument. *J Craniomaxillofac Surg* 2007;35:265-77.
8. Farkas LG. *Anthropometry of the head and face*. 2nd ed. New York: Raven Press; 1994.
9. Ferrario VF, Sforza C, Dellavia C, Vizzotto L, Carù A. Three-dimensional nasal morphology in cleft lip and palate operated adult patients. *Ann Plast Surg* 2003;51:390-7.
10. Ras F, Habets LL, van Ginkel FC, Prah-Andersen B. Method for quantifying facial asymmetry in three dimensions using stereophotogrammetry. *Angle Orthod* 1995;65:233-9.
11. van Loon B, Maal TJ, Plooi JM, Ingels KJ, Borstlap WA, et al. 3D Stereophotogrammetric assessment of pre- and postoperative volumetric changes in the cleft lip and palate nose. *Int J Oral Maxillofac Surg* 2010;39:534-40.
12. Russell KA, Waldman SD, Tompson B, Lee JM. Nasal morphology and shape parameters as predictors of nasal esthetics in individuals with complete unilateral cleft lip and palate. *Cleft Palate Craniofac J* 2001;38:476-85.
13. Russell KA, Milne AD, Varma D, Josephson K, Lee JM. Three-dimensional morphologic nasal surface characteristics that predict the extremes of esthetics in patients with repaired cleft lip and palate. *Cleft Palate Craniofac J* 2011;48:28-37.
14. Dusková M, Kristen M, Smahel Z. The anthropometric verification of corrective surgery outcome in cleft secondary deformities. *J Craniofac Surg* 2006;17:447-53.
15. Nagasao T, Miyamoto J, Hikosaka M, Yoshikawa K, Ishii N, et al. A new method to quantify subtle morphological deformities in nasal profile curvatures and its application for analysis of unilateral cleft lip noses. *J Craniomaxillofac Surg* 2008;36:321-34.
16. Yamada T, Mori Y, Minami K, Mishima K, Sugahara T, et al. Computer aided three-dimensional analysis of nostril forms: application in normal and operated cleft lip patients. *J Craniomaxillofac Surg* 1999;27:345-53.
17. Tanikawa C, Takada K, van Aalst J, Trotman CA. Objective three-dimensional assessment of lip form in patients with repaired cleft lip. *Cleft Palate Craniofac J* 2010;47:611-22.
18. Bookstein FL. Landmark methods for forms without landmarks: morphometrics of group differences in outline shape. *Med Image Anal* 1997;1:225-43.
19. Tanikawa C, Akcam MO, Takada K. Quantifying faces three-dimensionally in orthodontic practice. *J Cranio-maxillofacial Surgery*

- 2019;47:867-75.
20. Shintaku Y, Tanikawa C, Iida S, Aikawa T, Kogo M, et al. Maxillary expansion and midline correction by asymmetric transverse distraction osteogenesis in a patient with unilateral cleft lip/palate: a case report. *Cleft Palate Craniofac J* 2015;52:618-24.
21. Tanikawa C, Zere E, Takada K. Sexual dimorphism in the facial morphology of adult humans: a three-dimensional analysis. *Homo* 2016;67:23-49.
22. Kono K, Tanikawa C, Yanagita T, Kamioka H, Yamashiro T. A novel method to detect 3D mandibular changes related to soft-diet feeding. *Front Physiol* 2017;8:567.
23. Crouch DJM, Winney B, Koppen WP, Christmas WJ, Hutnik K, et al. Genetics of the human face: identification of large-effect single gene variants. *Proc Natl Acad Sci U S A* 2018;115:E676-85.
24. Claes P, Liberton DK, Daniels K, Rosana KM, Quillen EE, et al. Modeling 3D facial shape from DNA. *Proc Natl Acad Sci USA* 2018;115:E676-85.
25. Hammond P. The use of 3D face shape modelling in dysmorphology. *Arch Dis Child* 2007;92:1120-6.
26. Huber P, Hu G, Tena I R, Mortazavian P, Koppen WP, et al. A multiresolution 3D morphable face model and fitting framework. Available from: <http://www.ee.surrey.ac.uk/CVSSP/Publications/papers/Huber-VISAPP-2016.pdf>. [Last accessed on 7 Sep 2020]
27. Yang H, Zhu H, Wang Y, Huang M, Shen Q, et al. FaceScape: a large-scale high quality 3D face Dataset and detailed riggable 3D face prediction. Available from: <https://arxiv.org/abs/2003.13989>. [Last accessed on 25 Aug 2020]
28. Farkas LG, Hajnis K, Posnick JC. Anthropometric and anthroposcopic findings of the nasal and facial region in cleft patients before and after primary lip and palate repair. *Cleft Palate Craniofac J* 1993;30:1-12.
29. Bardach J, Mooney MP. The relationship between lip pressure following lip repair and craniofacial growth: an experimental study in beagles. *Plast Reconstr Surg* 1984;73:544-55.
30. Ayoub A, Garrahy A, Millett D, Bowman A, Siebert P, et al. Three-dimensional assessment of early surgical outcome in repaired unilateral cleft lip and palate: Part 1. Nasal changes. *Cleft Palate Craniofac J* 2011;48:571-7.

Review

Open Access



# Clinical application of mesenchymal stem cells for cartilage regeneration

Yu-Chun Chen<sup>1,2</sup>, Chih-Hung Chang<sup>1,3</sup>

<sup>1</sup>Department of Orthopedic Surgery, Far Eastern Memorial Hospital, New Taipei 220, Taiwan.

<sup>2</sup>College of General Studies, Yuan Ze University, Taoyuan 320, Taiwan.

<sup>3</sup>Graduate School of Biotechnology and Bioengineering, Yuan Ze University, Taoyuan 320, Taiwan.

**Correspondence to:** Prof. Chih-Hung Chang, Department of Orthopedic Surgery, Far Eastern Memorial Hospital, No. 21, Sec. 2, Nanya S. Rd., Banqiao Dist., New Taipei 220, Taiwan. E-mail: orthocch@mail.femh.org.tw

**How to cite this article:** Chen YC, Chang CH. Clinical application of mesenchymal stem cells for cartilage regeneration. *Plast Aesthet Res* 2020;7:49. <http://dx.doi.org/10.20517/2347-9264.2020.28>

**Received:** 27 Feb 2020 **First Decision:** 12 May 2020 **Revised:** 13 Jun 2020 **Accepted:** 17 Jul 2020 **Published:** 17 Sep 2020

**Academic Editor:** Yi-Lin Cao **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Cartilage has the ability to transmit and distribute loads, providing lubrication in the diarthrodial joints. Risk factors including age, gender, genetics, nutrition and bone density may predispose to osteoarthritis (OA) and cartilage defect formation. Appropriate treatment include sufficient rest and medical therapy. Intra-articular injections such as steroids, platelet-rich plasma, visco-supplementation and mesenchymal stem cells (MSCs) injections present as alternative options for non-operative treatments. For cartilage defects, microfracture (MF), osteochondral autograft transplantation (OAT) and autologous chondrocyte implantation (ACI) are the most common treatment procedures. MSCs have been identified as an ideal cell source for OA therapy because they are easily expanded in culture, generally non-tumorigenic, and can be readily obtained from patients. It may be harvested from bone marrow (BMSCs), adipose tissue (ADSCs), synovium (SDSCs) or peripheral blood. BMSCs features the most common source of stem cells, and infrapatellar fat pad (IPFP) is another popular stem cell source. A phase 1 clinical study entitled "Treatment of Knee OA with Autologous Mesenchymal Stromal Cell Product (RegStem®)" was conducted in Taiwan and utilized  $5 \times 10^7$  IPFP-MSCs in the study for OA therapy. Most of the existing clinical studies have shown that patients receiving MSCs treatment have improved clinical outcome, such as Visual Analogue Scale, International Knee Documentation Committee and Western Ontario and McMaster Universities Arthritis Index (WOMAC) score. Some studies have also found an improvement in cartilage volume by Magnetic Resonance Imaging evaluation. Furthermore, MSCs can also be used for cartilage defect treatment. Clinical outcomes such as IKDC, Lysholm, and Tegner scores showed significant improvement when the cartilage defects were repaired and regenerated by several millions of stem cells. A 10-year follow-up clinical research indicated that there was no apparent increased tumor formation risk when BMSCs were used for cartilage defect treatment.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





In addition, a BMSCs/collagen gel composite for cartilage repair clinical trial in Taiwan was conducted in 2008, and results suggested that there was an improvement in IKDC and MRI score at 9-years of follow-up. It appears that the use of MSCs for OA and cartilage defect treatment may be a promising method.

**Keywords:** Mesenchymal stem cell, osteoarthritis, cartilage defect, cartilage regeneration

## INTRODUCTION

Cartilage offers high compressive force and covers the surfaces of synovial joints<sup>[1]</sup>. Its main function is to transmit and distribute loads and to further provide lubrication in the diarthrodial joints<sup>[2]</sup>. Healthy articular surfaces in humans demonstrate a hyaline cartilage morphology with thickness of about 2 to 4 mm. Cartilage comprise of 65%-85% of water, 12%-24% of collagen, 3%-6% of glycosaminoglycans, and 16,000-90,000 chondrocytes per microgram of wet tissue<sup>[3]</sup>. The biomechanical properties of articular cartilage are related to the composition and integrity of its extracellular matrix (ECM)<sup>[4]</sup>. Cartilage may function well throughout life, but damage to this tissue is prominent and has been described to afflict more than 21 million patients each year in the United States alone.

Osteoarthritis (OA) is one of the most common joint disorders related to cartilage<sup>[5]</sup>. In the United States alone, several millions of patients suffer from OA and treatment of this condition costs about 185.5 billion dollars annually<sup>[6]</sup>. OA pathology also ranks as the fourth leading cause of disability in Asia<sup>[7]</sup>. In addition, OA has a 12% prevalence rate in patients more than 60 years of age, and this is forecasted to increase within the next 10 years<sup>[8]</sup>. It has also been reported that the incidence of OA has doubled in women, and tripled in men, in recent years<sup>[9]</sup>. Risk factors increasing its preponderance include that of age, gender, genetics, nutrition and bone density which lead to greater susceptibility in OA<sup>[5]</sup>.

Pro-inflammatory cytokines are also the critical mediators implicated in the pathophysiology of OA, where they affect both quantity and quality of the cartilage ECM. Interleukin 1 beta (IL-1 $\beta$ ), Tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin 6 (IL-6) are the main pro-inflammatory cytokines related to its pathogenesis. Elevated levels of IL-1 $\beta$  and TNF- $\alpha$  have been found in OA patient's synovial fluid, synovial membrane and subchondral bone. Several studies have also indicated that the presence of IL-1 $\beta$  and TNF- $\alpha$  down-regulated type II collagen and aggrecan expression in chondrocyte, subsequently stimulating the release of matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-3 (MMP-3) and matrix metalloproteinase-13 (MMP-13)<sup>[10,11]</sup>. IL-6 was also found to be elevated in OA patient's synovial fluid and sera<sup>[12]</sup>, and it up-regulated the expression of MMP-1 and MMP-13 in combination with IL-1 $\beta$  and oncostatin. These cytokines contribute to the pathogenesis of OA through down-regulation of anabolic events and up-regulation of catabolic and inflammatory responses, resulting in structural damage to the OA joint<sup>[13,14]</sup>.

Apart from OA, cartilage defects are another common source of joint disorders. Trauma, sports injuries, biomechanical imbalance and genetic disease are common causes of cartilage defect. Patients suffering from cartilage defects may experience pain and loss of articular function, with altered activities of daily living. According to the international cartilage repair society ICRS grading system<sup>[15]</sup>, cartilage defects can be ranked from grade 1 (mildest) to grade 4 (most severe) which implies the most serious cartilage defect. In grade 1, the cartilage lesions may be found within the superficial layers of the cartilage. Grade 2 lesions occur when its depth extends down to less than 50% of the cartilage depth. When the lesion extends down to more than 50% of the cartilage depth, this results in severely abnormal cartilage is classified as grade 3. In the most severe defect grade 4, the lesion extends to subchondral bone and the underlying bony structures are exposed. When the defect areas are large, pain evolves to become more severe, and limits patients' daily activities. Hence, treatment of OA and cartilage defects is critical to improve the quality of life.

**Table 1. Different clinical strategies for osteoarthritis treatment**

Treatment strategies	Steroid injections <sup>[77]</sup>	Visco-supplementation injections <sup>[78]</sup>	Prolotherapy	Platelet-rich plasma injections <sup>[19]</sup>	MSCs injections <sup>[20]</sup>
Functions	Symptom relief	Symptom relief	Symptom relief and tissue repair	Symptom relief and tissue repair	Symptom relief and tissue repair

MSCs: mesenchymal stem cells

## CLINICAL TREATMENT FOR OA AND CARTILAGE DEFECT

### OA treatment

Rest and medical therapy are the most common modalities of conservative treatment for OA, where the aim is to reduce the pain and not to repair the injury<sup>[16]</sup>. It is commonly advocated for patients with low grade OA. Intra-articular injections such as steroids, platelet-rich plasma and visco-supplementation have been used as alternative approaches to non-operative treatments<sup>[17]</sup>. However, there have been no evidence of structural improvement with the use of these conservative modalities to date. Several biologic adjuncts have been described to improve repair, including growth factors such as prolotherapy<sup>[18]</sup>, platelet rich plasma (PRP)<sup>[19]</sup> and mesenchymal stem cells (MSCs) injection<sup>[20]</sup> [Table 1].

### Cartilage defect treatment

Clinical treatment of cartilage injury is dependent on age, modality of sport activities, etiology, grade and quality of the lesion. Rest and medical therapy remain the most common conservative treatment, but its objective is to reduce the pain, not to regenerate the cartilage. For patients with severe cartilage injury, operative treatments are necessary. Operative treatment for cartilage injuries depends on the patient's age, size of the lesion, and the chronicity of the lesion. Fresh osteochondral allograft is not available in many countries, hence microfracture (MF), osteochondral autograft transplantation (OAT) and autologous chondrocyte implantation (ACI) remain the most common procedures for cartilage restoration. MF is a surgery which creates small holes within the subchondral bone to allow blood and marrow healing elements into the area of damaged cartilage<sup>[21]</sup>. The MF defect is occasionally covered with a scaffold known as matrix augmented micro fracture, or autologous matrix-induced chondrogenesis. OAT is a technique to transfer healthy osteochondral tissue from a non-weight bearing site to the defect site<sup>[22]</sup>. Certain biphasic scaffolds have also been developed for osteochondral regeneration. ACI is a technique which involves performing an arthroscopy, obtaining a small piece of cartilage from the injured knee, expanding the chondrocytes in a GTP lab, and subsequently implanting the cells into the defect site<sup>[23]</sup>. Another improved ACI known as matrix-induced autologous chondrocyte implantation (MACI) is a technique which obtains patients' cartilage from a non-weight bearing area for cell culture, expanding the chondrocytes in a GTP lab, thereafter seeded them onto a specific scaffold for damaged area repair<sup>[24]</sup> [Table 2].

Although commonly used, these treatments may have complications such as fibrocartilage formation in MF treatments, donor-site morbidity in OAT technique, and secondary surgery may be required in ACI and MACI procedures. Therefore, a simple and effective treatment based on the concept of tissue engineering for cartilage injury is needed.

## MSCS FOR OA AND CARTILAGE DEFECT TREATMENT

### MSCs

MSCs present as an ideal cell source for OA therapy because they are easily expanded in culture, generally non-tumorigenic, and can be readily obtain from patients. More importantly, they possess immunosuppressive properties after exposure to an inflammatory environment with the secretion of soluble factors<sup>[25]</sup>. MSCs may be harvested from several sites including bone marrow (BMSCs), adipose tissue (ADSCs), synovium (SDSCs) or peripheral blood. Clinical applications of MSCs should meet the minimal

**Table 2. Different clinical strategies for cartilage defect treatment**

Treatment strategies	MF <sup>[21]</sup>	AMIC	OAT <sup>[22]</sup>	Osteochondral composites scaffold implantation	ACI <sup>[23]</sup>	MACI <sup>[24]</sup>
Procedures	Using a small bone pick to punch into the subchondral bone causing microfractures	After microfracture, the defect site is covered with matrix	Taking cylindrical cartilage plugs from a donor site and inserting them into matching holes	Placing the composite scaffolds into the interface between cartilage and bone for osteochondral defect site repair	Cartilage tissue is taken from a non-weight bearing area for cell culture. When cell number is sufficient, the chondrocytes are applied on the damaged area	Cartilage tissue is taken from a non-weight bearing area for cell culture. When cell number is sufficient, the chondrocytes are seeded onto a scaffold for damaged area repair
Functions	Tissue repair	Tissue repair	Tissue repair	Tissue repair	Tissue repair	Tissue repair
Cell cultivation	No	No	No	No	Yes	Yes
Matrix	Without	With	Without	With	Without	With
Matrix examples	-	Chondro-Gide <sup>®[79]</sup> , BST-CarGel <sup>®[80]</sup>	-	PLGA/bioactive glass <sup>[81]</sup> , cartilage fragments combined with PLGA/beta-TCP composite <sup>[82]</sup> , porous PLGA/nano-hydroxyapatite hybrid scaffolds <sup>[83]</sup>	-	Chondro-Gide <sup>®[84]</sup> , CaReS <sup>®[85]</sup> , Hyalograft C <sup>®[86]</sup> , BioSeed-C <sup>®[87]</sup> , recycled cartilage auto/allo implantation (ClinicalTrials.gov Identifier: NCT03672825)

MF: micro-fracture; AMIC: matrix augmented micro fracture; OAT: osteochondral transplantation; ACI: autologous chondrocyte implantation; MACI: matrix-induced autologous chondrocyte implantation; PLGA: polylactide-co-glycolide; TCP: tricalcium phosphate

criteria established by International Society for Cellular Therapy including (1) being plastic-adherent in culture conditions; (2) expressing cluster of differentiation 105 (CD105), CD73, and CD90, lacking expression of CD45, CD34, CD14 or CD11b, CD79 or CD19, and human leukocyte antigen-DR isotype (HLA-DR) surface molecules; and (3) possessing tri-lineage differentiation into osteoblasts, adipocytes and chondroblasts<sup>[26]</sup>. Much of the recent literature has focused on BMSCs for chondrogenesis<sup>[27]</sup>. However, the clinical use of BMSCs has encountered challenges such as donor site morbidity, pain and low cell number upon harvest<sup>[28]</sup>. One issue is that only 0.001%-0.01% of the cells in bone marrow aspirate concentrate consist of BMSCs<sup>[29]</sup>. Thus, the ADSCs has become an attractive alternative source of MSCs because of its relatively easy accessibility and abundance during harvest<sup>[30]</sup>.

ADSCs can be isolated from the upper arm, medial thigh, buttocks, trochanteric, superficial deep abdominal depots, and even the infrapatellar fat pad (IPFP) within the knee joint. There are about 2 to 6 million cells in the stromal vascular fraction (SVF) which can be obtained in 1 mL lipoaspirate<sup>[31]</sup>. The number of ADSCs in 1 g of ADSCs may range from 5000 to 200,000<sup>[32-34]</sup>. In other words, if we isolated 100 g of ADSCs from patient, there would be 0.5 to 20 million ADSCs which can be extracted from the ADSCs. ADSCs have been reported to differentiate into adipocytes, osteoblasts<sup>[35]</sup>, chondrocytes<sup>[36]</sup>, and endothelial cells<sup>[37]</sup> in view of their mesodermal origin. In addition, they have been described to have the ability to differentiate into ectodermal, and endodermal origin cells, such as vascular smooth muscle cells<sup>[38]</sup>, keratinocytes<sup>[39]</sup>, hepatocytes, beta islet cells<sup>[40]</sup>, neuron-like cells<sup>[41]</sup> and glial lineages<sup>[42]</sup>. Both ADSCs and BMSCs exhibit a fibroblast-like morphology<sup>[35,43]</sup>, expressing CD29, CD44, CD73, CD90, CD105 while being absent for CD14, CD31, CD34, CD45, CD106 and HLA-DR and c-kit expression<sup>[35,36,43]</sup>. When comparing the cell differentiation ability between ADSCs and BMSCs *in vitro*, ADSCs demonstrated more prominent adipogenic differentiation ability, while BMSCs possessed stronger osteogenic differentiation ability compared to ADSCs<sup>[35,44]</sup>. Xu *et al.*<sup>[35]</sup> used bisulfite PCR analysis to examine the DNA methylation status of Runx2, PPAR $\gamma$ , and Sox9 from ADSCs and BMSCs. They described that the CpG sites of PPAR $\gamma$  promoter in BMSCs and the CpG sites of Runx2 promoter in ADSCs were hypermethylated. Nevertheless, the methylation status of Sox9 promoter in BMSCs was only slightly lower than that in ADSCs.

In specific orthopaedic procedures such as high tibial osteotomy or arthroscopy examination, a part of patients' tissue are removed, such as the SDSCs and IPFP. A significant number of MSCs exit within the deposited tissue and can be isolated by collagenase digestion. Sakaguchi *et al.*<sup>[45]</sup> compared the differentiation potential among BMSCs, SDSCs and ADSCs. They reported that the nucleated cell number of SDSCs was about 3000 per mg, and possessed the greatest chondrogenesis ability as compared with others. Moreover, they also found that proliferative potential of SDSCs and IPFP-MSC were greater than that of ADSC, and the pellets formed by SDSCs and IPFP-MSC could also produce more cartilage matrix than that in ADSCs pellets from another study<sup>[46]</sup>. Kouroupis *et al.*<sup>[47]</sup> demonstrated that IPFP-MSC exhibit higher clonogenicity and chondrogenic potential as compared with BMSC. Importantly, their findings showed that primed IPFP-MSC demonstrate sustained antagonism of activated human peripheral blood mononuclear cells proliferation. Considering its chondrogenic and anti-inflammation ability, it appears that IPFP-MSC may be the most promising MSC type for degenerative/inflammatory joint diseases treatment.

### MSCs for OA treatment

In early 2008, Centeno *et al.*<sup>[48]</sup> published their inaugural research findings about the use of autologous BMSCs for OA treatment, where they cultured BMSCs to passage 3 and injected about  $4.56 \times 10^7$  cells into a 36 years-old male's knee. After treatment for a 3-month period, the patient's VAS scores decreased from 3.33 to 0.13. Furthermore, his MRI results showed that the volume of meniscus increased. In 2011, Davatchi *et al.*<sup>[49]</sup> published their results on the use of autologous BMSCs for OA treatment ( $n = 4$ ), where they injected about  $8$  to  $9 \times 10^6$  cells into patients' knee cavity. They reported that the walking time for the pain to appear improved and patient's VAS scores decreased from 80~90 to 45~65. However, they were unable to find any improvement on X-rays. This study was continued from follow-up to post-treatment 5 years, and they found that the beneficial effects of BMSCs started to decline after 6 months, although this was still better at 5 years compared to the baseline<sup>[50]</sup>. In 2013, Orozco *et al.*<sup>[51]</sup> performed an OA clinical study ( $n = 12$ ), in which they injected  $4 \times 10^7$  BMSCs into Kellgren and Lawrence (KL) grade 2-4 patients' knee joints. They described that pain relief occurred by 3 months and improved for at least 1 year, and the Lequesne and WOMAC score were significantly increased. Moreover, the quantitative MRI results on cartilage quality showed improvement at the 2-year follow-up<sup>[51]</sup>. Leading on, the same research team also published a BMSCs study in 2016, where they used  $4 \times 10^7$  BMSCs to treat KL grade 2-3 OA treatment. Results showed that the daily activities VAS at the basal visit was about 58.27, and this value decreased to 19.47 at the 1-year a follow-up, with further reduction to  $14.62 \pm 14.93$  at the 4-year follow-up, and no serious adverse effects were reported<sup>[52]</sup>. In 2019, Chahal *et al.*<sup>[53]</sup> presented their research on using 1, 10, or 50 million BMSCs for KL grade 3-4 OA treatment. They found there were no improvements in morphological cartilage scores or decrease in T2 relaxation values. However, they showed possible chondroprotective effects based on cartilage catabolic biomarkers at 50 million BMSCs doses. They also found that IL12p40 within synovial fluid decreased with treatment, and the pro-inflammatory CD14<sup>+</sup>CD16<sup>+</sup> monocyte/macrophages maker tend to decrease as well after MSCs treatment.

Apart from bone marrow, ADSCs is another popular stem cell source. In Fodor's research, they treated OA knee with the use of 14.1 million viable, nucleated SVF cells, and found that there was a statistically significant improvement in WOMAC and VAS scores, which was maintained at 1 year<sup>[54]</sup>. Prof. Yokota Nakamura also conducted a clinical study recently to compare OA treatment effect of ADSCs or non-cultured SVF injection. Results showed that pain VAS and Knee injury and Osteoarthritis Outcome Score (KOOS) scores had improvement in both groups. Nonetheless, patients' symptoms improved earlier (at 3 months) and pain VAS decreased to a greater degree in the ADSCs injection group as compared with those in SVF group<sup>[55]</sup>. Adipose SVF contains a wide variety of cells including that of MSCs, pericytes, vascular adventitial cells, fibroblasts, pre-adipocytes, monocytes, macrophages, red blood cells, and fibrous tissue/matrix. The composition of these aforementioned cells or matrix may differ depending on individual differences or the preparation procedure of SVF. Thus, in some clinical studies evaluating the effects of

MSCs, they are isolated and expanded in the laboratory, thereafter being injected into the OA knee for treatment.

In a study from Jo *et al.*<sup>[9]</sup>, they present a 2-year follow-up result of IA injection of low ( $1 \times 10^7$ ), medium ( $5 \times 10^7$ ), and high ( $1 \times 10^8$ ) dose of ADSCs into the knee, respectively (NCT01300598). They report that MSCs improved knee function, as measured with the WOMAC, Knee Society clinical rating system, and KOOS, with patients experiencing reduced knee pain. In addition, there was a statistical significance of improvement found mainly in the high-dose group. However, in Pers's study (NCT01585857), they found the group of patients having injections of  $2 \times 10^6$  cells exhibiting the best response, and they had higher baseline pain and WOMAC scores compared with those receiving higher doses<sup>[56]</sup>. In 2019, Lee *et al.*<sup>[57]</sup> presented a prospective double-blinded, randomized controlled, phase IIb clinical trial, where they injected high-dose autologous ADSCs ( $1 \times 10^8$  cells) intra-articularly into the patients' knee, and found that a single injection of ADSCs led to a significant improvement of the WOMAC score at 6 months. Furthermore, there was no significant change in cartilage defect at 6 months in ADSCs group which contrasted with the increased defect size in the control group. Lu *et al.*<sup>[58]</sup> also conducted a double-blind, active-controlled, phase IIb knee OA clinical trial by using  $5 \times 10^7$  ADSCs. Results showed that most patients achieved a 70% improvement rate in the ADSCs receiving group after 12 months. Moreover, there was a notable increase in articular cartilage volume in the ADSC group, as compared with the hyaluronic acid (HA) group after 12 months as measured by MRI.

Recently, another type of fat tissue known as PFP has become a popular research topic due to its ability to diminish inflammation and cartilage degenerative grade. The IPFP is an intra-capsular structure within the anterior knee compartment, composed of approximately 20 cm<sup>3</sup> of ADSCs<sup>[59]</sup>, and may be easily harvested arthroscopically or during open knee surgery<sup>[60]</sup>. During embryonic development of the knee, researchers found that IPFP initiates from interzone formation between the femur and tibia, progressing to cavitation between this region, and finally a IPFP site formation. This is described to be a triangular space composed of a mesenchymal tissue formation below the patella at the 9th week of human development<sup>[61]</sup>. IPFP occupies space in the joint, maintaining the articular cavity, allowing the synovial fluid to circulate over the joint thus contributing to lubrication. In an experimental animal model of OA, Toghraie *et al.*<sup>[62]</sup> used direct IA injection of IPFP-MSCs into the OA knees of rabbits. The IPFP-MSCs used had been expanded and grown *in vitro* and were delivered 12 weeks after the operation in a single dose of 1 million cells suspended in 1 mL of medium. Twenty weeks after surgery, rabbits that received IF-MSCs demonstrated less cartilage degeneration, osteophyte formation, and subchondral sclerosis than did those in the control group.

In 2012, Koh published a Level III clinical study article with the use of IPFP-MSCs for OA therapy<sup>[60]</sup>, where they collected the IPFP (average weight, 9.4 g; range, 6.9-11.2 g) by skin incision extension, further isolating the IPFP-MSCs by tissue mincing, collagen digestion, and centrifugation. An average of  $1.89 \times 10^6$  stem cells were prepared with 3.0 mL of PRP and injected into the selected knees of patients in the study group. The mean Lysholm and VAS scores of patients in the study group improved significantly at the final follow-up (mean follow-up, 24.3 months; range, 24 to 26 months). Radiography demonstrated that the whole-organ MRI score had significantly improved from 60.0 points to 48.3 points<sup>[63]</sup>. Spasovski *et al.*<sup>[64]</sup> have also reported that the use of IPFP-MSCs in knee OA improves clinical symptoms and reduces pain at 3 months, obtaining the best results at 6 months. Currently, a phase 1 clinical study entitled "Treatment of Knee Osteoarthritis with Autologous Mesenchymal Stromal Cell Product (RegStem®)" is being conducted in Taiwan, which has been approved by Taiwan Food and Drug Administration on May, 2017 (ClinicalTrials.gov Identifier number: NCT03007576). The study has enrolled 12 subjects who have Kellgren-Lawrence grade 2~3 OA knee, and use  $5 \times 10^7$  IPFP-MSCs for therapy. At the culmination of 1, 3, 6, 12 and 24 months, the VAS, KOOS and IKDC scores of subjects will be further evaluated.



### MSCs for cartilage defect treatment

Even though the clinical outcome of MF, OAT and ACI for cartilage defect treatment has been shown to be desirable, there are some limitations, including that of low stem cell number and fibrocartilage formation in MF treatment, potential donor-site morbidity in OAT technique, and requirements for secondary surgery in ACI procedure. Thus, there are several research teams trying to isolate and proliferate the stem cell from patient's autologous tissue, re-seeding them into the tissue for cartilage defect treatment. They anticipate that high proliferation rate and chondro-differentiation potential of stem cells could potentially regenerate the cartilage tissue.

In 2002, Wakitani *et al.*<sup>[65]</sup> first presented using BMSCs for cartilage defect treatment, where they mixed  $1.3 \times 10^7$  cells into 2 mL of 0.25% type I collagen gel and placed the gel-cell composite onto the defect site. One year later, they discovered that the defects sites were covered with white soft hyaline cartilage-like tissue, and reported metachromasia within the cartilage tissue where there was presence of hyaline cartilage-like tissue forming. Recently, Nejadnik *et al.*<sup>[66]</sup> also compared the clinical results of cartilage defect repaired by 10-15 million chondrocytes or BMSCs. They found improvement in the quality of life of both patient groups, and there was no significant difference in IKDC, Lysholm, and Tegner scores. However, the use of BMSCs for cartilage defect treatment is a one stage surgery, and this modality of treatment may reduce costs, further minimizing the probability of donor-site morbidity. In another related research conducted by Haleem *et al.*<sup>[67]</sup>, they combined BMSCs and PRP for cartilage defect treatment, and the BMSCs seeding density was  $\sim 2 \times 10^6$  cells/cm<sup>2</sup>. They found after cell injection the Lysholm and RHSSK scores showed statistically significant improvement at 12-month follow-up, and MRI revealed complete defect filled with native cartilage. Considering long-term treatment outcome, Teo *et al.*<sup>[68]</sup> published his 10-year follow-up clinical research comparing patient-reported outcome between BMSCs and chondrocyte for cartilage repair. They found no significant differences between these two groups, and also no apparent increased tumor formation risk. However, cell isolation and cultivation are easier when using BMSCs for cartilage repair. Synovial MSCs are an alternative stem cell source for cartilage defect repair, where these cells have been extensively studied by Prof. Ichiro Sekiya. Research indicates that the SDSCs is a reservoir for MSCs which can contribute to intraarticular tissue repair<sup>[69]</sup>. In 2015, Sikiya's team conducted a synovial MSCs for cartilage defect treatment clinical study, where they isolated synovial MSCs and cultured them for 14 days, thereafter placing them on the cartilage defect site. Results showed that Lysholm scores were improved, and MRI score was increased at 18-months follow-up<sup>[70]</sup>. In 2015, Prof. Norimasa Nakamura developed a new method for cartilage repair, known as a scaffold-free tissue engineered construct (TEC). The construct was made by synovium-derived stem cells (SDSCs), where the team cultured cells in a medium with  $> 0.1$  mmol/L ascorbic acid-2 phosphate for a period, resulting in a stiff sheet-like TEC which was rich in collagen I and III<sup>[71]</sup>.

In Taiwan, there are several research teams which have tried to use MSCs for cartilage regeneration. Researchers developed an MSCs-derived chondrocyte implantation technique in 2005, and the technique obtained a US patent (patent number: US 20110189254 A1) entitled "Surgical grafts for repairing chondral defects". In this technique, BMSCs were isolated from patients' bone marrow and embedded in 3% type-I collagen solution in a  $2.6 \times 10^6$  cells/cm<sup>2</sup> cell density for cartilage repair. The gel/cell composite could gel in 12-well plates for an hour, and this was then overlaid with 2 mL chondrogenic differentiation medium for cartilage-like tissue induction. About 3 weeks later, the gel/cell composite reseeded into the cartilage defect site. This clinical study enrolled 12 human subjects and continued to follow up their clinical outcome and MRI results for about 9 years, results confirming that there were an improvement in IKDC and MRI score.

In 2011, Chang *et al.*<sup>[72]</sup> studied the possibility of using BMSCs containing tissue-engineering constructs for osteochondral defects repair in a porcine model. They used the gel/cell composite with a  $1 \times 10^6$  BMSCs/mL cell density for cartilage regeneration. They found that both undifferentiated MSCs and TGF- $\beta$ -induced

differentiated MSCs could be used for *in vivo* tissue engineering treatment of osteochondral defects. Six months after surgery, they discovered that the defects had smooth, fully repaired surfaces or partially repaired surfaces in both group, suggesting that the use of MSCs could be a viable approach for *in vivo* tissue engineered treatment of osteochondral defects.

Based on the concept that ECM may possess critical factors for MSC differentiation, some research groups have focused on combining cartilage matrix and MSC for cartilage repair. In 2012, Chen *et al.*<sup>[73]</sup> mixed  $6 \times 10^6$  BMSCs with cartilage fragment as a construct for cartilage regeneration and implanted it subcutaneously into nude mice. Results showed that the cells cultured in the constructs expressed type II collagen mRNA after 4 weeks of implantation. This implied that the cartilage fragments could promote chondrogenic differentiation of BMSCs. In a following study, they prepared the acellular cartilage matrix (ACM) from patients' cartilage tissue and mixed it with human SDSCs and collagen gel for *in vitro* culture. Results showed that SMSCs also express type II collagen and SOX-9 mRNA in an environment with growth factor absence. Thus such kind of ACM/stem cell composites may be beneficial to cartilage regeneration for future clinical applications<sup>[74]</sup>. In 2017, the group tried to compare the cartilage regeneration results between BMSCs and bone marrow concentrate (BMC). They mixed porcine cartilage, SDSCs fragments with BMSCs or BMC to form different constructs. Results showed that BMC-containing constructs could stimulate chondrogenesis and BMSCs-containing constructs could assist in ECM synthesis<sup>[75]</sup>.

### Challenges in using MSCs for cartilage regeneration

For cartilage regeneration, MSCs may be applied to knee joint injection or cartilage defect filling, but obtaining a high cell number remains a challenge. Patients are unable to receive their own high cell number MSCs immediately. Their MSC contained tissue would be sent to the qualified cell processing facility for cell isolation and expansion, and the expected cell receiving date might be up to three weeks later<sup>[57]</sup>. After MSCs are injected into knee joint, it is uncertain if the MSCs are well-distributed. Furthermore, in order to meet the high cell number, the MSCs are cultured *in vitro* for a long duration, where their phenotype may be changed, the cell population's doubling time would increase and cellular aging process occurs<sup>[76]</sup>.

## CONCLUSION

There are several biological factors related to OA and cartilage defect, which eventually lead to cartilage degeneration. The most common clinical treatment for cartilage degeneration involves the use of painkillers and HA injection. However, such kind of treatment may only serve to reduce the symptoms, and not to repair or regenerate the cartilage. Thus, several operative treatments were developed for cartilage repair, including MF, OAT and ACI. These operative surgeries are common in orthopedic surgery, but there is still room for advancement. Currently, several research groups have focused on the use of MSCs for cartilage repair, and most involve bone marrow and ADSCs as sources of MSCs. The majority of these have shown promising results in cartilage repair and OA treatment. Infrapatellar fat pads MSCs is a recent hot research topic as it possesses promising potential for OA and cartilage defect treatment.

## DECLARATIONS

### Acknowledgments

The authors would like to thank Ministry of Science and Technology (108-2314-B-418-010-MY3) and Far Eastern Memorial Hospital (FEMH -2019-C-004, FEMH-2019-C-080, FEMH-2017-C-007, FEMH-2017-C-046) for financial support and the Far Eastern Memorial Hospital Core Laboratories I & II for providing facilities and instruments.

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Chen YC, Chang CH

Performed data acquisition, as well as provided administrative, technical, and material support: Chen YC, Chang CH

### **Availability of data and materials**

Not applicable.

### **Financial support and sponsorship**

This work was supported by Ministry of Science and Technology (MOST-108-2314-B-418-010-MY3, MOST 109-2314-B-418-002-MY3) and Far Eastern Memorial Hospital (FEMH -2019-C-004, FEMH-2019-C-080, FEMH-2017-C-007, FEMH-2017-C-046).

### **Conflicts of interest**

All authors declared that there are no conflicts of interest.

### **Ethical approval and consent to participate**

Not applicable.

### **Consent for publication**

Not applicable.

### **Copyright**

© The Author(s) 2020.

## **REFERENCES**

1. Aigner T, Stove J. Collagens--major component of the physiological cartilage matrix, major target of cartilage degeneration, major tool in cartilage repair. *Adv Drug Deliv Rev* 2003;55:1569-93.
2. Mow VC, Setton LA. Mechanical properties of normal and osteoarthritic articular cartilage. In: Brandt KD, Doherty M, Lohmander LS, editors. *Osteoarthritis*. Oxford: Oxford University Press; 1998. pp. 108-22.
3. Mauck LR, Burdick JA. Engineering cartilage tissue. In: Pallua N, Suschek CV, editors. *Tissue Engineering, from lab to clinic*. UK: Springer-Verlag Berlin Heidelberg; 2011. pp. 493-520.
4. Bastiaansen-Jenniskens YM, Koevoet W, de Bart AC, van der Linden JC, Zuurmond AM, et al. Contribution of collagen network features to functional properties of engineered cartilage. *Osteoarthritis Cartilage* 2008;16:359-66.
5. Arden N, Nevitt MC. Osteoarthritis: epidemiology. *Best Pract Res Clin Rheumatol* 2006;20:3-25.
6. Kotlarz H, Gunnarsson CL, Fang H, Rizzo JA. Insurer and out-of-pocket costs of osteoarthritis in the US: evidence from national survey data. *Arthritis Rheum* 2009;60:3546-53.
7. Fransen M, Bridgett L, March L, Hoy D, Penserga E, et al. The epidemiology of osteoarthritis in Asia. *Int J Rheum Dis* 2011;14:113-21.
8. Neogi T, Zhang Y. Epidemiology of osteoarthritis. *Rheum Dis Clin North Am* 2013;39:1-19.
9. Jo CH, Chai JW, Jeong EC, Oh S, Shin JS, et al. Intra-articular injection of mesenchymal stem cells for the treatment of osteoarthritis of the Knee: a 2-year follow-up study. *Am J Sports Med* 2017;45:2774-83.
10. Saklatvala J. Tumour necrosis factor alpha stimulates resorption and inhibits synthesis of proteoglycan in cartilage. *Nature* 1986;322:547-9.
11. Lefebvre V, Peeters-Joris C, Vaes G. Modulation by interleukin 1 and tumor necrosis factor alpha of production of collagenase, tissue inhibitor of metalloproteinases and collagen types in differentiated and dedifferentiated articular chondrocytes. *Biochim Biophys Acta* 1990;22:366-78.
12. Kaneko S, Satoh T, Chiba J, Ju C, Inoue K, et al. Interleukin-6 and interleukin-8 levels in serum and synovial fluid of patients with osteoarthritis. *Cytokines Cell Mol Ther* 2000;6:71-9.
13. Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther Adv Musculoskelet Dis* 2013;5:77-94.
14. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol* 2011;7:33-42.
15. Harris DJ, Flanigan DC. Management of knee articular cartilage injuries. In: Dragoo JL, editor. *Modern Arthroscopy*. InTech; 2011. pp. 103-28.
16. Bhatia D, Bejarano T, Novo M. Current interventions in the management of knee osteoarthritis. *J Pharm Bioallied Sci* 2013;5:30-8.
17. Jones IA, Togashi R, Wilson ML, Heckmann N, Vangsness CT Jr. Intra-articular treatment options for knee osteoarthritis. *Nat Rev Rheumatol* 2019;15:77-90.

18. Rabago D, Patterson JJ, Mundt M, Kijowski R, Grettie J, et al. Dextrose prolotherapy for knee osteoarthritis: a randomized controlled trial. *Ann Fam Med* 2013;11:229-37.
19. Glynn LG, Mustafa A, Casey M, Krawczyk J, Blom J, et al. Platelet-rich plasma (PRP) therapy for knee arthritis: a feasibility study in primary care. *Pilot Feasibility Stud* 2018;4:93.
20. Mancuso P, Raman S, Glynn A, Barry F, Murphy JM. Mesenchymal stem cell therapy for osteoarthritis: the critical role of the cell secretome. *Front Bioeng Biotechnol* 2019;7:9.
21. Case JM, Scopp JM. Treatment of articular cartilage defects of the knee with microfracture and enhanced microfracture techniques. *Sports Med Arthrosc Rev* 2016;24:63-8.
22. Inderhaug E, Solheim E. Osteochondral autograft transplant (mosaicplasty) for knee articular cartilage defects. *JBJS Essent Surg Tech* 2019;9:e34.1-2.
23. Kim MK, Park JS, Jeon YM, Jeon YS. Clinical, radiological, and histological outcomes after the fibrin-matrix autologous chondrocyte implantation for chondral lesions of the knee in patients more than 50 years old: a prospective case series with minimum 2-year follow-up. *J Orthop Surg (Hong Kong)* 2020;28:2309499019893509.
24. Erickson BJ, Strickland SM, Gomoll AH. Indications, techniques, outcomes for matrix-induced autologous chondrocyte implantation (MACI). *Oper Tech Sports Med* 2018;26:175-82.
25. Pers YM, Ruiz M, Noel D, Jorgensen C. Mesenchymal stem cells for the management of inflammation in osteoarthritis: state of the art and perspectives. *Osteoarthritis Cartilage* 2015;23:2027-35.
26. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. *Cytotherapy* 2006;8:315-7.
27. Gupta PK, Das AK, Chullikana A, Majumdar AS. Mesenchymal stem cells for cartilage repair in osteoarthritis. *Stem Cell Res Ther* 2012;3:25.
28. Chen YC, Chen CH, Chen PL, Huang IY, Shen YS, et al. Donor site morbidity after harvesting of proximal tibia bone. *Head Neck* 2006;28:496-500.
29. Cotter EJ, Wang KC, Yanke AB, Chubinskaya S. Bone marrow aspirate concentrate for cartilage defects of the knee: from bench to bedside evidence. *Cartilage* 2018;9:161-70.
30. Nathan S, Das De S, Thambyah A, Fen C, Goh J, et al. Cell-based therapy in the repair of osteochondral defects: a novel use for adipose tissue. *Tissue Eng* 2003;9:733-44.
31. Si Z, Wang X, Sun C, Kang Y, Xu J, et al. Adipose-derived stem cells: Sources, potency, and implications for regenerative therapies. *Biomed Pharmacother* 2019;114:108765.
32. Baer PC, Geiger H. Adipose-derived mesenchymal stromal/stem cells: tissue localization, characterization, and heterogeneity. *Stem Cells Int* 2012;2012:812693.
33. Pak J, Lee JH, Park KS, Park M, Kang LW, et al. Current use of autologous adipose tissue-derived stromal vascular fraction cells for orthopedic applications. *J Biomed Sci* 2017;24:9.
34. De Ugarte DA, Morizono K, Elbarbary A, Alfonso Z, Zuk PA, et al. Comparison of multi-lineage cells from human adipose tissue and bone marrow. *Cells Tissues Organs* 2003;174:101-9.
35. Xu L, Liu Y, Sun Y, Wang B, Xiong Y, et al. Tissue source determines the differentiation potentials of mesenchymal stem cells: a comparative study of human mesenchymal stem cells from bone marrow and adipose tissue. *Stem Cell Res Ther* 2017;8:275.
36. Heo JS, Choi Y, Kim HS, Kim HO. Comparison of molecular profiles of human mesenchymal stem cells derived from bone marrow, umbilical cord blood, placenta and adipose tissue. *Int J Mol Med* 2016;37:115-25.
37. Deng M, Gu Y, Liu Z, Qi Y, Ma GE, et al. Endothelial differentiation of human adipose-derived stem cells on polyglycolic acid/polylactic acid mesh. *Stem Cells Int* 2015;2015:350718.
38. Lin J, Zhu Q, Huang J, Cai R, Kuang Y. Hypoxia promotes vascular smooth muscle cell (VSMC) differentiation of adipose-derived stem cell (ADSC) by regulating mettl3 and paracrine factors. *Stem Cells Int* 2020;2020:2830565.
39. Edwards NJ, Stone R, Christy R, Zhang CK, Pollok B, et al. Differentiation of adipose derived stem cells to keratinocyte-like cells on an advanced collagen wound matrix. *Tissue Cell* 2018;53:68-75.
40. Wada Y, Ikemoto T, Morine Y, Imura S, Saito Y, et al. The differences in the characteristics of insulin-producing cells using human adipose-tissue derived mesenchymal stem cells from subcutaneous and visceral tissues. *Sci Rep* 2019;9:13204.
41. Gao S, Guo X, Zhao S, Jin Y, Zhou F, et al. Differentiation of human adipose-derived stem cells into neuron/motoneuron-like cells for cell replacement therapy of spinal cord injury. *Cell Death Dis* 2019;10:597.
42. Tomita K, Madura T, Sakai Y, Yano K, Terenghi G, et al. Glial differentiation of human adipose-derived stem cells: implications for cell-based transplantation therapy. *Neuroscience* 2013;236:55-65.
43. Lee RH, Kim B, Choi I, Kim H, Choi HS, et al. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. *Cell Physiol Biochem* 2004;14:311-24.
44. Tsuji W, Rubin JP, Marra KG. Adipose-derived stem cells: implications in tissue regeneration. *World J Stem Cells* 2014;6:312-21.
45. Sakaguchi Y, Sekiya I, Yagishita K, Muneta T. Comparison of human stem cells derived from various mesenchymal tissues - Superiority of synovium as a cell source. *Arthritis Rheum* 2005;52:2521-9.
46. Mochizuki T, Muneta T, Sakaguchi Y, Nimura A, Yokoyama A, et al. Higher chondrogenic potential of fibrous synovium- and adipose synovium-derived cells compared with subcutaneous fat-derived cells: distinguishing properties of mesenchymal stem cells in humans. *Arthritis Rheum* 2006;54:843-53.
47. Kouroupis D, Bowles AC, Willman MA, Perucca Orfei C, Colombini A, et al. Infrapatellar fat pad-derived MSC response to inflammation

- and fibrosis induces an immunomodulatory phenotype involving CD10-mediated Substance P degradation. *Sci Rep* 2019;9:10864.
48. Centeno CJ, Busse D, Kisiday J, Keohan C, Freeman M, et al. Regeneration of meniscus cartilage in a knee treated with percutaneously implanted autologous mesenchymal stem cells. *Med Hypotheses* 2008;71:900-8.
  49. Davatchi F, Abdollahi BS, Mohyeddin M, Shahram F, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis. Preliminary report of four patients. *Int J Rheum Dis* 2011;14:211-5.
  50. Davatchi F, Abdollahi BS, Mohyeddin M, Nikbin B. Mesenchymal stem cell therapy for knee osteoarthritis: 5 years follow-up of three patients. *Int J Rheum Dis* 2016;19:219-25.
  51. Orozco L, Munar A, Soler R, Alberca M, Soler F, et al. Treatment of knee osteoarthritis with autologous mesenchymal stem cells: two-year follow-up results. *Transplantation* 2014;97:e66-8.
  52. Soler R, Orozco L, Munar A, Huguet M, Lopez R, et al. Final results of a phase I-II trial using ex vivo expanded autologous mesenchymal stromal cells for the treatment of osteoarthritis of the knee confirming safety and suggesting cartilage regeneration. *Knee* 2016;23:647-54.
  53. Chahal J, Gómez-Aristizábal A, Shestopaloff K, Bhatt S, Chaboureaud A, et al. Bone marrow mesenchymal stromal cell treatment in patients with osteoarthritis results in overall improvement in pain and symptoms and reduces synovial inflammation. *Stem Cells Transl Med* 2019;8:746-57.
  54. Fodor PB, Paulseth SG. Adipose derived stromal cell (ADSC) injections for pain management of osteoarthritis in the human knee joint. *Aesthet Surg J* 2016;36:229-36.
  55. Yokota N, Hattori N, Ohtsuru T, Otsuji M, Lyman S, et al. Comparative clinical outcomes after intra-articular injection with adipose-derived cultured stem cells or noncultured stromal vascular fraction for the treatment of knee osteoarthritis. *Am J Sports Med* 2019;47:2577-83.
  56. Pers YM, Rackwitz L, Ferreira R, Pullig O, Delfour C, et al. ADIPOA Consortium. Adipose mesenchymal stromal cell-based therapy for severe osteoarthritis of the knee: a phase I dose-escalation trial. *Stem Cells Transl Med* 2016;5:847-56.
  57. Lee WS, Kim HJ, Kim KI, Kim GB, Jin W. Intra-articular injection of autologous adipose tissue-derived mesenchymal stem cells for the treatment of knee osteoarthritis: a phase IIb, randomized, placebo-controlled clinical trial. *Stem Cells Transl Med* 2019;8:504-11.
  58. Lu L, Dai C, Zhang Z, Du H, Li S, et al. Treatment of knee osteoarthritis with intra-articular injection of autologous adipose-derived mesenchymal progenitor cells: a prospective, randomized, double-blind, active-controlled, phase IIb clinical trial. *Stem Cell Res Ther* 2019;10:143.
  59. Chuckpaiwong B, Charles HC, Kraus VB, Guilak F, Nunley JA. Age-associated increases in the size of the infrapatellar fat pad in knee osteoarthritis as measured by 3T MRI. *J Orthop Res* 2010;28:1149-54.
  60. Koh YG, Choi YJ. Infrapatellar fat pad-derived mesenchymal stem cell therapy for knee osteoarthritis. *Knee* 2012;19:902-7.
  61. do Amaral R, Almeida HV, Kelly DJ, O'Brien FJ, Kearney CJ. Infrapatellar fat pad stem cells: from developmental biology to cell therapy. *Stem Cells Int* 2017;2017:6843727.
  62. Toghraie FS, Chenari N, Gholipour MA, Faghil Z, Torabinejad S, et al. Treatment of osteoarthritis with infrapatellar fat pad derived mesenchymal stem cells in Rabbit. *Knee* 2011;18:71-5.
  63. Koh YG, Jo SB, Kwon OR, Suh DS, Lee SW, et al. Mesenchymal stem cell injections improve symptoms of knee osteoarthritis. *Arthroscopy* 2013;29:748-55.
  64. Spasovski D, Spasovski V, Bascarevic Z, Stojiljkovic M, Vreca M, et al. Intra-articular injection of autologous adipose-derived mesenchymal stem cells in the treatment of knee osteoarthritis. *J Gene Med* 2018;20:e3002.
  65. Wakitani S, Imoto K, Yamamoto T, Saito M, Murata N, et al. Human autologous culture expanded bone marrow mesenchymal cell transplantation for repair of cartilage defects in osteoarthritic knees. *Osteoarthritis Cartilage* 2002;10:199-206.
  66. Nejadnik H, Hui JH, Feng Choong EP, Tai BC, Lee EH. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. *Am J Sports Med* 2010;38:1110-6.
  67. Haleem AM, Singergy AAE, Sabry D, Atta HM, Rashed LA, et al. The clinical use of human culture-expanded autologous bone marrow mesenchymal stem cells transplanted on platelet-rich fibrin glue in the treatment of articular cartilage defects: a pilot study and preliminary results. *Cartilage* 2010;1:253-61.
  68. Teo AQA, Wong KL, Shen L, Lim JY, Toh WS, et al. Equivalent 10-year outcomes after implantation of autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation for chondral defects of the knee. *Am J Sports Med* 2019;47:2881-7.
  69. Koga H, Shimaya M, Muneta T, Nimura A, Morito T, et al. Local adherent technique for transplanting mesenchymal stem cells as a potential treatment of cartilage defect. *Arthritis Res Ther* 2008;10:R84.
  70. Sekiya I, Muneta T, Horie M, Koga H. Arthroscopic transplantation of synovial stem cells improves clinical outcomes in knees with cartilage defects. *Clin Orthop Relat Res* 2015;473:2316-26.
  71. Shimomura K, Ando W, Moriguchi Y, Sugita N, Yasui Y, et al. Next generation mesenchymal stem cell (MSC)-based cartilage repair using scaffold-free tissue engineered constructs generated with synovial mesenchymal stem cells. *Cartilage* 2015;6:13S-29S.
  72. Chang CH, Kuo TF, Lin FH, Wang JH, Hsu YM, et al. Tissue engineering-based cartilage repair with mesenchymal stem cells in a porcine model. *J Orthop Res* 2011;29:1874-80.
  73. Chen CC, Liao CH, Wang YH, Hsu YM, Huang SH, et al. Cartilage fragments from osteoarthritic knee promote chondrogenesis of mesenchymal stem cells without exogenous growth factor induction. *J Orthop Res* 2012;30:393-400.
  74. Chang CH, Chen CC, Liao CH, Lin FH, Hsu YM, et al. Human acellular cartilage matrix powders as a biological scaffold for cartilage tissue engineering with synovium-derived mesenchymal stem cells. *J Biomed Mater Res A* 2014;102:2248-57.
  75. Chen CC, Hsiao CY, Wang YH, Chen YC, Chang CH, et al. A comparison of distinct bone marrow-derived cells on cartilage tissue engineering. *J Taiwan Inst Chem Eng* 2017;78:32-8.



76. Yang YK, Ogando CR, Wang See C, Chang TY, Barabino GA. Changes in phenotype and differentiation potential of human mesenchymal stem cells aging in vitro. *Stem Cell Res Ther* 2018;9:131.
77. Raynauld JP, Buckland-Wright C, Ward R, Choquette D, Haraoui B, et al. Safety and efficacy of long-term intraarticular steroid injections in osteoarthritis of the knee: a randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 2003;48:370-7.
78. Ray TR. Using viscosupplementation to treat knee osteoarthritis. *Phys Sportsmed* 2013;41:16-24.
79. Volz M, Schaumburger J, Frick H, Grifka J, Anders S. A randomized controlled trial demonstrating sustained benefit of Autologous Matrix-Induced Chondrogenesis over microfracture at five years. *Int Orthop* 2017;41:797-804.
80. Shive MS, Stanish WD, McCormack R, Forriol F, Mohtadi N, et al. BST-CarGel® treatment maintains cartilage repair superiority over microfracture at 5 years in a multicenter randomized controlled trial. *Cartilage* 2015;6:62-72.
81. Jiang J, Tang A, Ateshian GA, Guo XE, Hung CT, et al. Bioactive stratified polymer ceramic-hydrogel scaffold for integrative osteochondral repair. *Ann Biomed Eng* 2010;38:2183-96.
82. Chiang H, Liao CJ, Hsieh CH, Shen CY, Huang YY, et al. Clinical feasibility of a novel biphasic osteochondral composite for matrix-associated autologous chondrocyte implantation. *Osteoarthritis Cartilage* 2013;21:589-98.
83. Xue D, Zheng Q, Zong C, Li Q, Li H, et al. Osteochondral repair using porous poly(lactide-co-glycolide)/nano-hydroxyapatite hybrid scaffolds with undifferentiated mesenchymal stem cells in a rat model. *J Biomed Mater Res A* 2010;94:259-70.
84. McCarthy HS, Roberts S. A histological comparison of the repair tissue formed when using either Chondrogide® or periosteum during autologous chondrocyte implantation. *Osteoarthritis Cartilage* 2013;21:2048-57.
85. Schneider U, Rackwitz L, Andereya S, Siebenlist S, Fensky F, et al. A prospective multicenter study on the outcome of type I collagen hydrogel-based autologous chondrocyte implantation (CaReS) for the repair of articular cartilage defects in the knee. *Am J Sports Med* 2011;39:2558-65.
86. Trattnig S, Pinker K, Krestan C, Plank C, Millington S, et al. Matrix-based autologous chondrocyte implantation for cartilage repair with Hyalograft®C: two-year follow-up by magnetic resonance imaging. *Eur J Radiol* 2006;57:9-15.
87. Erggelet C, Kreuz PC, Mrosek EH, Schagemann JC, Lahm A, et al. Autologous chondrocyte implantation versus ACI using 3D-bioresorbable graft for the treatment of large full-thickness cartilage lesions of the knee. *Arch Orthop Trauma Surg* 2010;130:957-64.

Review

Open Access



# 50+ years of replantation surgery experience: are we progressing or regressing?

Karen Noh, Jacques H. Hacquebord

Department of Orthopaedic Surgery, NYU Langone Health, New York, NY 10014, USA.

**Correspondence to:** Dr. Jacques H. Hacquebord, Department of Orthopaedic Surgery, NYU Langone Health, 530 1st Avenue, New York, NY 10016, USA. E-mail: Jacques.Hacquebord@nyulangone.org

**How to cite this article:** Noh K, Hacquebord JH. 50+ years of replantation surgery experience: are we progressing or regressing? *Plast Aesthet Res* 2020;7:50. <http://dx.doi.org/10.20517/2347-9264.2020.49>

**Received:** 15 Mar 2020 **First Decision:** 12 Aug 2020 **Revised:** 21 Aug 2020 **Accepted:** 4 Sep 2020 **Published:** 17 Sep 2020

**Academic Editor:** Alessandro Thione **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

The first successful digit replantation was reported in 1965 and accepted enthusiastically by hand surgeons. The decade that immediately followed saw a surge of interest in this complex surgery, fueling significant improvements in success rates and the rise of hand and microsurgeons who were highly proficient in replantation. The decades that followed, however, showed a stable field lacking any significant changes or advancements. More recently, and especially in the United States, the frequency with which surgeons even attempt replantation and the rate of survival have plummeted. If this trend continues, successful replantation surgery will become all too rare of an event. It is critical that we evaluate the state of replantation surgery today, identify the primary causes, and work to not only revive the field but allow it to advance similar to other areas of medicine.

**Keywords:** Digit, replantation, amputation, hand surgery, microsurgery

## INTRODUCTION

In 2017, 14.7% of primary diagnoses at emergency department visits in the United States were classified as an injury to the wrist, hand, and fingers<sup>[1]</sup>. Injuries to the hand and digits are exceedingly common - of these, traumatic amputations of the digit are among the most severe injuries possible. The hand surgeon is faced with two important options in treating this devastating injury: replantation or revision amputation.

Studies have demonstrated that replantation may be more desirable: a 2019 study reported significantly better functional outcomes in patients with successful replantation than revision outcomes, as measured



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



by nine-hole peg test times, Semmes-Weinstein monofilament test, and three-point pinch test. Patient-reported outcomes - the Michigan Hand Outcomes Questionnaire; Disabilities of the Arm, Shoulder, and Hand; and Patient-Reported Outcomes Measurement Information System upper-extremity module scores and functional outcomes - were significantly higher as well<sup>[2]</sup>. While successful replantation appears to be superior to revision amputation, there has been an alarming decrease in attempts to replant amputated digits.

We start with the history of digit replantation to better understand the current state of digit replantation and how our techniques have evolved - or not evolved.

## EARLY HISTORY

In 1894, Sadi Carnot, the president of France, was assassinated after a stab wound to the abdomen that lacerated his portal vein. Surgeons of the time claimed he could not have been saved due to the nature of his injuries. Alexis Carrel, then a young medical student at the University of Lyon, disagreed<sup>[3]</sup>. He began experimenting, and by 1902 he had published his first articles on vascular anastomosis<sup>[4]</sup>.

Carrel performed the first extremity replantation in 1906, the amputated hind limb of a dog<sup>[5]</sup>. Much of his work became pillars of organ transplantation and heart surgery, and he later became the recipient of the Nobel Prize in Physiology or Medicine for his contributions to vascular suture and transplantation of blood vessels and organs<sup>[6]</sup>. These early developments in vascular anastomosis made replantation of limbs, and eventually digits, a possibility.

## EARLY BARRIERS TO SUCCESSFUL REPLANTATION

There exists a nearly 60-year gap between Carrel's early experimentation with limb replantation in canines in 1907 and Ronald Malt's first limb replantation in a human in 1964<sup>[7]</sup>. This gap was at least in part due to the lack of appropriate equipment and technology. Antibiotics were not discovered until the late 1920s and were not standard postoperative protocol until even later. Anticoagulants such as heparin and coumadin were not discovered until 1936 and 1941<sup>[8]</sup>. The operating microscope was introduced in 1921, but it was not used for microvascular surgery until 1960<sup>[8]</sup>. Finally, Harry Buncke developed the first set of microsurgical instruments including microsutures and microsurgical needles<sup>[9]</sup>.

With these developments, the major technical challenges to digital replantation had been addressed: proper suture technique to minimize the risk of hemorrhage, stenosis, and thrombosis; antibiotics to prevent infection; anticoagulants to prevent clotting; the operating microscope for better visualization; and the appropriate tools for microvascular surgery. The stage was set.

## THE FIRST SUCCESSFUL DIGIT REPLANTATION

In 1965, Komatsu and Tamai<sup>[10]</sup> reported the first successful digital replantation. They had attempted several replantations of completely amputated digits over a year, none of which were successful. In a 4.5-h surgery, Komatsu and Tamai replanted a completely amputated thumb, performing an end-to-end anastomosis on two volar digital arteries and two volar dorsal veins. The patient was discharged 40 days after the landmark surgery, returning to his original occupation only four months after his injury. Remarkably, 200 days after the operation, the patient displayed only slight atrophy and loss of sensation. The case report was published in 1968, and it was received enthusiastically by the medical community.

Such great success of the world's first reported digit replantation begs the question: How much has improved since then? Is there more to be improved, or have we already reached the limits of success in digit replantation?

## EARLY CHALLENGES

Following the first reported successful replantation, there was a surge of interest in replantation surgery<sup>[11]</sup>. Hand/microsurgeons attempted replantation with enthusiasm. However, replanted digits often lacked function and sensation; some were even painful<sup>[11]</sup>. Reattaching the amputated digit simply because it was possible was not enough.

As it became clear that digit amputations were not all equal, hand surgeons first looked to case selection as a means of improving success rates. The first consideration was if the digit was in suitable condition for replantation. For instance, a severe crush injury causing comminuted fractures and significant soft tissue disruption may not be eligible for replantation. Fingers that were stored improperly during transport, such as in non-biologic solutions or on dry ice, were definite contraindications.

In 1978, a replantation team in Vienna compiled their three-year experience in replantation, which included a set of indications<sup>[12]</sup>. They stated replantation should always be attempted in any amputations in young patients, especially children. Thumbs should always be replanted but not the other fingers except in the case of multiple digit amputations causing loss of the ability to grip. They believed single finger amputations should be performed only if the patient required that digit for their profession, skills, or hobbies. In 1981, Dr. Zhong-Wei, Dr. Meyer, Dr. Kleinert, and Dr. Beasley, today regarded as pioneers in replantation, together compiled the experiences from the authors' home institutions in China, Switzerland, and the United States<sup>[13]</sup>. While they admit that firm indications for finger replantation are impossible, their experiences largely matched those published by the Viennese replantation team. Since then, the indications and contraindications have mostly remained unchanged<sup>[11,14,15]</sup>:

### 1. Thumb amputations

As the thumb is responsible for up to 40% of hand function, all thumb amputations should be considered for replantation. Factors that may constitute contraindications in any other single digit amputation are underemphasized relative to the importance of the thumb<sup>[16]</sup>.

### 2. Multiple digit amputations

Unsurprisingly, the more digits that are successfully replanted, the greater is the final function. Replantation is attempted starting with the digit with the greatest contribution to hand function and greatest chance of recovery<sup>[17]</sup>.

### 3. Mid-palm amputations

Amputations at the mid-palm level or more proximally are replanted more successfully than amputations at the level of the digital arteries. Following successful replantations at this level, function is far superior to any prosthetics<sup>[18]</sup>.

### 4. Single digit amputation distal to FDS tendon insertion

Reported as early as 1981 by Dr. Zhong-Wei, amputations distal to insertion of the FDS tendon were found to have superior outcomes<sup>[13]</sup>. Replanting digits proximal to the insertion of the FDS tendon often results in a stiff proximal interphalangeal joint.

### 5. Amputations in pediatric patients

Pediatric patients have superior healing potential compared to adults. However, their anatomy is even smaller than that of adult patients and thus cases in pediatric patients may be even more challenging.

While these guidelines were a good place to start, the exact details of the injury and the patient were determined to be equally, if not more, important. A detailed history - including the circumstances of the injury, past medical history, and social history were found to be critical in order to determine if replantation was worthwhile. Mechanism was also found to be a crucial component to the consideration that directly affects the zone of injury and likelihood for a successful outcome.

While these guidelines were a good place to start, the exact details of the injury and the patient were determined to be equally, if not more, important. A detailed history - including the circumstances of the

injury, past medical history, and social history were found to be critical in order to determine if replantation was worthwhile. Mechanism was also found to be a crucial component to the consideration that directly affects the zone of injury and likelihood for a successful outcome. A final consideration is ischemia time, especially in rural or medically underserved areas where a patient may need to travel significant distances or be transferred to a trauma center with microsurgical service availability. Traditionally, it has been taught that prolonged ischemia time negatively impacts success rates. However, recent literature has shown that ischemia time may not play a large role in digit replantation success. A meta-analysis found ischemia time failed to influence replant survival<sup>[19]</sup>. This is likely because digits do not have large muscle mass, and therefore are less vulnerable to rapid necrosis. However, reperfusion injury remains a significant risk and urgent, although perhaps not immediate, replantation is necessary. Current guidelines suggest limits of 12 h of warm ischemia time or 24 h of cold ischemia time, although this has increasingly been challenged<sup>[20]</sup>. Lin and colleagues reported a success rate of 64.0% in a small cohort of 14 patients who underwent replantation after 24 h of ischemia<sup>[21]</sup>. In as early as 1988, Wei and colleagues reported successful replantations after 84, 86, and 94 h of cold ischemia time<sup>[22]</sup>.

An interesting addendum to extending ischemia time is cryopreservation. A critical component of successfully reattaching a cryopreserved part is uniform freezing and thawing; because digits do not have significant muscle mass, they have potential to be successfully replanted following cryopreservation. Wang and colleagues reported two successful cases of digit replantation after cryopreservation - one left index finger cryopreserved in liquid nitrogen for 10 days before replantation, and one left thumb cryopreserved in liquid nitrogen for 30 days before replantation<sup>[23]</sup>.

Surgeons from early on appreciated how comorbidities were an important consideration, as they may influence vasculature quality. Hustedt and colleagues found that the rates of replant failure were highest in patients with psychotic disorders, peripheral vascular disease, and electrolyte imbalances<sup>[24]</sup>. As significant medical comorbidities were of concern, age quickly became a relative contraindication. Concern was certainly warranted in older patients as with any long and invasive surgery. However, Kwon and colleagues found that microsurgical success and satisfaction with the results of the procedure were comparable in elderly patients (70 years and older) versus younger patients<sup>[25]</sup>.

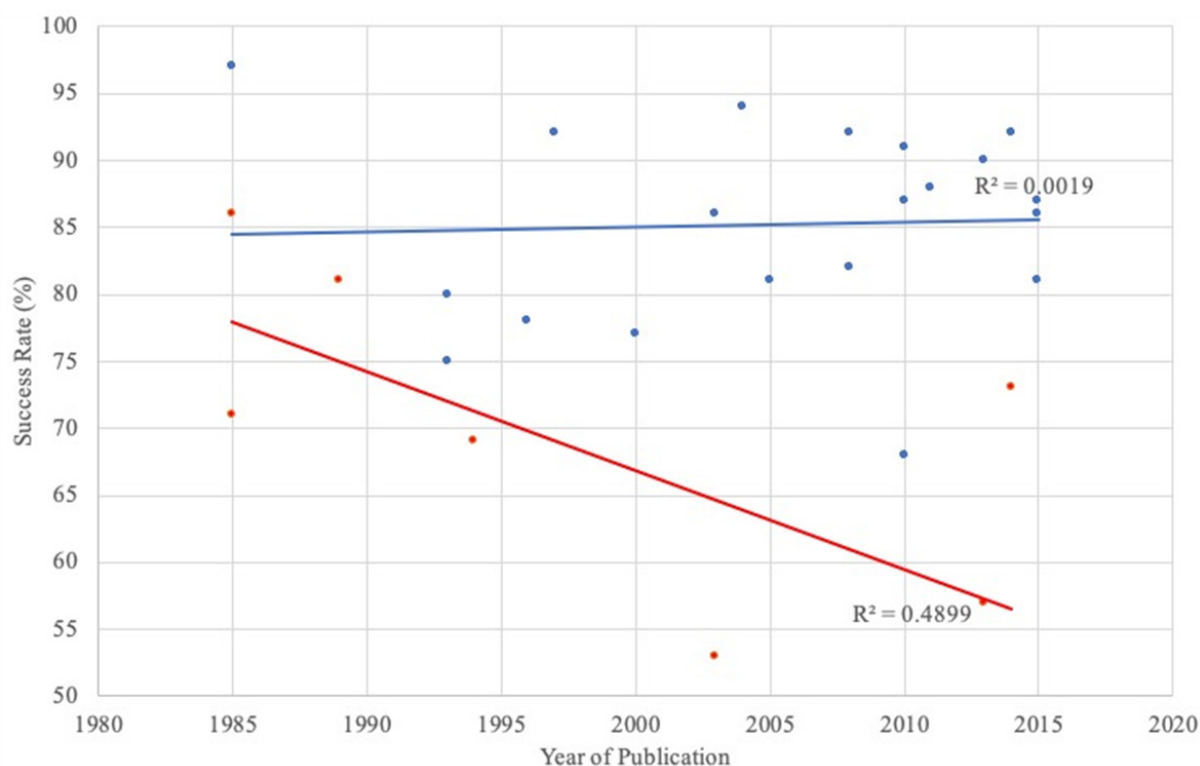
From early on, it was realized that many contraindications and indications for replantation were not absolute. Surgeons had the responsibility to carefully weigh the risks and benefits of a variety of factors - which included more than just the medical facts. As Dr. Zhong-Wei said<sup>[13]</sup>, “The psychological response to injury, the importance of body image, and the concern for disfigurement vary enormously among cultures but in many may be as important as the restriction of physical abilities.” He noted that, in some cultures, an intact but nonfunctional digit is of greater value than an absent digit that is functionally compensated for by other digits or a prosthetic, and vice versa in other cultures. He concluded that “function” cannot be measured solely by physiology and ability, but rather it must also embrace the patient’s very personal perception of a successful outcome.

## REPLANTATION TODAY

Success rates of replantation rapidly increased as surgeons gained experience and selected cases more prudently, with one study reporting a survival rate of 97% in as early as 1985<sup>[26]</sup>. However, the recent literature is less optimistic.

A recent meta-analysis compiled the results of 32 studies with comparable inclusion criteria and outcome measures to determine factors associated with increased survival<sup>[19]</sup>. An interesting trend emerged: during 1985-2015, survival attempts of studies done in the US ranged 48%-86%, while rates those coming from Asia, namely China, Japan, Korea, Taiwan, and India, ranged 68%-97%. While survival rates in Asian





**Figure 1.** The success rates of 28 studies in Asia versus the United States. We plotted the success rates reported in 28 studies, derived from the 2018 meta-analysis by Shaterian and colleagues. Linear regression in this case is not intended to suggest mathematically or statistically meaningful trends, but it demonstrates a simplified visual of the trend of success rates in Asian countries (China, Japan, Korea, Taiwan, and India) versus the United States

countries largely remained constant, survival rates in the US have plummeted [Figure 1]. In 2013, Fufa and colleagues reported only a 57% success rate for replantation of Tamai Level III or IV amputations performed at two large academic Level I hospitals<sup>[27]</sup>. Mulders and colleagues reported a success rate of 48%<sup>[28]</sup>. A large academic replantation center in 2019 reported a survival rate of only 50%<sup>[29]</sup>. This is in stark contrast to success rates in Asian countries. What is the cause of this discrepancy? More importantly, how can we improve?

## IN THE LITERATURE: UNCERTAINTY AND DISAGREEMENT

The field of replantation developed significantly in the early days, but this trend has not continued through the decades. There remains great variability in surgeons' preferred techniques in nearly all aspects of the treatment. The pioneers in replantation anticipated that survival rates would increase as techniques advanced. However, the literature seems to show more disagreement and a lack of definitive answers than advancements in techniques and innovation.

Even the sequence of steps for when vascular anastomoses is performed varies between surgeons. In Green's Operative Hand Surgery, the recommended sequence of repair is: osseous fixation, extensor tendons, flexor tendons, digital nerves, dorsal veins, and arteries<sup>[30]</sup>. Chung and Alderman, however, recommended the artery before the vein<sup>[31]</sup>. Anecdotally, high volume replantation surgeons will proceed only if good veins can be identified. Other surgeons have argued that venous outflow is not essential for successful surgery<sup>[29]</sup>. While these distinctions may seem minor, survival of the digit is solely dependent on establishing adequate arterial inflow and venous outflow.

The optimal osseous fixation remains debated. Lee *et al.*<sup>[32]</sup> retrospectively evaluated 103 replanted digits. Of their 32 digits with radiographic nonunion, the highest rate of nonunion was found after cross fixation and the lowest after intraosseous wire alone; however, this difference was not statistically significant. Hoffman and colleagues concluded that intraosseous wire was better for fracture fixation and early hand therapy as well<sup>[33]</sup>. However, Cheng *et al.*<sup>[34]</sup> concluded that rigid internal fixation with plate and screws were superior in their cohort. Anecdotally, k-wire fixation remains one of the most common techniques seen with many replantation surgeons. Further prospective studies are needed to determine the optimal method of bony fixation which minimizes complications while maximizing the possibility for early motion and successful replantation.

Uncertainty regarding the use of postoperative anticoagulants was reported as early as 1978, and remains an area of disagreement today<sup>[35]</sup>. Anticoagulants are used widely following replantation surgery. However, the appropriate type, dose, and timing between surgeons and institutions varies widely, ranging from no thromboprophylaxis at all to various doses of aspirin, dextran, and heparin<sup>[36]</sup>. Even the specifics of route, quantity, and what type of heparin is administered varies greatly among surgeons. A 2019 randomized and single-blinded study found no significant difference between the three groups: (1) no heparin; (2) low dose heparin (10,000 IU/day); and (3) high dose heparin (starting at 15,000 IU/day and adjusted to achieve activated partial thromboplastin time of 1.5-2.5 times the patient's baseline)<sup>[37]</sup>. Studies with various therapeutic evidence levels have supported these conclusions<sup>[38,39]</sup>. In addition to dose, one study found increased survival with progressive weaning of heparin rather than abruptly discontinuing it<sup>[40]</sup>. While systematic heparinization does not appear to impact success rates, two recent studies have supported digit salvage using local injections of heparin<sup>[41]</sup> or heparin calcium<sup>[42]</sup>. Preventing complications with arterial inflow and venous outflow is critical for obvious reasons and also because even if caught early salvage rates remain low<sup>[43]</sup>. Clearly, further research is needed to clarify the specific type, dose, and timing that is most effective.

As reflected in the literature, there remains significant disagreement about which techniques and interventions result in the highest success rates. More and higher quality research is urgently needed to answer pressing questions about each step of replantation surgery.

## IN PRACTICE: DWINDLING INTEREST AND ATTEMPTS

Discouraging rates of success in combination with the lack of consensus on the best techniques and postoperative protocols have likely contributed to decreasing interest and attempts at replantation surgery in the West. Some have suggested that this decrease in attempts is natural, caused by a decrease in industrial accidents. A 2018 study found, however, that, while workplace finger amputations decreased significantly between 2000 and 2010 in the US, overall finger amputation incidence did not change significantly. However, in that same period, replantation surgery decreased by more than 50%<sup>[44]</sup>. The conclusion is that attempts at replantation in the US have decreased despite consistent incidence of amputations. There are likely several factors that have contributed to this.

Peterson and colleagues found inconsistent availability of hand/microsurgeons at Level I and II trauma centers in the US, leading to inadequate evaluation of the amputated digit, and decreased likelihood that replantation would even be considered<sup>[45]</sup>. Related to this, Hustedt and colleagues theorized that decreasing success rates in the US are correlated with decentralization of replants away from high-volume hospitals<sup>[46]</sup>. They found that high-volume surgeons (more than five replants per year) at high-volume hospitals (more than 20 replants per year) had greater success rates than low-volume surgeons at low-volume hospitals - 92.0% compared to 72.1%. Their proposed solution was centralization of replantations. In distinction to this, Cho *et al.*<sup>[2]</sup> found that “neither hospital case volume nor hospital type was predictive of successful replantation”. Replantation attempts decreased at all hospital types: rural or urban, teaching or non-

teaching. They concluded that centralization alone would not be effective but that other factors required to be addressed.

An interesting finding in the Cho *et al.*<sup>[2]</sup> study was that patients with private insurance were twice as likely to receive replantation in comparison to patients with Medicare or Medicaid. As payer status directly influences reimbursement, they suggested that financial incentive may play a role. This is further supported by a recent analysis of reimbursement information for 51,716 patients by Hooper and colleagues, who determined that replantation reimburses at \$78/wRVU, which is significantly lower compared to revision amputation (\$108) or common procedures such as carpal tunnel release (\$101), trigger finger release (\$116), and extensor tendon repair (\$122)<sup>[47]</sup>. Physician work relative value units (wRVU) is a direct measure of physician reimbursement in the United States and, in this case, an indirect indicator of the perceived value of a procedure. For both surgeons and payers, a common misconception is that there is minimal value in replantations. This is in stark contrast to the literature, which shows good functional outcomes and high patient satisfaction<sup>[15,48]</sup>.

It has been proposed that surgeon experience with microsurgery and anastomoses of fine veins is more important to successful replantation than surgical technique<sup>[11]</sup>. A conclusion of the above evidence is that the stagnant or even decreasing survival rates result from a lack of experience. In the US, this has unfortunately led to a self-perpetuating cycle: lack of experience leads to decreasing success rates, which leads to decreased confidence and incentive, fewer attempts, and thus further lack of experience.

## CONCLUSION

The current literature reveals two problems. First, there is a stagnation of the techniques and knowledge associated with replantation in the literature. There are few conclusive statements that can be made of the intricacies of the surgery. Second, and more discouraging, there is a regression of the field in the Western hemisphere, most notably in the US. The rates of replantation and survival are both decreasing - this is evidence of regression of medical care in the US. These two problems paint a concerning picture for the current state of replantation surgery.

In 21st century medicine, we expect a continual and forward march in our knowledge, innovation, treatments, and solutions. The current state of replantation surgery is unfortunately not consistent with this. Can or should we expect any better in the future? If so, there are very important changes that need to take place. Below are four important points that, if appropriately implemented, can allow for progression in replantation surgery.

(1) Further research is urgently needed to better understand the barriers of successful replantation, specifically in the United States. The current literature on replantation varies widely. Length of follow up, how function is determined, and patient-reported outcomes vary from study to study. A coordinated effort with consistent measures of function and patient-reported outcomes, similar to the 1981 report by Dr. Zhong-Wei and other leaders in replantation, would be immensely valuable. Since injury patterns and techniques are heterogenous, well designed and large prospective multi-center studies are a necessary part of the solution.

(2) The evidence clearly supports the importance of technical skill, frequency of replantation, and clinical experience as critical for improved survival rates. It is no secret that there are centers with much higher success rates. Centralizing replantation surgery, especially in the United States, is an essential component of this and naturally fosters the formation for centers of excellence. The knowledge and skill gained at these centers of excellence must be shared and taught to younger replantation surgeons allowing for elevation of the entire field.

(3) Successful replantation relies on a team of people that work in concert. From the operating theatre, to the hospital ward, the therapists' suite, and the doctor's office-all members of the team must be knowledgeable, dedicated, and skilled at treating this unique patient population. Only with this team in place should replantation be embarked. The surgery is only one component of the patient's care.

(4) Replantation surgery is a unique surgical procedure and involves the treatment of multiple different tissue structures: osseous, nerve, skin, vasculature, and connective tissue. Survival of the digit focuses primarily on only one tissue structure - the vasculature. However, successful replantation, as discussed above, must include consideration of function. For this reason, equal attention must be placed on treating all tissue structures. When one tissue structure is either prioritized or neglected compared to the others, the function will suffer.

The future of replantation surgery is at a crossroads. If current trends in the United States remain, successful replantation will become an uncommon occurrence with ever increasing contraindications. As the advancements in partial hand and finger prosthetics have accelerated, some may begin to argue that digit replantation is never necessary. Whether or not the technology will ever develop to the point of being equal to a native and well-functioning digit is uncertain and debatable. Irrespective, technology is far from that point yet and our patients are still in need of successful replantation surgery. Especially in the United States, we must acknowledge that the state of replantation surgery has regressed and is inferior to some of our colleagues around the world. The field of hand surgery and our patients require that we not accept the current state. We must make the necessary changes to further the current standard.

## **DECLARATIONS**

### **Authors' contributions**

Made substantial contributions to review of literature, writing of article: Noh K, Hacquebord JH

### **Availability of data and materials**

Not applicable.

### **Financial support and sponsorship**

None.

### **Conflicts of interest**

Both authors declared that there are no conflicts of interest.

### **Ethical approval and consent to participate**

Not applicable.

### **Consent for publication**

Not applicable.

### **Copyright**

© The Author(s) 2020.

## **REFERENCES**

1. Rui P, Kang K. National hospital ambulatory medical care survey: 2017 emergency department summary tables. National Center for Health Statistics. Available from: [https://www.cdc.gov/nchs/data/nhamcs/web\\_tables/2017\\_ed\\_web\\_tables-508.pdg](https://www.cdc.gov/nchs/data/nhamcs/web_tables/2017_ed_web_tables-508.pdg). [Last accessed on 10 Sep 2020]
2. Cho HE, Zhong L, Kotsis SV, Chung KC. Finger replantation optimization study (FRONT): update on national trends. *J Hand Surg Am*

- 2018;43:903-12.e1.
3. Rothwell A. Alexis Carrel: innovator extraordinaire. *J Perioper Pract* 2011;21:73-6.
4. Dente CJ, Feliciano DV. Alexis Carrel (1873-1944): Nobel laureate, 1912. *Arch Surg* 2005;140:609-10.
5. Kocher MS. History of replantation: from miracle to microsurgery. *World J Surg* 1995;19:462-7.
6. The Nobel Prize. The nobel prize in physiology or medicine 1912: Alexis Carrel. Available from: <https://www.nobelprize.org/prizes/medicine/1912/summary/>. [Last accessed on 15 Mar 2020]
7. Malt R, McKhann CF. Replantation of severed arms. *JAMA* 1964;189:716-22.
8. Kriss TC, Kriss VM. History of the operating microscope: from magnifying glass to microneurosurgery. *Neurosurgery* 1998;42:899-908.
9. American Association of Plastic Surgeons. Memoirs: Harry J. Buncke, M.D. 1922-2008. Available from: <https://aaps1921.org/memoirs/HarryBuncke.cgi>. [Last accessed on 15 Mar 2020]
10. Komatsu S, Tamai S. Successful replantation of a completely cut-off thumb. *Plast Reconstr Surg* 1968;42:374-7.
11. Hadley SR, Capo JT. Digit replantation the first 50 years. *Bull Hosp Jt Dis* 2013;73:148-55.
12. Berger A, Millesi H, Mandl H, Freilinger G. Replantation and revascularization of amputated parts of extremities: a three-year report from the Viennese replantation team. *Clin Orthop Relat Res* 1978;133:212-4.
13. Zhong-Wei C, Meyer VE, Kleiner HE, Beasley RW. Present indications and contraindications for replantation as reflected by long-term functional results. *Orthop Clin North Am* 1981;12:849-70.
14. MacLeod AM, O'Brien BM, Morrison WA. Digital replantation: clinical experiences. *Clin Orthop Relat Res* 1978;133:26-34.
15. Pet MA, Morrison SD, Mack JS, Sears ED, Wright T, et al. Comparison of patient-reported outcomes after traumatic upper extremity amputation: Replantation versus prosthetic rehabilitation. *Injury* 2016;47:2783-8.
16. Soucacos PN, Beris AE, Malizos KN, Toulaitos AS. Bilateral thumb amputation. *J Hand Surg Am* 1982;7:549-56.
17. Salah MM, Khalid KN. Replantation of multiple digits and hand amputations: four case reports. *Cases J* 2008;1:266.
18. Beris AE, Lykissa MG, Korompilias AV, Mitsionis, GI, Vekris MD, et al. Digit and hand replantation. *Arch Orthop Trauma Surg* 2010;130:1141-7.
19. Shaterian A, Rajaii R, Kanack M, Evans G, Leis A. Predictors of digit survival following replantation: quantitative review and meta-analysis. *J Hand Microsurg* 2018;10:66-73.
20. Wolfe VM, Angela AW. Replantation of the upper extremity: current concepts. *J Am Acad Orthop Surg* 2015;23:373-81.
21. Lin CH, Aydyn N, Lin YT, Hsu CT, Lin CH, et al. Hand and finger replantation after protracted ischemia (more than 24 hours). *Ann Plast Surg* 2010;64:286-90.
22. Wei FC, Chang YL, Chen HC, Chuang CC. Three successful digital replantations in a patient after 84, 86, and 94 hours of cold ischemia time. *Plast Reconstr Surg* 1988;82:346-50.
23. Wang J, Lin J, Pei Y, Xu Q, Zhu, L. Cryopreservation and transplantation of amputated finger. *J Cryobiol* 2020;92:235-40.
24. Hustedt JW, Chung A, Bohl DD, Olmscheid N, Edwards S. Evaluating the effect of comorbidities on the success, risk, and cost of digital replantation. *J Hand Surg Am* 2016;41:1145-52.e1.
25. Kwon GD, Ahn JS, Park YG, Chang GW, Ha YC. The effect of patient age on the success rate of digital replantation. *Plast Reconstr Surg* 2017;139:420-6.
26. Cheng GL, Pan DD, Yang ZX, Qu ZY. Replantation of digits amputated at or about the distal interphalangeal joint. *Ann Plast Surg* 1985;15:465-73.
27. Fufa D, Calfee R, Wall L, Zeng W, Goldfarb C. Digit replantation: experience of two U.S. academic level-I trauma centers. *J Bone Joint Surg Am* 2013;95:2127-34.
28. Mulders MAM, Neuhaus V, Becker SJE, Lee SG, Ring DC. Replantation and Revascularization vs. Amputation in Injured Digits. *Hand* 2013;8:267-73.
29. Milone MT, Klifto CS, Lee ZH, Thanik V, Hacquebord JH. Relationships between vein repairs, postoperative transfusions, and survival in single digit replantation. *Hand (N Y)* 2020;15:488-94.
30. Wolfe SW, Hotchkiss RN, Pederson WC, Kozin SH, Cohen MS, et al. Replantation. In: *Green's operative hand surgery*. 7th ed. Philadelphia: Elsevier Health Sciences; 2016. pp. 1476-85.
31. Chung K, Alderman AK. Replantation of the upper extremity: indications and outcomes. *J Hand Surg Am* 2002;2:78-94.
32. Lee SW, Lee DC, Kim JS, Roh SY, Lee KJ. Analysis of bone fixation methods in digital replantation. *Arch Plast Surg* 2017;44:53-8.
33. Hoffmann R, Buck-Gramcko D. Osteosynthesis in digital replantation surgery. *Ann Chir Gynaecol* 1982;71:14-8.
34. Cheng HS, Wong LY, Chiang LF, Chan I, Yip TH, et al. Comparison of methods of skeletal fixation for severely injured digits. *Hand Surg* 2004;9:63-9.
35. Phelps D, Lilla JA, Boswick JA. Common problems in clinical replantation and revascularization in the upper extremity. *Clin Orthop Relat Res* 1978;133:11-25.
36. Buckley T, Hammert WC. Anticoagulation following digital replantation. *J Hand Surg Am* 2011;36:1374-6.
37. Nishijima A, Yamamoto N, Gosho M, Yanagibayashi S, Yoshida R. Appropriate use of intravenous unfractionated heparin after digital replantation: a randomized controlled trial involving three groups. *Plast Reconstr Surg* 2019;143:1224e-32e.
38. Chen YC, Chi CC, Chan FC, Wen YW. Low molecular weight heparin for prevention of microvascular occlusion in digital replantation. *Cochrane Database Syst Rev* 2013;7:CD009894.
39. Retrouvey H, Solaja O, Batlzer HL. Role of postoperative anticoagulation in predicting digit replantation and revascularization failure: a propensity-matched cohort study. *Ann Plast Surg* 2019;83:542-7.
40. Efanov JI, Khriguian J, Cassier S, Boghossian E, Harris PG, et al. Duration and cessation characteristics of heparinization after finger



- replantation: A retrospective analysis of outcomes. *Microsurgery* 2018;38:251-8.
41. Alfesky H, McArthur P, Helmy Y. Salvaging digital replantation and revascularisation: efficiency of heparin solution subcutaneous injection. *Surg Res Pract* 2018;2018:1601738.
  42. Kadota H, Imaizumi A, Ishida K, Sashida Y. Successful local use of heparin calcium for congested fingertip replants. *Arch Plast Surg* 2020;47:54-61.
  43. Tejedor Navarro A, Vendrell Jordà M, Puente Alonso C. Digital replantation/revascularization: predictive factors to microsurgery success-a single-center study. *Eur J Trauma Emerg Surg* 2019; doi: 10.1007/s00068-019-01226-x.
  44. Reavey PL, Stranix JT, Muresan H, Soares M, Thanik V. Disappearing digits: analysis of national trends in amputation and replantation in the united states. *Plast Reconstr Surg* 2018;141:857e-67e.
  45. Peterson BC, Mangiapani D, Kellog R, Leversedge FJ. Hand and microvascular replantation call availability study: a national real-time survey of Level-I and Level-II Trauma Centers. *J Bone Joint Surg Am* 2012;94:e185.
  46. Hustedt JW, Bohl DD, Champagne L. The detrimental effect of decentralization in digital replantation in the United States: 15 Years of evidence from the national inpatient sample. *J Hand Surg Am* 2016;41:593-601.
  47. Hooper RC, Sterbenz JM, Zhong L, Chung KC. An in-depth review of physician reimbursement for digit and thumb replantation. *J Hand Surg Am* 2019;44:443-53.
  48. Sebastin SJ, Chung KC. A systematic review of the outcomes of replantation of distal digital amputation. *Plast Reconstr Surg* 2011;128:723-37.

Review

Open Access



# Phalloplasty: understanding the chaos

Megan Lane<sup>1</sup>, Emily C. Sluiter<sup>1</sup>, Shane D. Morrison<sup>2</sup>, Devin Coon<sup>3</sup>, Katherine M. Gast<sup>4</sup>, Jens U. Berli<sup>5</sup>, William M. Kuzon<sup>1</sup>

<sup>1</sup>Section of Plastic Surgery, University of Michigan, Ann Arbor, MI 48109, USA.

<sup>2</sup>Division of Plastic Surgery, University of Washington School of Medicine, Seattle, WA 98195, USA.

<sup>3</sup>Department of Plastic and Reconstructive Surgery, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

<sup>4</sup>Division of Plastic Surgery, University of Wisconsin, Madison, WI 53792, USA.

<sup>5</sup>Division of Plastic and Reconstructive Surgery, Oregon Health and Science University, Portland, OR 97239, USA.

**Correspondence to:** Dr. William M. Kuzon, Section of Plastic Surgery, Michigan Medicine, 1500 E. Medical Center Drive, 2130 Taubman Center, SPC 5340, Ann Arbor, MI 48109, USA. E-mail: wkuzon@med.umich.edu

**How to cite this article:** Lane M, Sluiter EC, Morrison SD, Coon D, Gast KM, Berli JU, Kuzon WM. Phalloplasty: understanding the chaos. *Plast Aesthet Res* 2020;7:51. <http://dx.doi.org/10.20517/2347-9264.2020.106>

**Received:** 11 May 2020 **First Decision:** 2 Jun 2020 **Revised:** 20 Jun 2020 **Accepted:** 4 Aug 2020 **Published:** 27 Sep 2020

**Academic Editor:** Marlon E. Buncamper **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Wide variation in overall strategies and surgical specifics for masculinizing genital surgery has created a “phalloplasty chaos” that is confusing to both surgeons and patients seeking gender confirming surgery. The purpose of this article is to review masculinizing genital confirming surgery, or “phalloplasty”, focusing on specific goals and categorizing each component of the surgical process. Experienced surgeons from several high-volume centers review and categorize the commonly employed strategies and techniques for gender confirming phalloplasty, including the permutations of approaches to cutaneous flap for phallic construction, the sequence and staging of procedures, and strategies for urethral construction. There is no clear advantage or reduction in complications associated with particular sequences of urethral and phallic reconstruction. Because no single technique or staging strategy has proven superior for gender confirming genital surgery, it is paramount that surgeons are knowledgeable of all available options and the associated advantages, disadvantages, and risks.

**Keywords:** Transgender, phalloplasty, FtM, gender dysphoria, gender reassignment, transmasculine, gender confirmation surgery, gender confirming surgery, gender affirming surgery, transgender genital surgery

## INTRODUCTION

The purpose of this article is to review masculinizing genital gender confirming surgery (GCS) or “phalloplasty”. Experienced surgeons from several high-volume centers review and categorize the



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Reproductive organs	<ul style="list-style-type: none"> <li>•Hysterectomy</li> <li>•Salphingoophorectomy</li> <li>•Vaginectomy</li> </ul>
Creation of phallus	<ul style="list-style-type: none"> <li>•RFFF</li> <li>•ALT</li> <li>•Other</li> </ul>
voiding	<ul style="list-style-type: none"> <li>•Pars fixa reconstruction</li> <li>•Creation of pars pendulans</li> </ul>
sensation	<ul style="list-style-type: none"> <li>•Preservation of at least one dorsal clitoral nerve at the base</li> <li>•Coaptation of cutaneous nerve branch in chosen flap</li> </ul>
Aesthetics and erection	<ul style="list-style-type: none"> <li>•Coronaplasty</li> <li>•Glansplasty</li> <li>•Scrotal and penile implants</li> </ul>

**Figure 1.** Goals of phalloplasty and procedures to create a masculine phallus. As discussed throughout the paper, there are countless combinations of procedures that can be offered to patients on the basis of their specific goals and preferences in addition to chronologic order. Depicted are the core procedures involved in genital masculinization. RFFF: radial forearm free flap; ALT: anterolateral thigh

commonly employed strategies and techniques for gender confirming phalloplasty, focusing on specific goals and categorizing each component of the surgical process. There is wide variation among centers in the choice of cutaneous flap for phallic construction, the sequence and staging of procedures, and in particular, the strategies for urethral construction. All centers performing masculinizing genital GCS should ideally employ a consistent, local algorithm tailored to the available expertise of the participating surgeons while also allowing for appropriate patient preference.

### MASCULINIZING GENITAL GCS: INDICATIONS AND PATIENT SELECTION

In appropriately selected patients, GCS has been clearly demonstrated to improve body image<sup>[1]</sup>, psychosexual functioning<sup>[2]</sup>, and other quality of life measures<sup>[3]</sup> as well as reducing or eliminating gender dysphoria<sup>[4]</sup>. For transgender or non-binary patients who were assigned female at birth and who possess female genital anatomy, construction of a male penis and scrotum to affirm their male gender has been generically termed phalloplasty. However, the transformation of vulvar to penile genitalia is a complex surgical challenge requiring multiple staged procedures; [Figure 1](#) lists a typical sequence of procedures. The propagation of misinformation and unrealistic expectations in the patient population has compounded this “phalloplasty chaos”.

The primary indication for any GCS is to relieve the individual’s gender dysphoria by aligning their anatomy with their gender identity. Two things should be emphasized relative to that statement. First, human gender identity is complex. Although a majority of individuals identify as cis-gender male or cis-gender female, the spectrum of gender identity is broad, and the recent proliferation of descriptive terminology is still inadequate to describe many individuals’ internal sense of their gender. Therefore, masculinizing genital GCS should not be viewed as a cookie-cutter process to construct anatomically

analogous male genitalia for all patients. Each patient's surgical plan should be tailored to best confirm the individual's gender identity and to the individual's willingness to undergo staged procedures and to accept surgical risk, with the latter varying depending on preexisting comorbidities. This may mean that for a given patient the components of phalloplasty discussed below will vary, most commonly by omitting certain elements of a full transformation. This variability, of course, adds to the chaos.

Secondly, we recognize and understand that while the use of gender dysphoria as a diagnosis has helped many transgender patients gain access to medically necessary care, stating that the indication for GCS is to "treat" that diagnosis, and even the notion that surgery must have an indication and must "treat something" with direct benefit to the patient, has been criticized as "pathologizing" to non-cis identified individuals<sup>[5]</sup>. While acknowledging this debate, we emphasize the demonstrated health benefits of medically necessary GCS when applied appropriately<sup>[4,6,7]</sup>. As for any surgery, the indications for intervention and the proper selection of patients are critical to achieving these successful outcomes. Because surgeons alone do not have the expertise to determine which patients will see relief of gender dysphoria through surgical intervention, multidisciplinary collaboration is critical for patient selection. The World Professional Association for Transgender Health Standards of Care (WPATH SOC) has been criticized as pathologizing, paternalistic and authoritative "gatekeeping"<sup>[8-10]</sup>. Although the WPATH SOC may sometimes be applied in this manner, that is not the intent. Rather, the SOC should not be viewed as a set of rigid rules, but instead viewed as a statement of principles to structure communication among the multidisciplinary team working to achieve the best outcome for each patient. The overarching goal is to prevent patient harm through inappropriate intervention. As a result, we advocate adherence to the WPATH SOC when selecting patients for masculinizing GCS. Again, we acknowledge that this is not a universally held opinion.

## GOALS OF MASCULINIZING GCS

Once patients have been identified as a surgical candidate, their individual goals, tolerance for surgical risk, and their willingness to engage in multiple staged procedures will guide surgical planning. The general goals of masculinizing genital GCS include: (1) the preservation of erogenous and orgasmic sensation; (2) the anatomic transformation of the vulva and vagina to a penis and a scrotum; (3) achieving an appropriately directed urinary stream, including standing urination if the patient so desires; (4) achieving sensation in the neophallus; and (5) providing for erectile potential sufficient for penetrative intercourse. Of these goals, the preservation of erogenous and orgasmic potential is most important and, because preservation of an innervated clitoris at the base of the neophallus will preserve erogenous/orgasmic sensation in nearly all patients, is associated with the least variance in strategic approach from center to center<sup>[11]</sup>. The remaining goals will achieve varying significance for individual patients, dependent both on their specific gender identity and, perhaps more importantly, on their tolerance for surgical risk. Therefore, patient choice is a significant contributor to the wide variation in masculinizing genital GCS procedures. As for developing all surgical plans, patients should be made fully aware of the surgical options and, especially, the associated risks.

The surgical interventions necessary to achieve all the above goals when fully transitioning vulvar and vaginal anatomy to penile and scrotal anatomy are listed in [Figure 1](#); each will be considered individually along with variations in sequencing, staging, and approach. A comprehensive discussion of complications and outcomes is beyond the scope and purpose of this paper and will be mentioned only when relevant to understanding variations in surgical strategy.

## Hysterectomy, salpingo-oophorectomy, vaginectomy

The removal of the uterus, ovaries, Fallopian tubes, and vagina is important to many transmasculine patients. Patients should receive counseling on options for fertility preservation; some patients may opt to delay these procedures until after they have had children<sup>[12]</sup>. The option to retain female reproductive

organs while still creating a neophallus and/or scrotum is surgically possible but should be carefully evaluated in concert with mental health professionals who have expertise in gender-related care and know the patient well. Specifically, retention of one or both ovaries is offered at some institutions as an option for fertility preservation and/or for maintenance of bone density.

At most centers offering phalloplasty, hysterectomy is indicated whenever a patient desires to have urethral lengthening. The sequencing of hysterectomy, salpingo-oophorectomy, and vaginectomy can vary. Hysterectomy and salpingo-oophorectomy are commonly performed using a transvaginal or standard laparoscopic approach during a separate stage in advance of masculinizing genital surgical procedures<sup>[13]</sup>. A primary advantage of this staging is to avoid the longer operative times, increased procedural blood loss, and greater physiologic disturbance when combining these with other procedures<sup>[14]</sup>. Note that chest recontouring can be performed at the same time as hysterectomy and salpingo-oophorectomy.

For vaginectomy, there is variation in the specific technique used to ablate the vaginal mucosa. Although direct surgical excision may entail more blood loss, and although fulgarization may result in a higher chance of retained vaginal mucosa, at present, there appear to be limited data<sup>[14]</sup> indicating the superiority of any one of the three commonly employed methods: fulgarization<sup>[15]</sup>, direct surgical excision<sup>[16]</sup>, or dual-approach, robotically assisted excision<sup>[17]</sup>. The selected method depends on surgeon expertise and preference and on the staging and specific method of pars fixa construction.

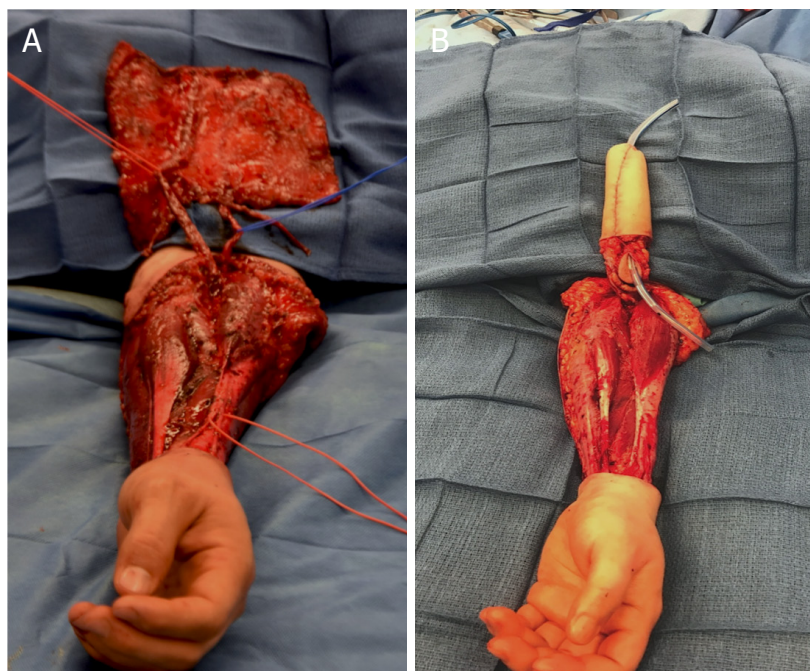
For patients desiring urethral lengthening, pars fixa construction with concomitant vaginectomy provides additional layers of vascularized tissue that may prevent urologic complications<sup>[15]</sup>. However, due to anecdotal reports of urinary leakage and chronic accumulation in the vaginectomy cavity, which can require complex, secondary correction, some centers opt to perform vaginectomy at the time of hysterectomy and salpingo-oophorectomy<sup>[17,18]</sup>. Still, many high-volume centers prefer to perform vaginectomy at the time of pars fixa construction to allow the use of vascularized anterior vaginal myomucosal flaps to create a portion of the pars fixa urethra, hypothesizing that this may reduce the incidence of urinary strictures and fistulas<sup>[15,19-21]</sup>. Note that although pars fixa construction has been described in patients who choose not to undergo vaginectomy, the significant risk profile for urethral complications with this approach leads most centers, current authors included, to advise against it.

### **Tactile and erogenous sensation**

There is also variability in the specific peripheral nerves used to restore tactile sensation in the neophallus. However, restoration of cutaneous sensation in the phallus is always via straightforward nerve coaptation between a cutaneous nerve of the flap and a sensory nerve in the recipient site. This aspect of phalloplasty has been well covered in detail in two recent reviews and we will not elaborate further here<sup>[22-24]</sup>.

Despite variability in specifics to restore cutaneous sensation in the neophallus, one aspect of masculinizing genital surgery that varies little among centers is that the strategy is to preserve rather than ablate and then try to restore erogenous and orgasmic sensation. To that end, virtually all centers performing phalloplasty leave a de-epithelialized clitoris with at least one dorsal clitoral nerve intact at the base of the neophallus<sup>[11,25-28]</sup>. Although there are isolated centers that divide both clitoral nerves to reinnervate the cutaneous skin of the neophallus<sup>[29]</sup>, considerable data confirming the poor recovery of cutaneous sensation after any manner of peripheral nerve division and repair has led to the overwhelming consensus not to rely on the reinnervation of the cutaneous surface of the phallus achieving sufficient reinnervation for erogenous sensation. This consensus is also based on data demonstrating that retention of an innervated, de-epithelialized clitoris will preserve erogenous and orgasmic sensation in nearly 100% of patients undergoing masculinizing GCS<sup>[25,26,30]</sup>.





**Figure 2.** Radial forearm free flap (RFFF)<sup>[31]</sup>. A-B: tube-in-a-tube. The flap is then folded in to make two tubes, with epithelium lining the urethra as well as the surface of the phallus. There is a resultant large donor site defect requiring skin grafting. The RFFF is based upon the radial artery with the original flap dimensions of 10 cm × 11-12 cm. The length of this flap is determined in part by the length of the forearm, which will limit the length of the reconstructed phallus. Photos courtesy of Dr. Jens Berli

## PHALLOPLASTY

The large cutaneous surface area of the male phallus dictates the use of a large cutaneous flap for phallic construction. In the past, random-pattern cutaneous flaps were most commonly used, but axial pedicled or microvascular free flaps dominate at present<sup>[29-31]</sup>. The free radial forearm flap (RFFF) and the anterolateral thigh pedicled or free flap (ALT) are, by a considerable margin, the most common donor site choices currently. The latissimus dorsi (LD) myocutaneous flap<sup>[32,33]</sup> and the superficial circumflex iliac artery flap (SCIP)<sup>[34,35]</sup> are used at select centers. The free fibula osteocutaneous flap<sup>[35-37]</sup> and the lower abdominal pedicled flap<sup>[29,38]</sup> are used less frequently. To add to this heterogeneity, the method of penile urethra construction will influence flap choice and, at times, entail “composite phalloplasty” which is the use of two separate flaps: one for the phallus itself and a second flap for the pars pendulans urethra.

At present, the most commonly employed flap for phallic construction in transmasculine patients is the RFFF, first reported by Chang and Hwang<sup>[28]</sup> in 1984 [Figure 2]. Advantages of the RFFF are: (1) extremely reliable vascular and peripheral neural anatomy<sup>[39]</sup>; (2) the forearm is thin and pliable in most individuals, allowing for simultaneous creation of the pars pendulans urethra via the “tube-in-a-tube” technique<sup>[28,40]</sup>; and (3) the RFFF having the highest innervation density of all the available flaps, providing the best potential for cutaneous reinnervation, and the ulnar, volar surface of the forearm being hair-free in many individuals, lessening or sometimes even obviating the need for hair removal prior to phalloplasty. In addition, the RFFF donor site results in no significant functional disturbance of the arm and hand for the large majority of patients<sup>[41]</sup>. The primary disadvantages of the RFFF are the large donor defect in forearm that must be skin grafted, occasional complications including edema in the hand or sensory neuromas<sup>[42]</sup>, and, if the patient’s forearm is short, a limitation on phallic length [Figure 2]. Despite strategies to reduce donor site deformity<sup>[43]</sup>, some patients refuse the RFFF as they consider the forearm scar to be stigmatizing<sup>[44]</sup>. Regardless, most surgeons performing gender confirming phalloplasties consider the RFFF to be the best flap donor site at the present time.



**Figure 3.** Anterolateral thigh flap (ALT) design. The ALT is based on the lateral femoral circumflex system<sup>[48]</sup>. The dimensions of this flap are determined by the available perforators. Generally, it should be approximately 20 cm in width for a tube-in-a-tube design and can vary in length. The flap should be as distal as possible to ensure pedicle length. Given the dimensions of this flap, the donor site must be skin grafted. Photos courtesy of Dr. Jens Berli

Because the donor site is easily concealed beneath clothing, the ALT flap has gained popularity for phallic construction since first reported for that purpose in 2005 [Figure 3]<sup>[45]</sup>. The ALT can often be performed as a pedicled, rather than as a microvascular, flap, avoiding the potential for complete flap failure secondary to anastomotic thrombosis. The ALT also has a very large cutaneous territory, and in very thin patients, a tube-in-a-tube technique to construct the penile urethra is possible. Although phallic length is generally not limited by the cutaneous territory of the ALT, and although patients may request this, it is generally inadvisable to create a phallus beyond the “standard” dimensions of 5-6 inches. The reason for this is that the thigh often has a thick layer of subcutaneous fat, so tubing the flap into a phallus can be difficult, increasing the chances of partial or total flap loss and sometimes resulting in an unnaturally thick and heavy, “Coke-can” penis pre-disposed to long-term mons pubis and phallic ptosis. This ptosis is difficult to remedy since subsequent debulking of the phallus jeopardizes both the vascularity and innervation of the flap. Furthermore, except in very thin patients as already noted, the bulkiness of the flap frequently precludes creating the penile urethra using the tube-in-a-tube strategy, another strategy for penile urethra construction is often necessary, resulting in additional staged procedures (see Pars Pendulans Construction below)<sup>[46]</sup>. In addition, the ALT donor site must be skin grafted with a significant scar burden [Figure 3]. Lastly, the innervation density of the thigh is low and the single nerve available to restore cutaneous sensation, the lateral cutaneous nerve of the thigh, is often small and has variable anatomy limiting the return cutaneous sensation after ALT<sup>[47]</sup>. For all these reasons, the ALT is a second-choice option usually reserved for thin patients who are adamant that they cannot accept a forearm donor site. Of note, tissue expansion and liposuction of the ALT flap several months in advance of phalloplasty may both delay its vascular territory and obviate the issue with flap bulk<sup>[48]</sup>.

In considering other flaps, most, as already mentioned, are used preferentially in single centers, used infrequently, or mainly of historical interest. Djordjevic *et al.*<sup>[32]</sup> have advocated phalloplasty using the latissimus dorsi myocutaneous flap. Advantages include the reliable donor site anatomy, the inconspicuous donor site that, with adjacent tissue rearrangement, can be closed primarily, and the large amount of tissue available. The authors also speculate that a reinnervated latissimus muscle can aid phallic rigidity and allow penetrative intercourse, although data to confirm this are lacking<sup>[32]</sup>. Significant disadvantages, however,

have limited the wide use of the LD flap for phalloplasty; most significant are the inability to restore cutaneous sensation in the phallus and the need to construct a penile urethra using a second flap or staged skin grafting. The SCIP has also seen limited use for both neophallic and penile urethral construction<sup>[34,35]</sup>. Limitations in the cutaneous skin paddle size, the arc of rotation of the flap, and the difficulty in restoring cutaneous sensation have restricted its use to isolated centers<sup>[34,35]</sup>. The fibula osteoseptocutaneous flap was also conceptualized as a way to provide for penile rigidity, but its use is limited by the complex dissection necessary, the morbidity of the donor site, an insufficient cutaneous island to construct a penile urethra, and the inconvenience and complications resulting from a permanently rigid neophallus<sup>[36]</sup>.

Lastly, it should be noted that “shaft-only phalloplasty” is an option chosen by some patients<sup>[29]</sup>. By constructing only the phallus to affirm male gender, this approach avoids the multi-stage complexity and many of the risks inherent to a complete female-to-male anatomic transformation. Although any cutaneous flap large enough for phallic reconstruction can be used<sup>[49]</sup>, the ALT flap is a more common choice for flap-only phalloplasty than it is for full male genital construction. In addition, local pedicled flaps, in particular the lower abdominal “butterfly” flap, can be used since robust axial flap vascularity to support urethral construction is not necessary<sup>[50]</sup>. If a patient chooses a shaft-only phalloplasty, there are then three distinct options regarding the management of the vulva and vagina: (1) leave genital/vulvar and reproductive anatomy unchanged; (2) perform a hysterectomy, vaginectomy, scrotoplasty and perineal urostomy; or (3) perform a vaginal preservation vulvo-scrotoplasty. In this technique the vaginal canal and native urethra are preserved while the clitoris is buried and the labia majora are used to create a neo-scrotum.

Note that the considerable variation in flap choice and approach to phalloplasty exists not only between, but even within high-volume centers<sup>[51]</sup>. The major advantages and disadvantages of each flap choice described above are listed in [Table 1](#).

### Glansplasty/coronaplasty

Many patients undergoing phalloplasty will request a glansplasty (also called coronaplasty) to mimic the coronal groove and coronal ridge present in circumcised cis-males<sup>[52]</sup>. Despite minor variations, this is nearly universally done by incising the neophallus along the planned coronal groove, elevating a short distally-based flap, and either imbricating the flap back to itself or skin grafting the underside to form the coronal ridge. The proximal raw area of the coronal groove can be left to heal secondarily or skin grafted. More complex methods using diced cartilage grafts<sup>[53]</sup>, hyaluronidase injections<sup>[54]</sup>, or even two separate free flaps, one for the glans penis and one for the penile shaft<sup>[55]</sup>, have been reported as isolated case series but have not been widely adopted. Regardless of the technique used, the coronal ridge has a tendency to flatten over time and repeat coronaplasty is sometimes necessary<sup>[55,56]</sup>. In addition, there have been reports of distal flap necrosis when coronaplasty is done primarily, at the time of phallic construction<sup>[57]</sup>. For this reason, and since masculinizing genital GCS is almost always a series of staged operations, it may be advisable to perform coronaplasty as a delayed procedure, especially for the ALT where the perforators are usually situated more proximally in the flap. Lastly, tattooing can improve the appearance of the glans created by any of the methods noted above.

### Urethral construction

The female urethral orifice terminates in the perineum just anterior to the vaginal introitus. Urination from the tip of a neophallus, therefore, requires construction of both the pars fixa and pars pendulans urethra. Because of both the complexity of constructing the pars fixa urethra and the reported 30%-70% rate of subsequent urethral strictures and fistulae<sup>[27,58]</sup>, specific expertise is required to perform these procedures and manage the complications. Therefore, in centers performing phalloplasty with full urethral reconstruction, a collaborative team that includes expertise in reconstructive microsurgery and in reconstructive urology is essential.

**Table 1. Flap selection for phalloplasty**

Staging	Advantages	Disadvantages
Radial forearm free flap	Phallus and pars pendula reconstruction with one flap Long pedicle Reliable anatomy High density of nerve innervation Thin flap in obese and overweight patients	Aesthetically displeasing donor site Skin graft needed for donor site Donor site sensory disturbance and neuroma possible Phallus length limited by forearm length
Anterolateral thigh flap	Phallus and pars pendula reconstruction with one flap Long pedicle Flap can be pedicled Easily concealable donor site	Thick flap in overweight and obese patients Skin graft needed for donor site Variable perforator anatomy
Latissimus flap	Reliable anatomy Long pedicle Muscle can be reinnervated and cause “pseudo-erection” Concealable donor site	No cutaneous sensation Must perform pars pendula reconstruction in second stage
Superior circumflex iliac perforator flap	Can be used as a pedicled flap Minimal donor site morbidity	Two flaps needed for phallus and pars pendula reconstruction Thick abdominal flaps in overweight patients Limited ability to restore sensory innervation in the phallic skin
Free fibula flap	Has rigidity for sexual intercourse	There is constant phallus rigidity Short pedicle Morbidity donor site Phallus length limited by length of fibula harvest to preserve ankle mortise and fibular head

There are a variety of donor sites for phalloplasty, with the most common being the radial forearm free flap. There is variation based on advantages and disadvantages according to the individual patient and institution

### Pars fixa urethra (also referred to as the perineal urethra)

Among centers that perform masculinizing GCS, there is a wide diversity of specific technical details for pars fixa construction. The specific techniques for pars fixa reconstruction have been reported elsewhere (see references below) and are beyond our purpose and scope here. That said, the nearly universal strategy is to use adjacent tissues, sometimes augmented with skin or buccal mucosal grafts, to construct a hairless, epithelial-lined tube from the location of the female urethral orifice to the base of the neophallus. The variations in specific techniques include (1) the degree of clitoral chordee release and resection of clitoral skin performed when repositioning the clitoral body<sup>[38,59-61]</sup>; (2) the timing of vaginectomy and the use (or not) of an anterior vaginal myomucosal turnover flap to augment the proximal pars fixa<sup>[20,21,62]</sup>; (3) the use (or not) of skin or buccal mucosal grafts to form the dorsal “floor” of the pars fixa<sup>[61-65]</sup>; (4) the specifics of labia minora pedicled flaps to construct the ventral “roof” of the pars fixa<sup>[60-65]</sup>; (5) the timing of pars fixa construction (discussed below); and (6) the use (or not) of a pedicled gracilis muscle or other vascularized flap to augment healing of the multiple anastomotic suture lines necessary<sup>[66,67]</sup>. These variations have been employed in virtually every combination at various centers. Most importantly, the reported urethral complication rates vary most between centers, and not between specific surgical techniques or from differences in staging. That is, no single specific technique or staging strategy has convincingly demonstrated reduced urethral complications.

### Pars pendulans urethra (also referred to as the penile urethra)

Variations in reconstruction of the penile urethra are largely a function of the specific flap used to construct the neophallus. As already mentioned, an advantage of the RFFF is that it is thin and pliable with robust vascularity, allowing construction of the penile urethra at the time of free-flap phalloplasty using the tube-in-a-tube technique [Figure 3]<sup>[28]</sup>. It has been hypothesized that the very large skin island necessary to do a one-stage penile urethral construction at the time of phalloplasty requires pushing the limits of the vascular territory of the RFFF and that this may account for the high rates of urethral complications; specific technical modifications may improve vascularity in the final RFFF territory<sup>[68]</sup>. Although using the ALT in a similar one-stage fashion has been described, the bulkiness of the flap precludes this approach in the majority of patients<sup>[69]</sup>.



Two general strategies have been employed to construct the penile urethra in flaps that do not lend themselves to a primary, tube-in-a-tube pars pendulans constructions. The first is to employ two separate flaps, one for the phallus and a second flap for the penile urethra. Phallic construction using an ALT or LD flap and urethral construction at the same time using a RFFF, ulnar artery forearm flap, SCIP flap, or pedicled labia minora flaps have been described<sup>[69-72]</sup>. The principle advantages of this approach are freedom of design, lessened donor site deformity, and the robust vascularity in both flaps. The disadvantages are the morbidity of two separate donor sites and the cumulative risk of doing two separate pedicled or microvascular flaps simultaneously.

The second strategy is to perform a staged urethral fabrication using skin, vaginal mucosal, buccal mucosal or even uterine or colonic grafts<sup>[21,73,74]</sup>. This strategy has been employed in two ways. The first, mentioned for completeness sake only, is to pre-fabricate a urethra within the phalloplasty flap as an initial stage<sup>[73,74]</sup>. Skin, vaginal mucosal, and/or buccal mucosal grafts are harvested and tubed around a Foley catheter. The flap, usually the ALT or RFFF, is then partially elevated and the Foley/graft construct is implanted within the substance of the flap. After a delay of 3 or more months to allow healing and vascularization of the graft, the flap, now with the pre-fabricated urethra, is elevated for phallic construction. Another described option is to place two buried skin grafts opposing one another. This makes postoperative care and hygiene easier. At the second surgery, the posterior skin graft is left in place while the anterior one is tubularized. Despite the initial enthusiasm<sup>[71]</sup>, this approach has been largely abandoned due to high rates of complex urethral complications, especially long-segment strictures<sup>[71]</sup>.

As an alternative, described primarily for the ALT, the phallic flap can be performed first, with construction of the pars pendulans urethra done in two subsequent stages<sup>[75]</sup>. In this approach, a flap-only ALT phalloplasty is performed without either pars fixa or pars pendulans construction. At a second stage, the phallus is split along its ventral surface and the graft material of choice, usually a skin graft, is laid into the cleft. After several months for graft healing, the penile urethra is formed by tubing the skin graft, usually with a simultaneous pars fixa construction. This strategy has the advantage of allowing full visualization of graft healing prior to tubing the penile urethra with the obvious disadvantage of necessitating a minimum of three stages to construct the penile urethra.

### Staging of urethral construction

As already noted above, there is variation in the staging of both pars fixa and pars pendulans urethral construction; this accounts for the majority of the variance between centers doing masculinizing GCS. Some centers perform phallic pars fixa and pars pendulans construction in a single operation<sup>[19]</sup>. Some centers perform a pars fixa urethra construction, often along with a formal metoidioplasty as an initial step, followed by a phalloplasty with simultaneous construction of the penile urethra<sup>[75]</sup>. This sequence can also be reversed, with the phallic flap and penile urethral construction performed as a first stage, followed by pars fixa construction as a second procedure<sup>[75]</sup>.

Staging urethral construction has the obvious disadvantage of requiring more than one operation to establish urethral continuity. Advantages, however, are shortened operating times for each stage, the preservation of critical anatomy should the phallic flap fail, and, from first principle reasoning, a lessened amount of surgical swelling and inflammation at the time of pars fixa to pars pendulans urethra anastomosis. An additional advantage to staging may be improved predictability for the recovery and return to work for the patient after smaller, staged operations rather than a single, much larger procedure. It is critical to note, however, that although staging has been purported to reduce urethral complications, the variation between centers and case-series in reported rates of urethral strictures and fistulas is greater than the variation between urethral construction techniques and strategies. That is, no single technique or staging strategy has proven superior in reducing overall urethral complication rates. That said, the current





**Figure 4.** Status after second stage in anterolateral thigh phalloplasty (tube within a tube) with scrotoplasty. Scrotoplasty is performed using anteriorly based labia majora flaps, which are advanced to the midline. The perineum posterior to the scrotoplasty is then closed. Photo courtesy of Dr. Jens Berli

literature presents vastly heterogeneous data and often lacks granular details. Complication rate as measure of superiority of procedures may fall short as it omits details on complication severity and impact on patient reported outcomes. For example, a small fistula may be amenable to a short outpatient procedure, whereas a long stricture may require a two-stage repair and longer urinary diversion. Future research should therefore also focus on complication severity and not simply on overall urethral complication rates.

### Scrotal construction and testicular implants

Several specific techniques of scrotal reconstruction have been described, but all rely on a common strategy, the use of adjacent tissue flaps to form the scrotum, and following a suitable delay for healing, the placement of testicular implants<sup>[19]</sup>. Most commonly, bilateral, anteriorly based labia majora flaps are used for the skin of the scrotum [Figure 4]<sup>[19]</sup>. The placement of testicular implants is straightforward and has been well described elsewhere<sup>[76]</sup>.

### Erectile prostheses

Although a few centers use osteocutaneous flaps or place cartilage grafts primarily at the time of phallic construction<sup>[21,74]</sup>, for most patients, the ability to achieve penile rigidity sufficient for penetrative intercourse will rely on the placement of an erectile prosthesis. Despite enthusiastic demand from some patients and short-term data indicating efficacy, considerable variation in philosophy and the technical specifics regarding erectile prostheses are driven by high rates of eventual prosthetic infection and/or extrusion and of prosthesis malfunction<sup>[76]</sup>. Analysis of existing data by some centers leads them to conclude that the risks outweigh the benefits, and they advise patients in advance that they do not recommend erectile prosthesis placement. For centers that do place prostheses, there is considerable variation in surgical specifics. A thorough review of the various types of penile prosthesis is beyond our scope, but the surgeon and patient will need to choose between the two general categories: semi-rigid or inflatable prostheses<sup>[77]</sup>. When placing inflatable prosthesis in cis-males, two cylinder devices are employed, with one cylinder placed within each corpora cavernosa. For trans-males, some centers place a single inflatable cylinder or semi-rigid element, especially for RFFF phalloplasties. Other centers may place dual-cylindrical devices, especially for ALT phalloplasties, where the weight and bulk of the flap makes rigidity difficult with a single chamber<sup>[76]</sup>. In addition, attempts to reduce the extrusion rate have focused on anchoring the prosthesis to the pubis with

**Table 2. Phalloplasty: common staging strategies for construction of pars fixa and pendulans urethra**

Staging	Procedural sequence
Single stage reconstruction <sup>[28]</sup>	Phallus and pars pendulans created from a single, "tube-in-a-tube" flap (usually RFFF), simultaneous vaginectomy and pars fixa construction (specific methods vary), simultaneous scrotal construction
Two stages: pars pendulans first <sup>[73]</sup>	Stage one: phallic flap with pars pendulans created via "tube-in-a-tube" strategy; no disturbance of female urethra or labia (i.e., a discontinuous urethra without pars fixa is created) Stage two: pars fixa construction (multiple specific techniques), anastomosis to previously created pars pendulans, simultaneous scrotal construction
Two stages: pars fixa first <sup>[73]</sup>	Stage one: pars fixa construction, often combined with vaginectomy, sometimes as a formal metoidioplasty Stage two: phallic flap with pars pendulans created via "tube-in-a-tube" strategy; anastomosis to previously created pars fixa urethra, simultaneous scrotal construction
Three stages: pars pendulans first, but requiring two stages <sup>[73]</sup>	Stage one: phallic construction without pars pendulans (often ALT flap), no disturbance of female urethra or labia Stage two: incise ventral surface of the phallus, skin graft to the ventral portion of the phallus Stage three: tubularization of the grafted, ventral surface of the phallus to create pars pendulans, simultaneous pars fixa construction (specific methods vary), anastomosis of pars fixa and pendulans, simultaneous scrotal construction

Like all aspects of phalloplasty, there are countless variations in the construction and staging of the pars pendula and pars fixa. The most common permutations are presented. RFFF: radial forearm free flap; ALT: anterolateral thigh

Mitek anchors<sup>[77]</sup> and/or encasing the prosthesis within a sleeve of acellularized dermal matrix, or other biocompatible material<sup>[76]</sup>.

## SUMMARY AND CONCLUSION

The wide variation in both overall strategies and in surgical specifics between surgeons and centers underscore the complexity and difficulty of masculinizing GCS. The multiple options discussed above, along with the paucity of multicenter, prospective data to guide surgical practice, has created phalloplasty chaos, which is confusing to both surgeons and patients. When guiding a patient desiring masculinizing GCS, it is paramount that surgeons are knowledgeable of all available options and the associated advantages, disadvantages, and risks. Because constructing an algorithm encompassing the multiple variations in virtually every aspect of phalloplasty discussed above would include a dozen or more specific decision points and many dozens of specific surgical pathways, each center performing masculinizing GCS should ideally employ a consistent, local algorithm tailored to the available expertise of the participating surgeons while also allowing for appropriate patient preference. All algorithms for masculinizing GCS, however, should determine first if the patient desires standing urination and if their own profile of comorbidities would make this possible. If a patient does not desire standing urination, a shaft only-phalloplasty is a reasonable option. If a patient does desire standing urination, institutional preferences for staging and surgical specifics come into play and should be explained to the patient to provide for appropriately informed consent. The most commonly employed combinations of the multiple options are presented in [Table 2](#).

All that said, from our interpretation of available, data we draw the following conclusions and make the following recommendations:

- (1) Data strongly support the maintenance of an innervated clitoris to preserve erogenous and orgasmic potential. We strongly advise against the division of both clitoral nerves to neurotize the neophallus.
- (2) From available data and from the advantages and disadvantages of each choice discussed above, the RFFF should be considered the best current flap for phallic construction. There should be compelling reasons to use other flaps in individual patients.
- (3) The most frequent and most difficult complications from masculinizing GCS arise from reconstruction of the pars fixa and pars pendulans urethra. We strongly advocate close collaboration between a reconstructive microsurgeon and a reconstructive urologist to achieve the best patient outcomes.

(4) Although vaginectomy at the time of pars fixa urethral construction is done successfully at many centers, anecdotal reports of the colpectomy cavity becoming a urinary diverticulum should be considered when performing these procedures simultaneously.

(5) There is considerable variation among centers in reported rates of minor and major urethral complications, even those using nearly identical techniques. Most importantly, there are no data at present demonstrating that any given staging of procedures, or that any of many variations in surgical specifics for urethral construction result in consistent reductions in subsequent rates of urethral strictures or fistulas.

(6) The placement of erectile prostheses in transmasculine patients after phalloplasty will be guided by the expertise, philosophy, and outcomes of each surgical team. However, all phalloplasty patients should be informed in advance that the majority of long-term studies report high rates of infection and extrusion of erectile devices.

Despite this phalloplasty chaos, we emphasize that masculinizing genital GCS is medically necessary with well-demonstrated health benefits for properly selected patients. With an increased volume of these procedures being performed<sup>[78]</sup>, and with further research in all domains, we anticipate continual advances in this difficult and challenging area of surgery.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception, design and intellectual content of this article, provided administrative, technical, and material support: Lane M, Sluiter EC, Morrison SD, Coon D, Gast KM, Berli JU, Kuzon WM

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Written informed consent was obtained for publication of patient images used in this manuscript. Consent for use in publication was obtained by Dr. Berli.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. van de Grift TC, Elaut E, Cerwenka SC, Cohen-Kettenis PT, De Cuypere G, et al. Effects of medical interventions on gender dysphoria and body image: a follow-up study. *Psychosom Med* 2017;79:815-23.
2. Wernick JA, Busa S, Matouk K, Nicholson J, Janssen A. A systematic review of the psychological benefits of gender-affirming surgery. *Urol Clin North Am* 2019;46:475-86.
3. Manieri C, Castellano E, Crespi C, Di Bisceglie C, Dell'aquila C, et al. Medical treatment of subjects with gender identity disorder: the experience in an Italian public health center. *Int J Transgend* 2014;15:53-65.
4. Byne W, Bradley SJ, Coleman E, Eyler AE, Green R, et al; American psychiatric association task force on treatment of gender

- identity disorder. Report of the American psychiatric association task force on treatment of gender identity disorder. *Arch Sex Behav* 2012;41:759-96.
5. Davy Z. The DSM-5 and the politics of diagnosing transpeople. *Arch Sex Behav* 2015;44:1165-76.
  6. Weissler JM, Chang BL, Carney MJ, Rengifo D, Messa CA 4th, et al. Gender-affirming surgery in persons with gender dysphoria. *Plast Reconstr Surg* 2018;141:388-96e.
  7. World Professional Association for Transgender Health. Standards of care for transsexual, transgender, and gender nonconforming people. *Int J Transgend* 2012;13:165-232.
  8. Riggs DW, Pearce R, Pfeffer CA, Hines S, White F, et al. Transnormativity in the psy disciplines: constructing pathology in the diagnostic and statistical manual of mental disorders and standards of care. *Am Psychol* 2019;74:912-24.
  9. Budge SL. Psychotherapists as gatekeepers: an evidence-based case study highlighting the role and process of letter writing for transgender clients. *Psychotherapy (Chic)* 2015;52:287-97.
  10. Puckett JA, Cleary P, Rossman K, Newcomb ME, Mustanski B. Barriers to gender-affirming care for transgender and gender nonconforming individuals. *Sex Res Social Policy* 2018;15:48-59.
  11. Selvaggi G, Monstrey S, Ceulemans P, T'Sjoen G, De Cuyper G, et al. Genital sensitivity after sex reassignment surgery in transsexual patients. *Ann Plast Surg* 2007;58:427-33.
  12. Abern L, Maguire K. Fertility preservation among transgender individuals. *Fertil Steril* 2018;110:e281.
  13. Jevtovic M, Stojanovic B, Bizic M, Stanojevic D, Kisic J, et al. Hysterectomy with bilateral salpingo-oophorectomy in female-to-male gender affirmation surgery: comparison of two methods. *Biomed Res Int* 2018;2018:3472471.
  14. Hougen HY, Dugi DD 3rd, Berli JU, Sajadi KP. Outcomes of transperineal gender-affirming vaginectomy and colpocleisis. *Female Pelvic Med Reconstr Surg* 2020; doi: 10.1097/SPV.0000000000000843.
  15. Massie JP, Morrison SD, Wilson SC, Crane CN, Chen ML. Phalloplasty with urethral lengthening: addition of a vascularized bulbospongiosus flap from vaginectomy reduces postoperative urethral complications. *Plast Reconstr Surg* 2017;140:551-8e.
  16. Nikkels C, van Trotsenburg M, Huirne J, Bouman MB, de Leeuw R, et al. Vaginal colpectomy in transgender men: a retrospective cohort study on surgical procedure and outcomes. *J Sex Med* 2019;16:924-33.
  17. Groenman F, Nikkels C, Huirne J, van Trotsenburg M, Trum H. Robot-assisted laparoscopic colpectomy in female-to-male transgender patients; technique and outcomes of a prospective cohort study. *Surg Endosc* 2017;31:3363-9.
  18. Dy GW, Sun J, Granieri MA, Zhao LC. Reconstructive management pearls for the transgender patient. *Curr Urol Rep* 2018;19:36.
  19. Chen ML, Safa B. Single-stage phalloplasty. *Urol Clin North Am* 2019;46:567-80.
  20. Zhang YF, Liu CY, Qu CY, Lu LX, Liu AT, et al. Is vaginal mucosal graft the excellent substitute material for urethral reconstruction in female-to-male transsexuals? *World J Urol* 2015;33:2115-23.
  21. Medina CA, Fein LA, Salgado CJ. Total vaginectomy and urethral lengthening at time of neourethral prelamination in transgender men. *Int Urogynecol J* 2018;29:1463-8.
  22. Morrison SD, Massie JP, Dellon AL. Genital sensibility in the neophallus: getting a sense of the current literature and techniques. *J Reconstr Microsurg* 2019;35:129-37.
  23. Safa B, Lin WC, Salim AM, Deschamps-Braly JC, Poh MM. Current concepts in masculinizing gender surgery. *Plast Reconstr Surg* 2019;143:857-71e.
  24. Hadj-Moussa M, Agarwal S, Ohl DA, Kuzon WM Jr. Masculinizing genital gender confirmation surgery. *Sex Med Rev* 2019;7:141-55.
  25. Vukadinovic V, Stojanovic B, Majstorovic M, Milosevic A. The role of clitoral anatomy in female to male sex reassignment surgery. *ScientificWorldJournal* 2014;2014:437378.
  26. Morrison SD, Shakir A, Vyas KS, Kirby J, Crane CN, et al. Phalloplasty: a review of techniques and outcomes. *Plast Reconstr Surg* 2016;138:594-615.
  27. Monstrey S, Hoebeke P, Selvaggi G, Ceulemans P, Van Landuyt K, et al. Penile reconstruction: is the radial forearm flap really the standard technique? *Plast Reconstr Surg* 2009;124:510-8.
  28. Chang TS, Hwang WY. Forearm flap in one-stage reconstruction of the penis. *Plast Reconstr Surg* 1984;74:251-8.
  29. Garcia MM, Christopher NA, De Luca F, Spilotros M, Ralph DJ. Overall satisfaction, sexual function, and the durability of neophallus dimensions following staged female to male genital gender confirming surgery: the Institute of Urology, London U.K. experience. *Transl Androl Urol* 2014;3:156-62.
  30. Frey JD, Poudrier G, Chiodo MV, Hazen A. A systematic review of metoidioplasty and radial forearm flap phalloplasty in female-to-male transgender genital reconstruction: is the "ideal" neophallus an achievable goal? *Plast Reconstr Surg Glob Open* 2016;4:e1131.
  31. Heston AL, Esmonde NO, Dugi DD 3rd, Berli JU. Phalloplasty: techniques and outcomes. *Transl Androl Urol* 2019;8:254-65.
  32. Djordjevic ML, Bencic M, Kojovic V, Stojanovic B, Bizic M, et al. Musculocutaneous latissimus dorsi flap for phalloplasty in female to male gender affirmation surgery. *World J Urol* 2019;37:631-7.
  33. Vesely J, Hyza P, Ranno R, Cigna E, Monni N, et al. New technique of total phalloplasty with reinnervated latissimus dorsi myocutaneous free flap in female-to-male transsexuals. *Ann Plast Surg* 2007;58:544-50.
  34. Veerman H, de Rooij FPW, Al-Tamimi M, Ronkes BL, Mullender MG, et al. Functional outcomes and urological complications after genital gender affirming surgery with urethral lengthening in transgender men. *J Urol* 2020;204:104-9.
  35. Koshima I, Nanba Y, Nagai A, Nakatsuka M, Sato T, et al. Penile reconstruction with bilateral superficial circumflex iliac artery perforator (SCIP) flaps. *J Reconstr Microsurg* 2006;22:137-42.
  36. Sengezer M, Oztürk S, Deveci M, Odabaşı Z. Long-term follow-up of total penile reconstruction with sensate osteocutaneous free fibula flap in 18 biological male patients. *Plast Reconstr Surg* 2004;114:439-50; discussion 451-2.

37. Mcroberts JW, Sadove RC. Penile reconstruction with a free sensate osteocutaneous fibula flap in the surgical management of the intersex patient. In: Zderic SA, Canning DA, Carr MC, Snyder HM, editors. *Pediatric gender assignment*. Boston: Springer US; 2002. pp. 283-8.
38. Dabernig J. Pedicled pubic phalloplasty in females with gender dysphoria. *BJU Int* 2005;96:1422-3.
39. Djordjevic ML. Novel surgical techniques in female to male gender confirming surgery. *Transl Androl Urol* 2018;7:628-38.
40. Colebunders B, Brondeel S, D'Arpa S, Hoebeke P, Monstrey S. An update on the surgical treatment for transgender patients. *Sex Med Rev* 2017;5:103-9.
41. Selvaggi G, Monstrey S, Hoebeke P, Ceulemans P, Van Landuyt K, et al. Donor-site morbidity of the radial forearm free flap after 125 phalloplasties in gender identity disorder. *Plast Reconstr Surg* 2006;118:1171-7.
42. Kovar A, Choi S, Iorio ML. Donor site morbidity in phalloplasty reconstructions: outcomes of the radial forearm free flap. *Plast Reconstr Surg Glob Open* 2019;7:e2442.
43. Garaffa G, Christopher NA, Ralph DJ. Total phallic reconstruction in female-to-male transsexuals. *Eur Urol* 2010;57:715-22.
44. Tran BNN, Epstein S, Singhal D, Lee BT, Tobias AM, et al. Gender affirmation surgery: a synopsis using American College of Surgeons National Surgery Quality Improvement Program and National Inpatient Sample databases. *Ann Plast Surg* 2018;80:S229-35.
45. Felici N, Felici A. A new phalloplasty technique: the free anterolateral thigh flap phalloplasty. *J Plast Reconstr Aesthet Surg* 2006;59:153-7.
46. Xu KY, Watt AJ. The pedicled anterolateral thigh phalloplasty. *Clin Plast Surg* 2018;45:399-406.
47. Mardini S, Chim H, Wei FC. Anterolateral and anteromedial thigh flaps. In: Wei FC, Mardini S, editors. *Flaps and reconstructive surgery*. 2nd ed. Elsevier; 2016. pp. 700-16.e5.
48. D'Arpa S, Colebunders B, Stillaert F, Monstrey S. Pre-expanded anterolateral thigh perforator flap for phalloplasty. *Clin Plast Surg* 2017;44:129-41.
49. Esmonde N, Bluebond-Langner R, Berli JU. Phalloplasty flap-related complication. *Clin Plast Surg* 2018;45:415-24.
50. Bajpai M. "Bird-Wing" abdominal phalloplasty: a novel surgical technique for penile reconstruction. *J Indian Assoc Pediatr Surg* 2013;18:49-52.
51. Hage J, Bouman F, de Graaf F, Bloem J. Construction of the neophallus in female-to-male transsexuals: the Amsterdam experience. *J Urol* 1993;149:1463-8.
52. Hage JJ, Bout CA, Bloem JJ, Megens JA. Phalloplasty in female-to-male transsexuals: what do our patients ask for? *Ann Plast Surg* 1993;30:323-6.
53. Ma S, Cheng K, Liu Y, Chen F. A new surgical procedure for penile reconstruction by combined free radial forearm flap and dorsalis pedis flap. *Urology* 2016;97:232-7.
54. Moon DG, Kwak TI, Cho HY, Bae JH, Park HS, et al. Augmentation of glans penis using injectable hyaluronic acid gel. *Int J Impot Res* 2003;15:456-60.
55. Sommeling CE, De Wolf EJ, Salim A, Monstrey S, Opsomer D, et al. A new technique for coronaplasty in penile reconstruction. *J Sex Med* 2018;15:920-3.
56. Besteiro J, Caterina R, Ishida L, Aki F. Microsurgical phalloplasty. *J Reconstr Microsurg* 2006;22.
57. Dubin BJ, Sato RM, Laub DR. Results of phalloplasty. *Plast Reconstr Surg* 1979;64:163-70.
58. Al-Tamimi M, Pigot GL, van der Sluis WB, van de Grift TC, Mullender MG, et al. Colpectomy significantly reduces the risk of urethral fistula formation after urethral lengthening in transgender men undergoing genital gender affirming surgery. *J Urol* 2018;200:1315-22.
59. Selvaggi G, Bellringer J. Gender reassignment surgery: an overview. *Nat Rev Urol* 2011;8:274-82.
60. Djordjevic ML, Bizic M, Stanojevic D, Bumbasirevic M, Kojovic V, et al. Urethral lengthening in metoidioplasty (female-to-male sex reassignment surgery) by combined buccal mucosa graft and labia minora flap. *Urology* 2009;74:349-53.
61. Djordjevic ML, Bizic MR. Comparison of two different methods for urethral lengthening in female to male (metoidioplasty) surgery. *J Sex Med* 2013;10:1431-8.
62. Chen ML, Reyblat P, Poh MM, Chi AC. Overview of surgical techniques in gender-affirming genital surgery. *Transl Androl Urol* 2019;8:191-208.
63. Wilson SC, Stranix JT, Khurana K, Morrison SD, Levine JP, et al. Fasciocutaneous flap reinforcement of ventral onlay buccal mucosa grafts enables neophallus revision urethroplasty. *Ther Adv Urol* 2016;8:331-7.
64. Chauhan A, Sham E, Chee J. Microsurgical urethroplasty for complex bulbar urethral strictures using the radial forearm free flap prelined with buccal mucosa. *J Reconstr Microsurg* 2016;32:378-85.
65. Hage JJ. Metoidioplasty: an alternative phalloplasty technique in transsexuals. *Plast Reconstr Surg* 1996;97:161-7.
66. Kim SK, Moon JB, Heo J, Kwon YS, Lee KC. A new method of urethroplasty for prevention of fistula in female-to-male gender reassignment surgery. *Ann Plast Surg* 2010;64:759-64.
67. Cohen O, Stranix JT, Zhao L, Levine J, Bluebond-Langner R. Use of a split pedicled gracilis muscle flap in robotically assisted vaginectomy and urethral lengthening for phalloplasty: a novel technique for female-to-male genital reconstruction. *Plast Reconstr Surg* 2020;145:1512-5.
68. Safa B. "Phalloplasty." Presented for American Society of Plastic Surgeons. Virtual Grand Rounds. April 2020. Available from: <https://ednet.plasticsurgery.org/diweb/catalog/item/id/5085154/pid/5037869>. [Last accessed on 17 Sep 2020]
69. Morrison SD, Son J, Song J, Berger A, Kirby J, et al. Modification of the tube-in-tube pedicled anterolateral thigh flap for total phalloplasty: the mushroom flap. *Ann Plast Surg* 2014;72 Suppl 1:S22-6.
70. Al-Tamimi M, Pigot GL, Ronkes B, de Haseth KB, van de Grift TC, et al. The first experience of using the pedicled labia minora flap for urethral lengthening in transgender men undergoing anterolateral thigh and superficial circumflex iliac artery perforator flap phalloplasty:



- a multicenter study on clinical outcomes. *Urology* 2020;138:179-87.
71. van der Sluis WB, Smit JM, Pigot GLS, Buncamper ME, Winters HAH, et al. Double flap phalloplasty in transgender men: surgical technique and outcome of pedicled anterolateral thigh flap phalloplasty combined with radial forearm free flap urethral reconstruction. *Microsurgery* 2017;37:917-23.
72. D'Arpa S, Claes K, Lumen N, Oieni S, Hoebeke P, et al. Urethral reconstruction in anterolateral thigh flap phalloplasty: a 93-case experience. *Plast Reconstr Surg* 2019;143:382-92e.
73. Liu CY, Wei ZR, Jiang H, Zhao YZ, Zhang YF. Preconstruction of the pars pendulans urethrae for phalloplasty with digestive mucosa using a prefabricated anterolateral thigh flap in a one-arm patient. *Plast Reconstr Surg Glob Open* 2013;1:e53.
74. Salgado CJ, Fein LA, Chim J, Medina CA, Demaso S, et al. Prelamination of neourethra with uterine mucosa in radial forearm osteocutaneous free flap phalloplasty in the female-to-male transgender patient. *Case Rep Urol* 2016;2016:8742531.
75. Danker S, Esmonde N, Berli JU. "Staging" in Phalloplasty. *Urol Clin North Am* 2019;46:581-90.
76. Kang A, Aizen JM, Cohen AJ, Bales GT, Pariser JJ. Techniques and considerations of prosthetic surgery after phalloplasty in the transgender male. *Transl Androl Urol* 2019;8:273-82.
77. Kocjancic E, Iacovelli V. Penile prostheses. *Clin Plast Surg* 2018;45:407-14.
78. Lane M, Ives GC, Sluiter EC, Waljee JF, Yao TH, et al. Trends in gender-affirming surgery in insured patients in the United States. *Plast Reconstr Surg Glob Open* 2018;6:e1738.

Review

Open Access



# Skin grafting for penile skin loss

Alysen Demzik<sup>1</sup>, Charles Peterson<sup>2</sup>, Bradley D. Figler<sup>1</sup>

<sup>1</sup>Department of Urology, University of North Carolina-Chapel Hill, Chapel Hill, NC 27599, USA.

<sup>2</sup>University of North Carolina School of Medicine, Chapel Hill, NC 27599, USA.

**Correspondence to:** Dr. Bradley D. Figler, Department of Urology, University of North Carolina-Chapel Hill, 2105 Physician's Office Building, 170 Manning Drive, Chapel Hill, NC 27599, USA. E-mail: figler@unc.edu

**How to cite this article:** Demzik A, Peterson C, Figler BD. Skin grafting for penile skin loss. *Plast Aesthet Res* 2020;7:52. <http://dx.doi.org/10.20517/2347-9264.2020.93>

**Received:** 24 Apr 2020 **First Decision:** 11 Aug 2020 **Revised:** 1 Sep 2020 **Accepted:** 17 Sep 2020 **Published:** 12 Oct 2020

**Academic Editor:** Marlon E. Buncamper **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Penile skin grafting is an effective technique for managing skin deficiency resulting from a variety of causes. A thorough understanding of penile anatomy and the pathophysiology of the underlying condition being treated are essential. We provide an overview of penile anatomy as well as the pathophysiology of conditions that may lead to penile skin deficiency, as a result of either the underlying condition or its management. The conditions discussed include lichen sclerosus, buried penis, hidradenitis suppurativa, lymphedema, necrotizing fasciitis, cancer, and trauma. We also discuss surgical technique for penile skin grafting with an emphasis on technical considerations unique to the penis. Finally, we review the available literature on penile skin grafting.

**Keywords:** Skin grafting, penile reconstruction, buried penis, hidradenitis suppurativa, Fournier gangrene, penile lymphedema, penile cancer, penis, lichen sclerosus et atrophicus

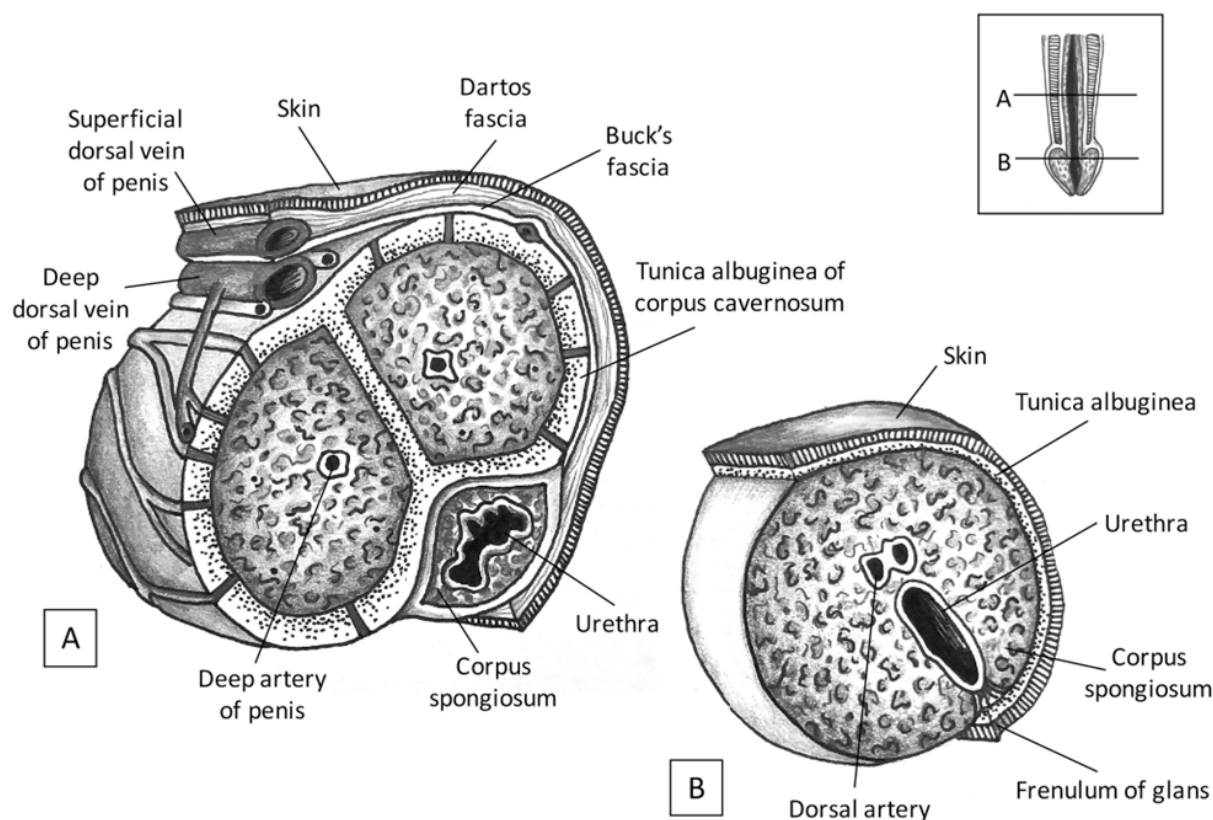
## INTRODUCTION

Penile skin grafting is an effective technique for managing skin deficiency resulting from a variety of causes, including trauma, infection/inflammation, surgery, and cancer treatment. A thorough understanding of penile anatomy and the pathophysiology of the underlying condition being treated are essential to achieving acceptable functional and aesthetic results. In this review, we provide an overview of penile anatomy as well as the pathophysiology of conditions that may lead to penile skin deficiency, either as a result of the underlying condition or its management. We also discuss surgical technique for penile



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** Penis cross sectional anatomy: (A) penile shaft and (B) penile glans

skin grafting with an emphasis on technical considerations unique to the penis and review the available literature on penile skin grafting.

## PENILE ANATOMY

The penis consists of paired erectile bodies (corpora cavernosa) and the urethra, which is surrounded by the corpus spongiosum [Figure 1]. Distally, the corpus cavernosa terminate, and the corpus spongiosum expands to form the glans penis. Each corpus is surrounded by tunica albuginea, a tough fibrous connective tissue. In the shaft of the penis, all three corpora are surrounded by Buck's fascia - a dense non-mobile fascial layer that is in continuity with the deep suspensory ligament of the penis and anterior rectus fascia in the abdominal wall. Dorsally, the artery, veins, and nerves for the glans traverse longitudinally along the penis deep to Buck's fascia. Dartos fascia, which is a loose areolar layer in continuity with Scarpa's fascia of the abdominal wall, is superficial to Buck's fascia and allows for movement of the penile shaft skin relative to Buck's fascia and the deeper penile structures. Dermal adhesions in the distal penile shaft skin result in a fold that allows the preputial skin to drape over the glans penis. The tunica albuginea of the glans penis is covered by a thin densely adherent layer of skin with no underlying fascia.

The common penile artery, which is the terminal branch of the internal pudendal artery, divides into the bulbourethral artery (which supplies the corpus spongiosum and glans penis), the cavernosal arteries (which supplies the corpora cavernosa), and the dorsal artery (which supplies the glans penis). Dartos fascia and skin are supplied by the superficial external pudendal artery (a branch of the femoral artery). The superficial and deep arterial systems communicate at the coronal sulcus. Following circumcision, the remaining preputial skin is supplied by Dartos fascia that was not divided during the circumcision and from retrograde flow from the glans. Venous drainage in the penis mirrors the arterial supply. Lymphatic drainage occurs via the superficial inguinal lymph nodes.

## **PATHOPHYSIOLOGY**

Successful management of penile skin deficiencies requires a thorough understanding of the underlying disease process. Common causes of penile skin loss and scarring are summarized below.

### **Lichen sclerosus**

Lichen sclerosus (LS) is a chronic, inflammatory condition of unknown etiology that results in scarring and contraction of the affected tissue. In men, LS typically involves the prepuce and skin of the glans but can also extend to the penile shaft skin and urethra. Scarring and contracture of this tissue can result in difficulty with urination, sexual function, and exposure of the penis. Malignant transformation is estimated to occur in 2%-8% of cases<sup>[1]</sup>, thus it is essential to ensure that patients with LS can adequately expose and monitor the penis for malignant changes. While there is no cure for LS, topical steroids may halt progression of the disease and resolve symptoms such as itching, burning, and pain related to inflammation<sup>[2]</sup>. Pain, urinary obstruction, and sexual dysfunction related to scarring and contracture of the penile or peri-penile tissues should be managed with excision of the affected tissue. In un-circumcised men with phimosis, circumcision alone is typically sufficient. In circumcised men, skin grafting is typically necessary.

### **Buried penis**

The term “buried penis” is vague but typically refers to a condition in which a prominent mons pannus hangs over the genital skin, causing inflammation and scarring/contracture of the penile and adjacent skin (stage “2b” in a classification system proposed by Tausch *et al.*<sup>[3]</sup>). In these cases, treatment involves panniculectomy to remove the source of inflammation, removal of the diseased and contracted penile shaft skin, and skin grafting of the resulting defect<sup>[4]</sup>.

### **Hidradenitis suppurativa**

Hidradenitis suppurativa is a chronic inflammatory disease of hair follicles. If untreated, follicular inflammation results in hyperkeratinization and ultimately occlusion of the hair follicle, resulting in formation of sinus tracts and fistulas within the dermis. In severe cases, chronic inflammation and infection of the dermis lead to abscess formation and scarring/contracture of the surrounding skin. Mild cases can be treated with topical and/or intralesional therapy, whereas severe cases require excision of the scarred and chronically inflamed skin<sup>[5]</sup>.

### **Lymphedema**

Penile lymphedema occurs as a result of abnormal retention of lymphatic fluid secondary to obstruction. Impaired lymphatic drainage of the penis may be idiopathic or secondary to surgery, malignancy, parasitic infection, or radiation. Early changes include soft, pitting edema. Chronic lymphedema results in inflammation, thickening, and fibrosis of the skin and subcutaneous tissues, which may lead to disfigurement, pain, and urinary or sexual dysfunction. While compression and manual lymphatic drainage may be helpful in managing symptoms of early lymphedema, these techniques are not curative and will not reverse secondary changes such as fibrosis. Restoration of lymphatic drainage with procedures such as lymphaticovenous anastomosis and vascularized lymph node transfer may be useful in certain cases of extremity lymphedema, but they have not proven efficacious for genital lymphedema<sup>[6]</sup>. The dual lymphatic drainage of the penis allows for treatment of chronic lymphedema with complete debulking of the affected skin and Dartos fascia followed by skin grafting directly to the deeper structures of the penis in order to bypass the obstructed lymphatic system.

### **Necrotizing fasciitis**

Necrotizing fasciitis of the genitals, also referred to as “Fournier’s Gangrene”, is a rapidly necrotizing infection of the skin and fascia. Predisposing factors include diabetes, alcoholism, immunosuppression,

recent surgical intervention, trauma, and morbid obesity. Infections are frequently polymicrobial, with synergistic involvement of both aerobic and anaerobic organisms found commonly in the perineal and genital area including Clostridia, Klebsiella, Streptococci, Coliforms, Staphylococci, Bacteriodes, and Corynebacteria. Its hallmark is thrombosis of small arteries, which leads to tissue ischemia, necrosis, and further proliferation of the infection<sup>[7]</sup>. Treatment includes medical management of sepsis (fluid resuscitation and broad-spectrum antibiotics) as well as emergent debridement of affected tissue. In the penis, the process is nearly always limited to skin and fascia so debridement of the corpus cavernosum, corpus spongiosum, and glans penis is not required. Once the affected tissue has been adequately debrided, the infection is controlled, and the patient is stable (usually 48-72 h after initial debridement), reconstruction can occur. While small skin defects can be closed primarily, larger defects require skin grafting.

### Cancer

Squamous cell carcinoma is the most common form of penile cancer, representing up to 95% of penile cancer cases. Risk factors include poor hygiene, phimosis, human papillomavirus, and smoking. Treatment options for non-invasive disease include topical therapy and wide local excision. Following excision, small lesions on the penile shaft may be closed primarily while larger lesions will likely require skin grafting. Glans skin is densely adherent to underlying corpus spongiosum and cannot be closed primarily; following excision, these wounds are best managed with skin grafting.

### Trauma

Penile skin loss due to trauma is rare, but can occur as a result of burns, animal bites, or farm equipment accidents (e.g., penile skin avulsion from a tractor's power take off mechanism). If the deeper structures of the penis are preserved, the skin deficiency should be treated with skin grafting.

## SURGICAL TECHNIQUE

The primary goal of penile reconstruction is to maintain or restore urinary and sexual function with acceptable cosmesis. The penis has several unique anatomic characteristics that should be considered during reconstruction. First, the penis consists of non-hair bearing thin skin that easily translates over the deeper tissues. Second, the penis enlarges with stimulation, requiring elasticity of the penile skin to accommodate the growth. Small wounds can often be closed primarily. Penile wounds that are too large to be closed primarily are best managed with skin grafting.

When possible, healthy Dartos tissue is maintained and used as a graft bed in order to allow for translocation of the penile skin over the deeper structures. In cases of penile lymphedema, it is essential to completely remove Dartos fascia and graft directly to Buck's fascia or tunica albuginea in order to bypass the obstructed lymphatics. While staging is not necessary for most indications, it is preferable when the excised tissue is grossly infected or colonized, such as in hidradenitis suppurativa or necrotizing fasciitis. In these cases, we typically excise the diseased skin, irrigate the wound copiously, secure the skin edges to the base of the penis, and return after one week of wet to dry dressing changes to perform a skin graft.

Various techniques have been used for penile skin grafting, which are summarized in [Table 1](#). Both full thickness skin grafts (FTSGs) and split thickness skin grafts (STSGs), which contain epidermis and a portion of dermis, have been successfully utilized on the penis. STSGs are typically harvested at a depth of 0.012-0.018 inches, with thinner grafts associated with improved graft take.

STSGs are typically harvested from the anterolateral or medial thigh, although STSG can be harvested from the pannus in patients with buried penis<sup>[8-11]</sup>. Compared to FTSGs, the advantages of STSGs include a thin graft that more closely resembles native penile skin, lack of hair follicles, improved graft take due



**Table 1. Published studies with  $\geq 10$  patients undergoing penile skin grafting since 2005**

Author	Years	n	Location	Indication	Skin graft	Failure, n (%)	Bedrest (days)
Parnham <i>et al.</i> <sup>[21]</sup> (2018)	2005-2016	172	Glans	CA	STSG (0.014-0.018) non-meshed	Partial 29 (17%) Complete 5 (3%)	None
Smith <i>et al.</i> <sup>[22]</sup> (2007)	NR	72	Glans	CA	STSG, non-meshed	Partial: 2 (3%)	4
Pariser <i>et al.</i> <sup>[17]</sup> (2018)	2007-2017	61	Shaft	BP	STSG (0.012-0.015) fenestrated	NR	2
*Tang <i>et al.</i> <sup>[18]</sup> (2008)							
Hampson <i>et al.</i> <sup>[19]</sup> (2017)	2005-2016	42	Shaft	BP	STSG (0.015) 1:1 meshed or unmeshed	Partial: 6 (14%)	5
Figler <i>et al.</i> <sup>[4]</sup> (2015)							
Jun <i>et al.</i> <sup>[16]</sup> (2018)	2007-2017	36	Shaft	BP	STSG (0.015) Non-meshed	NR	2
Tausch <i>et al.</i> <sup>[3]</sup> (2016)	2007-2015	31	Shaft	BP	STSG, non-meshed	3 (10%)	NR
Garaffa <i>et al.</i> <sup>[23]</sup> (2011)	1997-2010	31	Glans	LS	STSG (0.00-0.016)	Partial: 1 (3%)	2
Shabbir <i>et al.</i> <sup>[24]</sup> (2011)	2001-2010	25	Glans	CA	STSG (0.008-0.016) non-meshed	1 (4%)	2
Harris <i>et al.</i> <sup>[25]</sup> (2020)	NR	23	Shaft	Pediatrics Exstrophy Epispadias	STSG (0.016-0.018) FTSG, fenestrated	5 (22%)	NR
Cocci <i>et al.</i> <sup>[9]</sup> (2019)	2006-2016	23	Shaft	BP	STSG (0.016 or "thick")	NR	
Figler <i>et al.</i> <sup>[8]</sup> (2020)	2016-2019	19	Shaft	BP	STSG (0.018), non-meshed	0	None
Chertin <i>et al.</i> <sup>[26]</sup> (2016)	NR	17	Shaft	Pediatrics Prior surgery Trauma	STSG (0.012) fenestrated or meshed	1 (6%)	NR
Modolin <i>et al.</i> <sup>[27]</sup> (2006)	NR	17	Shaft	LE	STSG	NR	3
Palminteri <i>et al.</i> <sup>[28]</sup> (2007)	1998-2004	17	Glans	CA, LS	STSG, non-meshed	Partial: 2 (12%)	3
Theisen <i>et al.</i> <sup>[14]</sup> (2018)	2015-2017	16	Shaft	BP	STSG (0.016) fenestrated	NR	2
*Fuller <i>et al.</i> <sup>[15]</sup> (2017)							
Erpelding <i>et al.</i> <sup>[13]</sup> (2019)	2014-2017	16	Shaft	BP	STSG, meshed	0	None
Monn <i>et al.</i> <sup>[10]</sup> (2019)	2013-2018	13	Shaft	LE	FTSG, non-fenestrated	0	NR
Boonjindasup <i>et al.</i> <sup>[29]</sup> (2016)	2000-2013	11	Shaft	LE, LS Prior surgery	STSG (0.012-0.018) meshed 1.5:1	NR	
Voznesensky <i>et al.</i> <sup>[11]</sup> (2016)	2011-2015	11	Shaft	BP	STSG meshed and non-meshed	NR	2
Thompson <i>et al.</i> <sup>[30]</sup> (2006)		11	Shaft	Pediatrics Prior surgery, LE	FTSG	0	NR
Rybak <i>et al.</i> <sup>[31]</sup> (2014)	2007-2011	10	Shaft	BP	STSG (0.016-0.018) fenestrated	NR	
Hadway <i>et al.</i> <sup>[20]</sup> (2006)	NR	10	Glans	CA	STSG, non-meshed	0	5

\*Indicates technique paper. N: number of patients in study with penile skin graft; LE: lymphedema; HS: hidradenitis suppurativa; BP: buried penis; LS: lichen sclerosus; NF: necrotizing fasciitis; CA: cancer

to reduced metabolic requirements, and the ability to easily mesh and expand the graft to cover a larger recipient site. Additional benefits of meshing are preventing accumulation of fluid under the graft (which can interfere with graft take) and easier accommodation of the graft to the contours of an irregular graft bed. In non-meshed STSGs and FTSGs, fluid accumulation under the graft can be achieved by fenestration of the graft with an 11 blade or hollow bore needle. While meshed regions typically heal via epithelial ingrowth from the surrounding skin graft, mesh lines may persist and be aesthetically displeasing [Figure 2]. When meshing is performed on the penis, it is usually at a ratio of 1.5:1 to 2:1, although the use of non-expanded 1:1 meshing has been suggested as a way to achieve appealing cosmesis while preserving other benefits of meshing<sup>[12]</sup>.



**Figure 2.** Penile skin grafts at the time of surgery (A,C) and one year postoperatively (B,D) showing smooth appearance of unmeshed graft (D) and stippled appearance of meshed graft (B)

FTSGs are typically harvested from the inguinal region, where large amounts of hairless skin with high elasticity can be harvested relatively easily. Compared to STSGs, FTSGs experience more primary contracture (immediate recoil of elastin fibers in the dermis) and less secondary contracture (delayed shrinkage due to myofibroblast activity). FTSGs typically contain sweat glands, whereas STSGs do not contain sweat glands and require periodic application of a moisturizer or emollient. Since hidradenitis suppurativa results from dysregulation of apocrine glands, STSGs are preferred to FTSGs in these patients.

Immobilization of the skin graft on its bed is essential for graft survival. To avoid sheering or displacement of the graft, many surgeons place patients on 2-7 days of bed rest after a skin graft. However, prolonged bed rest after surgery is associated with an increased risk of deep vein thrombosis, which can lead to pulmonary embolus and death, thus it should be avoided if possible. As a result, recent studies have reported their experience using a bolster dressing without bed rest to immobilize penile skin grafts. These studies have reported excellent outcomes, suggesting that bed rest is not necessary if an appropriate bolster dressing is used after penile skin grafting<sup>[8,13]</sup>. The use of fibrin sealant to immobilize grafts has also been reported<sup>[14-18]</sup>.

Essential characteristics of penile bolster dressings are the use of non-stick gauze and a mechanism to keep the penis on full stretch so that there is adequate skin during an erection. This can be successfully accomplished by creating a tie-over bolster while the penis is on full stretch, by suturing the dressing to the penis while on full stretch, or by applying a negative pressure dressing while the penis is on full stretch. It is important to keep the graft moist; this can be accomplished with frequent application of a liquid solution (e.g., “sulfamylon slurry”) or by soaking the dressing in mineral oil at the time of surgery.

## OUTCOMES

Outcomes after penile skin grafting are generally excellent, with partial and complete graft loss occurring in 8% and 3% of patients, respectively [Table 1]. Patient-specific risk factors for poor graft take include obesity, diabetes mellitus/hyperglycemia, poor nutritional status, and the presence of an infected or colonized wound bed. Patients with an infected or colonized wound bed (e.g., those with hidradenitis suppurativa) typically benefit from a staged approach in which the wound is treated with wet to dry dressing changes or negative pressure therapy for 3-10 days before attempting skin grafting<sup>[5]</sup>.

Patient reported outcomes after buried penis repair indicate significant improvement in quality of life. At 13 months follow-up, Theisen *et al.*<sup>[14]</sup> reported a significant improvement in 10/12 domains of urinary function and 10/13 domains of sexual function. They also reported improvements in overall urinary and sexual bother in 88% and 94% of patients, respectively. At 39 months follow-up, Hampson *et al.*<sup>[19]</sup> reported improvement in all functional domains that were assessed (ability to see penis, ability to stand to urinate, ability to perform genital hygiene, erectile function, and sexual function). In their series, 85% of patients reported they would undergo buried penis surgery again, 74% that surgery led to a positive change in their lives, and 85% that surgery had remained a long-term success. Voznesensky *et al.*<sup>[11]</sup> reported similar results: patients reported improvement in hygiene (100%), urination (91%), and sexual function (41%), with 92% of patients reporting that they would choose to have the surgery again and 83% reporting that surgery led to a positive change in their lives. They also found that over 90% of men had lost additional body weight at their last clinical follow-up.

While patient reported quality of life outcomes have not been thoroughly explored after penile skin grafting for other indications, Hadway *et al.*<sup>[20]</sup> assessed a number of patient-reported quality of life outcomes after glans resurfacing for premalignant lesions. Among seven patients who completed the questionnaires, all seven stated that sensation at the tip of the penis was no different or better after surgery; five felt that their sex life had improved; and two felt it had not changed. All patients rated overall satisfaction as a 4 or 5 on a five-point scale.

## CONCLUSION

Penile skin grafting is an effective technique for managing skin deficiency resulting from a variety of causes. With a thorough understanding of penile anatomy and the pathophysiology of the condition being treated, successful outcomes can be reliably achieved.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study: Demzik A, Figler BD  
Performed data acquisition and provided technical support: Peterson C, Figler BD

### Availability of data and materials

Not applicable.

## Financial support and sponsorship

None.

## Conflicts of interest

All authors declared that there are no conflicts of interest.

## Ethical approval and consent to participate

A written informed consent to participate in the study was obtained from participants.

## Consent for publication

Written informed consent for publication of images was obtained.

## Copyright

© The Author(s) 2020.

## REFERENCES

1. Fergus KB, Lee AW, Baradaran N, Cohen AJ, Stohr BA, et al. Pathophysiology, clinical manifestations, and treatment of lichen sclerosus: a systematic review. *Urology* 2020;135:11-9.
2. Dahlman-ghozlan K, Hedblad M, von Krogh G. Penile lichen sclerosus et atrophicus treated with clobetasol dipropionate 0.05% cream: a retrospective clinical and histopathologic study. *J Am Acad Dermatol* 1999;40:451-7.
3. Tausch TJ, Tachibana I, Siegel JA, Hoxworth R, Scott JM, et al. Classification system for individualized treatment of adult buried penis syndrome. *Plast Reconstr Surg* 2016;138:703-11.
4. Figler BD, Chery L, Friedrich JB, Wessells H, Voelzke BB. Limited panniculectomy for adult buried penis repair. *Plast Reconstr Surg* 2015;136:1090-2.
5. Hamad J, McCormick BJ, Sayed CS, Paci K, Overton M, et al. Multidisciplinary update on genital hidradenitis suppurativa: a review. *JAMA Surg* 2020; doi: 10.1001/jamasurg.2020.2611.
6. Allen RJ Jr, Cheng MH. Lymphedema surgery: patient selection and an overview of surgical techniques. *J Surg Oncol* 2016;113:923-31.
7. Tessier JM, Sanders J, Sartelli M, Ulrych J, De Simone B, et al. Necrotizing soft tissue infections: a focused review of pathophysiology, diagnosis, operative management, antimicrobial therapy, and pediatrics. *Surg Infect (Larchmt)* 2020;21:81-93.
8. Figler BD, Gan ZS, Mohan CS, Zhang Y, Filippou P. Outpatient panniculectomy and skin graft for adult buried penis. *Urology* 2020;143:255-6.
9. Cocci A, Cito G, Falcone M, Capece M, Di Maida F, et al. Subjective and objective results in surgical correction of adult acquired buried penis: a single-centre observational study. *Arch Ital Urol Androl* 2019;91:25-9.
10. Monn MF, Socas J, Mellon MJ. The use of full thickness skin graft phalloplasty during adult acquired buried penis repair. *Urology* 2019;129:223-7.
11. Voznesensky MA, Lawrence WT, Keith JN, Erickson BA. Patient-reported social, psychological, and urologic outcomes after adult buried penis repair. *Urology* 2017;103:240-4.
12. Black PC, Friedrich JB, Engrav LH, Wessells H. Meshed unexpanded split-thickness skin grafting for reconstruction of penile skin loss. *J Urol* 2004;172:976-9.
13. Erpelding SG, Hopkins M, Dugan A, Liao JY, Gupta S. Outpatient surgical management for acquired buried penis. *Urology* 2019;123:247-51.
14. Theisen KM, Fuller TW, Rusilko P. Surgical management of adult-acquired buried penis: impact on urinary and sexual quality of life outcomes. *Urology* 2018;116:180-4.
15. Fuller TW, Theisen KM, Shah A, Rusilko PJ. Surgical management of adult acquired buried penis. *Curr Urol Rep* 2018;19:22.
16. Jun MS, Gallegos MA, Santucci RA. Contemporary management of adult-acquired buried penis. *BJU Int* 2018;122:713-5.
17. Pariser JJ, Soto-Aviles OE, Miller B, Husainat M, Santucci RA. A simplified adult acquired buried penis repair classification system with an analysis of perioperative complications and urethral stricture disease. *Urology* 2018;120:248-52.
18. Tang SH, Kamat D, Santucci RA. Modern management of adult-acquired buried penis. *Urology* 2008;72:124-7.
19. Hampson LA, Muncy W, Chung PH, Ma CC, Friedrich J, et al. Surgical and functional outcomes following buried penis repair with limited panniculectomy and split-thickness skin graft. *Urology* 2017;110:234-8.
20. Hadway P, Corbishley CM, Watkin NA. Total glans resurfacing for premalignant lesions of the penis: initial outcome data. *BJU Int* 2006;98:532-6.
21. Parnham AS, Albersen M, Sahdev V, Christodoulidou M, Nigam R, et al. Glansectomy and split-thickness skin graft for penile cancer. *Eur Urol* 2018;73:284-9.
22. Smith Y, Hadway P, Biedrzycki O, Perry MJ, Corbishley C, et al. Reconstructive surgery for invasive squamous carcinoma of the glans penis. *Eur Urol* 2007;52:1179-85.

23. Garaffa G, Shabbir M, Christopher N, Minhas S, Ralph DJ. The surgical management of lichen sclerosus of the glans penis: our experience and review of the literature. *J Sex Med* 2011;8:1246-53.
24. Shabbir M, Muneer A, Kalsi J, Shukla CJ, Zacharakis E, et al. Glans resurfacing for the treatment of carcinoma in situ of the penis: surgical technique and outcomes. *Eur Urol* 2011;59:142-7.
25. Harris TGW, Maruf M, Leto Barone AA, Redett RJ 3rd, Gearhart JP. Utility of skin grafting and tissue expansion in penile reconstruction for the exstrophy-epispadias complex. *Urology* 2020;136:231-7.
26. Chertin B, Kocherov S, Binenboym R, Gronovich Y, Tuchman I, et al. Fenestrated sheet split-thickness skin grafting for reconstruction of penile skin loss in pediatric population. *J Pediatr Surg* 2016;51:1362-5.
27. Modolin M, Mitre AI, da Silva JC, Cintra W, Quagliano AP, et al. Surgical treatment of lymphedema of the penis and scrotum. *Clinics (Sao Paulo)* 2006;61:289-94.
28. Palminteri E, Berdondini E, Lazzeri M, Mirri F, Barbagli G. Resurfacing and reconstruction of the glans penis. *Eur Urol* 2007;52:893-8.
29. Boonjindasup A, Pinsky M, Stewart C, Trost L, Chaffin A, et al. Management of adult concealed penis using a meshed, split-thickness skin graft. *Can Urol Assoc J* 2016;10:E407-11.
30. Thompson JH, Zmaj P, Cummings JM, Steinhart GF. An approach for using full thickness skin grafts for complex penile surgeries in children. *J Urol* 2006;175:1869-71.
31. Rybak J, Larsen S, Yu M, Levine LA. Single center outcomes after reconstructive surgical correction of adult acquired buried penis: measurements of erectile function, depression, and quality of life. *J Sex Med* 2014;11:1086-91.



Review

Open Access



# Periocular rejuvenation using hyaluronic acid fillers

Kasra Ziai<sup>1</sup>, Jessyka G. Lighthall<sup>1,2</sup>

<sup>1</sup>Department of Otolaryngology-Head and Neck Surgery, The Pennsylvania State University, College of Medicine, Hershey, PA 17033, USA.

<sup>2</sup>Facial Plastic and Reconstructive Surgery Division, Department of Otolaryngology-Head and Neck Surgery, The Pennsylvania State University, College of Medicine, Hershey, PA 17033, USA.

**Correspondence to:** Dr. Jessyka G. Lighthall, Facial Plastic and Reconstructive Surgery Division, Department of Otolaryngology-Head and Neck Surgery, The Pennsylvania State University, College of Medicine, 500 University Drive, H091, Hershey, PA 17033, USA. E-mail: [jlighthall@pennstatehealth.psu.edu](mailto:jlighthall@pennstatehealth.psu.edu)

**How to cite this article:** Ziai K, Lighthall JG. Periocular rejuvenation using hyaluronic acid fillers. *Plast Aesthet Res* 2020;7:53. <http://dx.doi.org/10.20517/2347-9264.2020.151>

**Received:** 16 Jul 2020 **First Decision:** 24 Aug 2020 **Revised:** 27 Aug 2020 **Accepted:** 14 Sep 2020 **Published:** 12 Oct 2020

**Academic Editor:** Wen-Guo Cui **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

The eyes and periocular region are critical for emotive display and play a key role in social interactions. This region includes the upper and lower eyelids, brow-lid complex, and lid-cheek complex. Perturbances in this area can lead to a prematurely aged appearance and patients complain of emotive misinterpretation. It often shows the earliest signs of facial aging, leading to a tired, sad, or angry appearance. With the evolution of medical and surgical knowledge on facial aging, there has been a shift from isolated volume reducing interventions for periorbital aging to volume replacement techniques. The treatment of periocular aging is multifactorial and often includes resurfacing, chemodenervation, surgical interventions, and volumization. The minimally-invasive, office-based nature of fillers has resulted in their increased popularity and filler placement has become one of the most commonly performed cosmetic oculoplastic interventions. With a multitude of fillers emerging over the past decade or so, facial plastic surgeons have been equipped with the means to address age-related periorbital hollowing and skeletonization in an outpatient setting. An appropriate knowledge of periocular anatomy, types of fillers, proper injection technique, and management of potential complications is required for safe injection and to achieve optimal aesthetic outcomes. This paper reviews the use of hyaluronic acid fillers for periocular rejuvenation.

**Keywords:** Filler, hyaluronic acid, periocular, facial aging



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



## INTRODUCTION

The periocular region is essential for social interactions and conveying emotion. It is one of the first subunits of the face to show facial aging. The youthful periocular region is defined by the fullness of the periorbital area, low or no skin excess, minimal pigmentation or rhytidosis of the skin, and appropriate brow height and shape<sup>[1]</sup>. Facial aging is the result of facial fat loss, decrease in skin elasticity, deepening dynamic rhytids, static rhytids, change in bony structure, and muscular changes. With the evolution of medical and surgical knowledge over the past few decades on facial aging, we now have a better understanding of the underlying pathophysiology. This has resulted in a shift from volume reducing interventions for periorbital aging such as blepharoplasty with fat resection to volume replacement techniques<sup>[2]</sup>. Originally, soft tissue ptosis was hypothesized to be the main underlying etiology of periocular aging. However, facial volume loss and redistribution as well as bony resorption have been proposed as contributing factors<sup>[2,3]</sup>. Since 2004, hyaluronic acid (HA) filler has been used for periorbital rejuvenation<sup>[4,5]</sup>. The minimally-invasive, office-based nature of HA filler use without the need for general anesthesia, the low complication rate, and the lack of downtime have resulted in increased popularity, and it has replaced blepharoplasty as the most commonly performed cosmetic procedure for periocular rejuvenation<sup>[6]</sup>.

In this review, we focus on the use of HA fillers in the periocular region. However, it should be noted that the treatment of periocular aging is often multifactorial. Younger patients with early hollowing may only require HA filler for adequate rejuvenation. Older patients may benefit from multiple surgical interventions such as a brow lift, upper and/or lower blepharoplasty with or without fat transposition, or a blepharoptosis repair. Many patients also require neuromodulation for dynamic rhytids, resurfacing with laser or peels, light- or energy-based therapies, or might be better suited for surgical volumization with abdominal fat transfer alone or in combination with other treatments. Therefore, periocular rejuvenation should be individualized to patient need and surgeon experience.

## ANATOMY

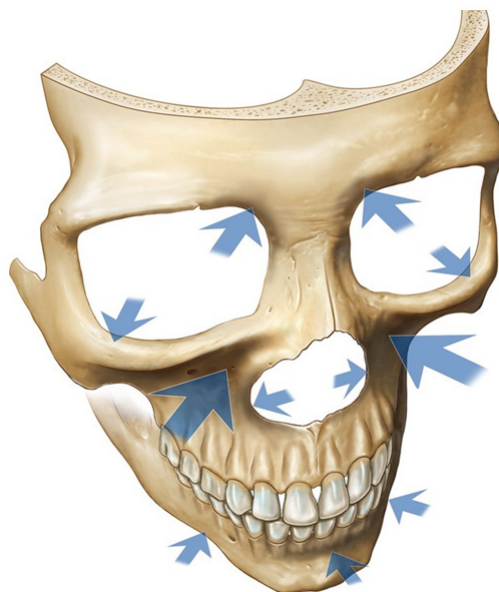
Knowledge of periocular anatomy as well as changes that occur during aging is critical for the appropriate treatment of patients with periocular aging and the selection of ideal candidates for HA fillers. In the evaluation of the periorbital subunit, the entire brow-lid-cheek complex should be assessed and treated<sup>[7]</sup>. The eye is roughly almond-shaped and the upper and lower eyelid margins should be at or near the superior and inferior limbus, respectively. Abnormal eyelid position or an excessively high supratarsal crease may require additional workup and treatment prior to volumization with filler. Although an in-depth discussion is beyond the scope of this paper, we briefly review the changes on each periorbital component including skin, bony structure, musculature apparatus, and fat pads.

### Skin

The skin of the periorbital area and specifically the tear trough region is thin and closely attached to the underlying orbicularis oculi muscle. This results in a transparent appearance and a bluish hue at baseline related to the underlying muscular structure and vasculature<sup>[8]</sup>. Additionally, hyperpigmentation and telangiectasias may occur due to long-term sun damage. Skin aging varies among different ethnicities with different Fitzpatrick skin types. Both layers of skin, dermis and epidermis, undergo age-related changes. These changes are more pronounced in the deep layer of the dermis where the collagen fibers are strongly connected to proteoglycans. With aging, this network becomes loose and less organized, resulting in fine rhytids and crow's feet lines.

### Bony compartment

The facial skeleton is believed to grow and expand with aging<sup>[9]</sup>. Recent studies have shown that certain portions of the facial bones undergo resorption with aging. The superomedial and inferolateral aspects of



**Figure 1.** Bone resorption with age. In this figure, the authors demonstrate the areas of facial skeleton susceptible to bony resorption by aging. The areas with a higher degree of bony resorption are illustrated with larger arrows. Reproduced with permission from Mendelson and Wong<sup>[3]</sup>

orbital rim undergo bony resorption [Figure 1]. The inferolateral quadrant resorption of the periorbital subunit normally manifests earlier and by middle age<sup>[3]</sup>. This age-related bone loss can result in a hollow appearance of the inferolateral subunit.

In males, the supraorbital rim is more prominent and the brow is flatter and located at the level of rim, resulting in a more linear appearance<sup>[10]</sup>. In females, the aesthetic brow is arched and located above the supraorbital rim. The female brow tapers laterally<sup>[11]</sup>. In general, the highest peak of the brow should be located at the junction of the middle and lateral third at either the lateral limbus or lateral canthus.

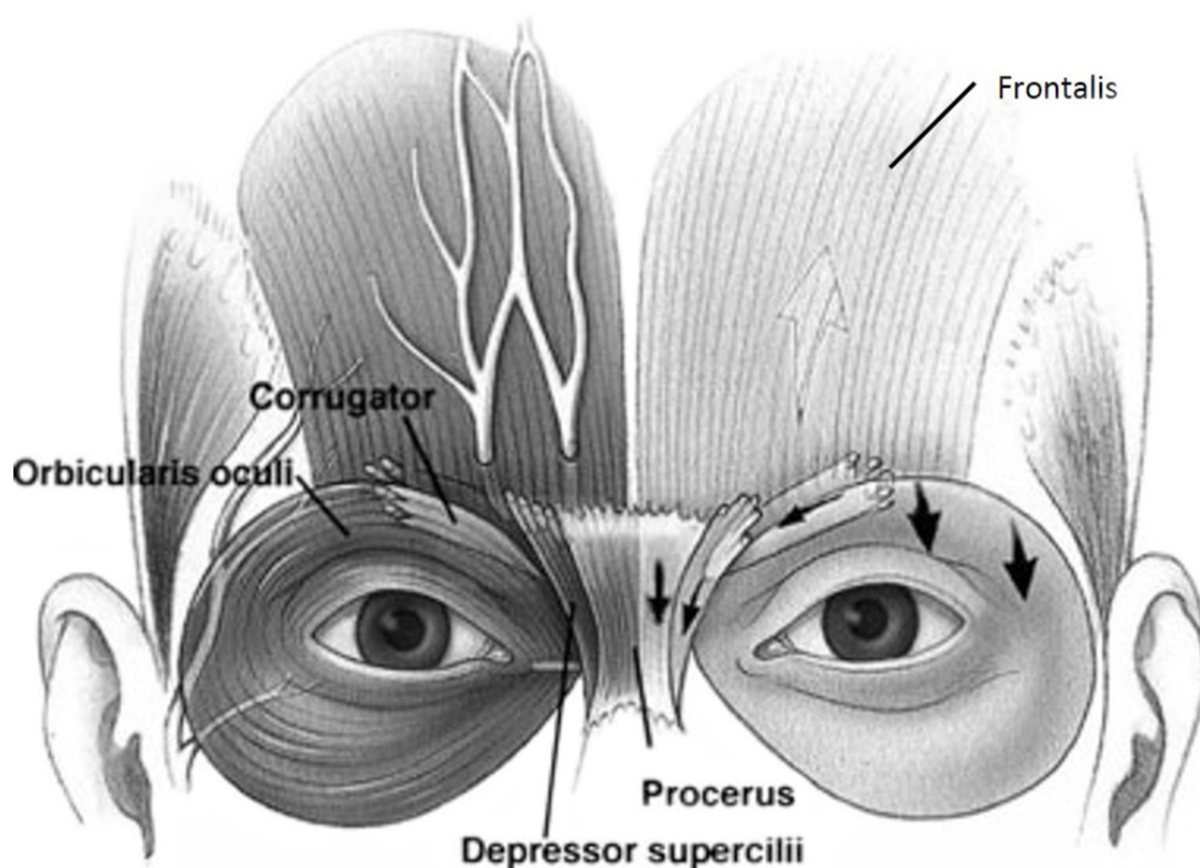
### Muscles

The periorbital muscles include the frontalis that elevates the brow and the depressor muscles which include the orbital portion of orbicularis oculi, corrugator, procerus, and depressor supercilii [Figure 2]<sup>[12]</sup>. The corrugator muscles result in vertical glabellar rhytids and the procerus results in the horizontal glabellar rhytid. These muscles are of importance when using neuromodulators in conjunction with fillers for deep static rhytids. Additionally, the orbital portion of the orbicularis oculi will contribute to the nasojugal groove due to attachments inferomedially.

### Fat pads

The upper periorbital area contains the preaponeurotic fat pad, preseptal fat pad, and galea fat pad or retro-orbicularis oculi fat (ROOF) pad. In periorbital aging, the preseptal and the ROOF fat descends and may lose volume, facilitating gravitational descent of the unsupported lateral brow, which causes brow ptosis and produces the appearance of a heavy lid with or without dermatochalasis. This is also attributable to the weakening of the orbital septum and subsequent pseudoherniation of the fat pads<sup>[13,14]</sup>. This will result in the deflation of the upper eyelid as well as hollowing and increased visibility of the supraorbital rim prominence. These changes produce a deep-set, hollow, and skeletonized orbit with tired, sad, or angry appearing eyes<sup>[1]</sup>.

Deep to the orbicularis is the orbital septum, which weakens with age allowing for orbital contents to bulge, producing a deepened appearance of the infraorbital hollow. Fascial extensions from the dermis to the



**Figure 2.** Periocular musculature. Reproduced with permission from Maas<sup>[11]</sup>

orbital rim (orbital retaining ligament) loosen with age, further accentuating this hollow<sup>[15,16]</sup>. These changes contribute to the classic “double convexity deformity” at the lid-cheek complex. Adding volume with HA fillers in this region can restore the youthful appearance of the periorbital area with a minimally invasive procedure. However, severe pseudoherniation of fat may limit the amount of correction obtained without surgical intervention.

### Periorbital blood supply

One of the uncommon but major complications of filler injections is vascular occlusion or embolization that can result in devastating outcomes including blindness and skin necrosis. Appropriate knowledge of periorbital vascular anatomy and treatment of vascular compromise is critical for any provider injecting fillers.

The periorbital blood supply arises from both internal and external carotid arterial branches. The supratrochlear and supraorbital arteries are branches of the ophthalmic artery that arises from the internal carotid artery. The supratrochlear artery passes anteriorly through the superomedial orbit. The supraorbital artery terminal branches anastomose with the supratrochlear artery and the frontal branch of the superficial temporal artery. The angular artery is a terminal branch of the facial artery that arises from the external carotid artery. The angular artery travels along the medial angle of the orbit. Another branch of the external carotid artery is the infraorbital artery, which travels along the orbital floor and exits from the infraorbital foramen below the orbital rim<sup>[17]</sup>. The periorbital veins drain into the internal and external jugular veins as well as the cavernous sinus via the post-tarsal venous system.

## PATIENT EVALUATION

Prior to any filler use, a thorough history of any prior aesthetic procedures including prior filler or neuromodulator use, surgical interventions, lasers, or light therapy should be clarified. If a patient has a history of prior filler use, the amount and dosage (if known), as well as the type of material used should be discussed. This discussion should also include any complications or dissatisfactions. Any functional concerns such as visual field obstruction or poor eye closure should be assessed.

Additionally, a basic medical history should be obtained with specific attention to a history of inflammatory, autoimmune, or infectious disease. An ocular history including vision complaints, eye surgeries, or dry eye symptoms should be assessed. Medications should be reviewed specifically for ocular lubricant use and any prescription or over the counter anticoagulants. It is pertinent to review and clarify any allergies to medications including local anesthetics, as many HA fillers include lidocaine and topical anesthetics are commonly used prior to filler injections. Patients should also be asked about pregnancy or breastfeeding status.

A physical exam should be performed with specific attention to volume loss, volume descent, periorbital hollowing and skeletonization, pseudo herniation of fat, skin quality, Fitzpatrick skin type, dynamic and static rhytids, and globe and brow position.

A discussion with the patient regarding realistic goals and expectations is important to produce a satisfactory result. For example, a patient with significant pseudofat herniation, severe midfacial volume descent, and a deep infraorbital groove may benefit more from surgical intervention if their goals are to have a youthful, smooth lid-cheek junction. However, this patient may not be a surgical candidate or just want a mild effacement of the groove without dramatic results. As many patients are not aware of the proper treatment for their condition, it is the physician's responsibility to provide them with appropriate insight.

Ultimately, a detailed conversation of the potential risks, benefits, and complications, temporary nature of HA fillers, and alternatives must be performed prior to treatment, and obtaining written consent is recommended.

## Indications

Periocular structural changes with aging are categorized into dynamic and static changes. The dynamic component is related to muscular activities and changes in resting muscle tone. The static component is defined as the changes related to volume loss as well as bony and fat pad changes<sup>[14]</sup>. Fillers are ideal to address the static component such as deep furrows and lines as well as age-related volume loss and skeletonization. Filling material can also be used for postsurgical or posttraumatic deformities in the periocular region.

## Contraindications

Any active skin or localized infection at the site of injection is an absolute contraindication to filler injection. Other absolute contraindications include hypersensitivity/allergy to filler components, including lidocaine, and active collagenoses, such as mixed connective tissue disease, active systemic lupus, and active morphea [Table 1]<sup>[18]</sup>. Although active anticoagulation use is not an absolute contraindication, patients should be asked to stop the anticoagulation medication one week prior to the injection if possible. If they are not able to suspend their therapy, they should be counseled on the possibility of extensive bruising.

## HA FILLERS

Prior to the availability of HA Fillers, autologous fat was predominantly used to restore volume around the eye. Autologous fat requires a donor site, is more invasive, and requires more downtime with a higher



**Table 1. Contraindications to filler use<sup>[18]</sup>**

Condition	Contraindication	Caution
Active skin infection such impetigo or herpes simplex	x	
Hypersensitivity to filler components	x	
Active collagenoses conditions	x	
Graft versus host disease	x	
Systemic bacterial infections such Tuberculosis	x	
Pregnancy and breastfeeding	x	
Coagulation disorders such as hemophilia		x
Active anticoagulant medication use		x
Marfan Syndrome, Ehler-Danlos Syndrome		x
HIV		x
Transplant patients		x

rate of complications<sup>[19,20]</sup>. Although other fillers had been available, in 2003, the first HA filler (Restylane; Galderma) was approved by the FDA, and, in 2004, it was used for periorbital rejuvenation<sup>[20]</sup>. Since the introduction of the first filler, a multitude of synthetic fillers have been introduced to the market. Volume replacement occurs primarily through the hydrophilic biomaterial properties of hyaluronic acids that act as a spacer within the tissue planes. Synthetic fillers such as calcium hydroxyapatite (CHA: Radiesse; MerzAesthetics), polymethyl methacrylate (PMMA; Artefill; Suneva Medical), and poly-L-lactic acid (PLLA: Sculptra Aesthetic) have additional biostimulatory effect in addition to spacer effect<sup>[21]</sup>.

Currently, there are a multitude of synthetic HA fillers available in the market and each has different properties and characteristics [Table 2]. These properties include: concentration of the HA, particle size, viscous modulus ( $G''$ ), elastic modulus ( $G'$ ), and the percentage of cross-linking. Elastic modulus ( $G'$ ) is essentially a measure of the fillers' firmness, with a higher  $G'$  indicating a stiffer product and a lower  $G'$  indicating a softer filler<sup>[22]</sup>. Other factors that may affect physician's and patient's decisions on choosing the type of filler are needle size, cost, duration, and presence of lidocaine in the filler. These differences make each HA filler unique and ideal for a specific region of the face that is aligned with the patient's goal. In general, fillers with smaller particle size, lower concentration, and lower  $G'$  are more appropriate for fine lines and wrinkles that require more superficial injection, while fillers with higher particle size and elastic modulus are more appropriate for deeper injections. A detailed discussion of each filler and its properties are beyond the scope of this article. However, when selecting a filler for periorbital volumization, one should consider these properties. A patient with skeletonizing and significant hollowness over the infraorbital region and rim requires deeper injection compared to a patient who wants to address crow's feet, glabellar, or fine periorbital rhytids. Fillers with low affinity to water such as Restylane and lower  $G'$  such as Belotero, Voluma, and Volbella are preferred in periocular rejuvenation.

## PREPARATION AND TECHNIQUE

A topical anesthetic such as a combination of 1% lidocaine/4% tetracaine cream is applied widely over and around the planned site of injection. This cream should be left in place for at least 20 min for adequate anesthesia. In our experience, this will provide satisfactory anesthesia without the need for injectable local anesthetics. Prior to injection, skin is cleansed with alcohol and/or 0.5% chlorhexidine based on surgeon preference with care to avoid the eye. The sites of injection may be marked if desired. HA filler may be injected with either a needle or a cannula, based on physician preference, although many feel that there is less risk of vascular occlusion when injecting with a blunt-tipped cannula. The senior author prefers the use of cannulas in the periorbital region. Key factors to consider while injecting are the depth of the desired injection, the speed of injection, the location of the periocular vasculature, and aspiration prior to injection.

**Table 2. Commonly used hyaluronic acid fillers and their properties with recommended needle size for administration**

Filler	Manufacturer	HA composition (mg/mL)	Presence of lidocaine	Needle size (Gauge)	G' (PG)
Restylane-L	Galderma	20	+	29-30	864
Restylane Lyft (Perlane)	Galderma	24	-	27-29	977
Restylane Silk	Galderma	20	+	30	-
Belotero	Merz North America	22.5	-	30	128
Juvederm Ultra XC	Allergan	20	+	27	-
Juvederm Ultra Plus XC	Allergan	24	+	27	207
Juvederm Voluma XC	Allergan	20	+	27	398
Juvederm Vollure XC	Allergan	17.5	+	30	-
Juvederm Volbella XC	Allergan	15	+	30	240
Versa	Revanesse	22-28	+	-	-

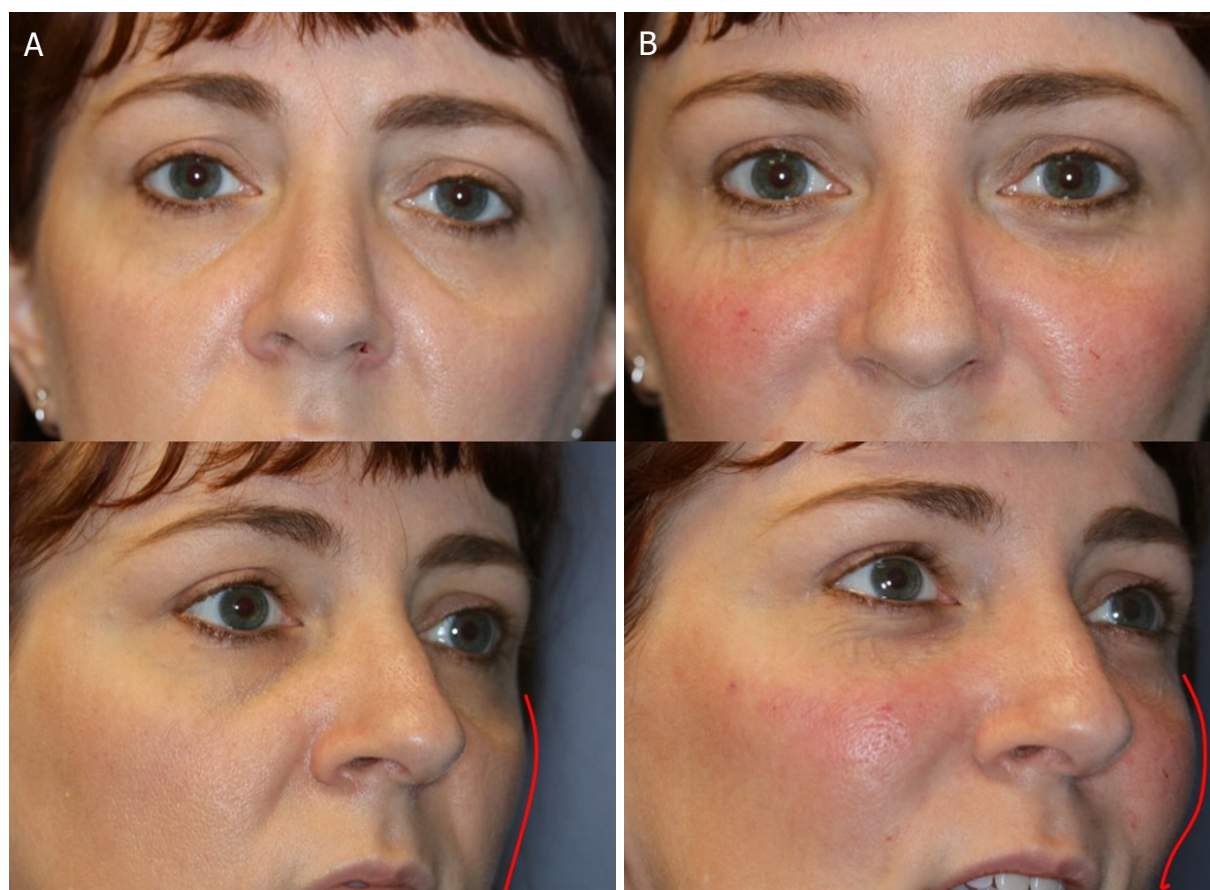
This is not an exhaustive list of all HA fillers. The G' properties and HA composition are adapted from Greene and Sidle<sup>[22]</sup> and Cho *et al.*<sup>[32]</sup>. HA: hyaluronic acid; G': elastic modulus



**Figure 3.** Tear trough deformity injections: (A) pre-injection; and (B) four months post-injection of 0.5 mL each side into the infraorbital hollows and 0.1 mL to the glabellar rhytids with Restylane-L

### Tear trough

Different injection techniques exist. If using a cannula, an entry hole is made with a 21- or 25-gauge needle depending on the cannula size below the infraorbital hollow. A cannula is then passed to the orbital retaining ligament and dry tunnels may be created along the length of the hollow followed by injection while withdrawing the cannula until the desired contour is obtained. If using a needle, one typically enters superior to the upper nasolabial fold, again passing superiorly along the orbital retaining ligament until meeting the resistance of the orbital rim, and injecting in a supraperiosteal plan. In younger patients or those with shallow folds, as little as 0.2-0.3 mL of filler per side is adequate for restoring volume. Caution should be made not to enter the orbit, injure the globe, and not to inject too superficial as this will result in increased bruising, a grayish or bluish discoloration, or irregularity. The area should be massaged after the injection to achieve a smooth contour [Figure 3]. If necessary to create a more rejuvenated appearance, injections into the malar eminence with a high G' filler may be combined with tear trough injections to create a more natural lid-cheek junction and restore the Ogee line [Figure 4]. These injections should be supraperiosteal and are typically injected in aliquots then massaged to provide a smooth contour. Examples of HA fillers commonly used in the malar region include Juvederm Voluma (Allergan) or Restylane Lyft (Galderma).



**Figure 4.** Hyaluronic acid injection to the tear trough and malar eminences: (A) pre-injection; and (B) post-injection to the infraorbital hollows with 1 mL of Volbella XC on each side as well as 1 mL of Voluma XC to each malar and infraorbital region

### Superior sulcus

The hollowing of the superior sulcus with deep supratarsal crease can be addressed by injection into the sub-orbicularis plane. A low G' filler such as Belotero (Merz North America), Restylane Silk (Galderma), or Volbella (Allergan) should be utilized. Prior to injection, the superior orbital rim and location of the supraorbital notch should be identified to avoid injury to the supraorbital nerve or vascular compromise. The needle or cannula should enter along the superior orbital rim at a 30° angle. After the needle has reached the bone, it should be slightly withdrawn to the preperiosteal space. An injection of as little as 0.1 mL on each side may be sufficient for mild cases and additional filler may be incrementally injected for more severe cases [Figure 5].

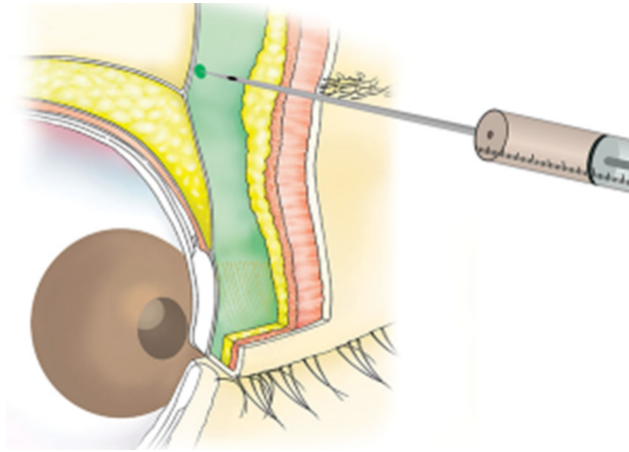
### Brow/temple complex

A similar injection technique may be used to subtly elevate the brow by injecting preperiosteally just below the brow along the supraorbital rim laterally. This is also a useful technique to decrease a skeletonized or hollowed appearance with a visible rim. This injection is often performed in conjunction with superior sulcus injections. In such cases, to optimize the appearance of the periorbital region, supraperiosteal injections along the medial temple at the lateral brow may also help produce a more youthful appearance and provide subtle but powerful volumization. A high G' filler should be used in the temple to restore volume loss.

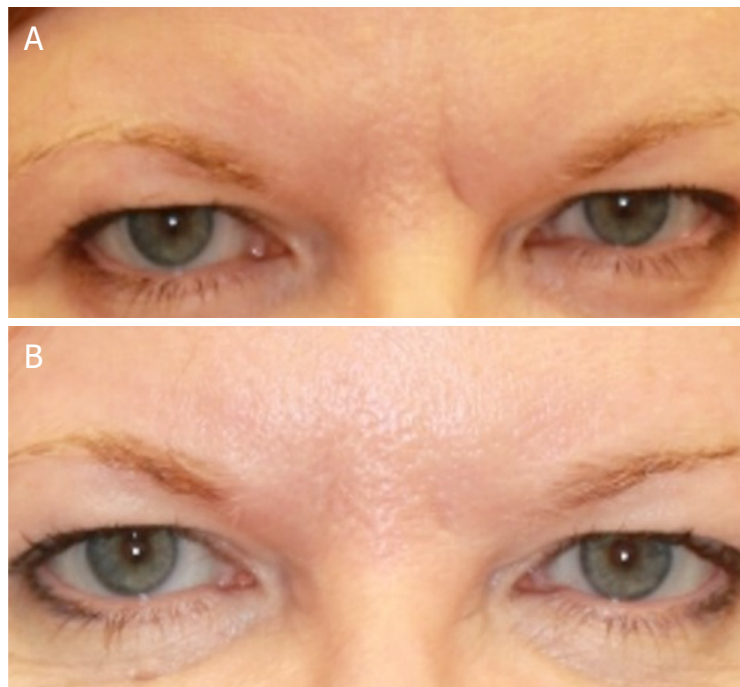
### Crow's feet and glabellar lines

In many instances, the combination of neuromodulators and fillers provide a superior outcome in addressing the crow's feet, glabella, and fine periorbital rhytids. Very small aliquots of intradermal filler





**Figure 5.** Hyaluronic acid injection to the superior sulcus. The needle or cannula should enter along the superior orbital rim at a 30° angle. After the needle has reached the bone, it should be slightly withdrawn to the preperiosteal space to inject into the suborbicularis plane. Adapted with permission from Looi *et al.* <sup>[31]</sup>



**Figure 6.** Combination of hyaluronic acid injection into the glabellar rhytids with chemodenervation: (A) pre-injection; and (B) two months after the glabellar injection of 0.1 mL on the right and 0.2 mL on the left of Restylane-L

with a low  $G'$  are typically required to efface these lines. The senior author prefers using a 30-gauge needle with bevel down for these injections. The filler should be injected in the subcutaneous plane and immediately massaged after injection to achieve an even contour and avoid early-onset nodules or visibility [Figure 6].

## COMPLICATIONS

### Vascular occlusion

Vascular occlusion is one of the most devastating complications of filler injections. In the periocular area, this can result in intra-arterial embolization or vascular compression and subsequent vision loss or skin

necrosis. The rate of vascular occlusion and subsequent ischemic complications has been reported up to 3 in every 1,000 injections<sup>[23]</sup>. Ophthalmic artery occlusion can result from intra-arterial injection in the periorbital region due to the multiple anastomosis between the ophthalmic artery and the dorsal nasal, supratrochlear, and supraorbital arteries with possible blindness<sup>[24]</sup>. A patient presenting with increased pain at the site of injection, visual loss, or visible signs of skin ischemia should undergo emergent evaluation for possible vascular occlusion.

Knowledge of appropriate techniques and preventive measures is important to decrease the risk of vascular complications. Aspiration prior to injection is extremely important to prevent inadvertent injection into the vessel lumen. Slow and steady injection has been shown to decrease the risk of vascular complications. It is also important to use an appropriate sized needle for injection. When the needle's gauge is too small, the risk of arterial wall damage and cannulation is higher. Consideration should be taken for the use of cannulas to decrease this risk. If severe pain occurs during injection, the procedure needs to be stopped immediately and the patient should be evaluated for possible vascular complication.

One of the advantages of HA fillers is the ability to provide reversal when complications such as skin ischemia, nodule formation, Tyndall effect, or subpar aesthetic outcomes occur by injection of hyaluronidase in a timely fashion. Although uncommon, if arterial embolization or vascular occlusion is suspected, immediate intervention is required. Treatments discussed in the literature are heat, topical nitroglycerin, hyaluronidase injection in the affected region, massage, and hyperbaric oxygen<sup>[23]</sup>. For any vision loss, immediate retrobulbar hyaluronidase injection is recommended. However, this treatment modality remains controversial. In a single case report by Chestnut<sup>[25]</sup>, injection of 450 units of hyaluronidase in the retrobulbar area was reported to reverse the vision loss caused by periorbital HA filler injection. However, the nature of the vision loss was not documented.

In a study by Lee *et al.*<sup>[26]</sup>, the effectiveness of retrobulbar hyaluronidase was investigated in rabbit models for possible reversal of HA filler related blindness. The authors found that in three out of four experimented eyes, the retinal perfusion was increased with normal electroretinography after retrobulbar hyaluronidase injection.

In another study by Paap *et al.*<sup>[27]</sup> the effectiveness of retrobulbar hyaluronidase was investigated in a human cadaver model. In this study, the authors demonstrated that retrobulbar hyaluronidase injection is unlikely to be effective in management of HA filler induced central retinal artery occlusion and blindness due to its inability to cross dural sheet of the optic nerve. A review of the medical literature by Paap *et al.*<sup>[28]</sup> regarding the efficacy of retrobulbar hyaluronidase as a treatment for filler related blindness concluded that to date this is an unproven therapy and largely unsuccessful.

Although this treatment modality has not been proven successful and further studies are needed to evaluate the effectiveness, an immediate retrobulbar hyaluronidase injection may be performed; however, patients must be informed that there is no current evidence-based, effective, and consistent treatment to reverse this devastating complication.

### **Bruising, redness, and swelling**

Redness, bruising, and swelling are the most common side effects related to filler injection. The redness and bruising usually happen immediately with periorbital injections caused by direct puncture of blood vessels upon entry of the needle. It has been proposed that higher gauge needles can result in less bruising and redness due to the lower risk of damage to blood vessel walls<sup>[24]</sup>. The use of a cannula may also cause less bruising due to fewer injection holes required and the blunt tip of the cannula. All patients should be advised to stop any anticoagulant medications, if possible, one week prior to treatment to decrease the





**Figure 7.** Antero-posterior and oblique view of a 76-year-old female who presented for a blepharoplasty consult. She had undergone blepharoplasty and hyaluronic acid injection to the tear trough by another provider years prior and noted a chronic swelling and discoloration in the under-eye. The exam showed a mild chronic inflammatory reaction with palpable curvilinear nodule with overlying edema. Small aliquots of hyaluronidase were injected with the resolution of the inflammation

risk of bruising and hematoma formation. Occasionally, patients develop more prominence of their native vessels after injection that may benefit from laser treatment.

### **Tyndall effect (blue-grey dyschromia)**

Superficial injections of fillers can result in bluish hue caused by light passing through colloid. Injections into the tear trough are more prone to Tyndall effect due to the thin, almost translucent nature of the skin at this location. This blue-grey discoloration can happen weeks to months after HA injection into the periorbital region and may persist long term<sup>[29]</sup>. To decrease the risk of Tyndall effect, the needle or cannula bevel should be pointed inferiorly at the time of injection and the product may be injected in a supraperiosteal plane. Makeup and phototherapy can be used for the management of blue-grey dyschromia as well as hyaluronidase injections.

### **Nodules**

Another side effect of fillers is delayed-onset nodules. These nodules are thought to be caused by local or systemic infections or inflammatory reactions and the incidence has been reported to be higher during winter months, which is thought to be related to a higher incidence of upper respiratory infections<sup>[30]</sup>. These nodules can appear years after injections and have also been postulated to be due to biofilm formation. These may appear in the tear trough region as a long, curvilinear swelling or irregularity along the infraorbital hollow that is mobile and may have surrounding edema [Figure 7]. Treatment with a prolonged course of empirical antibiotics such as macrolides has been recommended for their anti-inflammatory properties in addition to the antimicrobial effect. Injection of steroids into the nodule, a short-course of systematic steroids, and hyaluronidase are other potential options for management<sup>[24]</sup>.

### **CONCLUSION**

HA fillers provide an excellent option for a minimally invasive approach to periocular rejuvenation. With the emergence of a multitude of HA fillers over the past decade, facial and oculoplastic surgery has been equipped with the means to address age-related periorbital hollowing and skeletonization in an outpatient setting. Appropriate knowledge of periocular region anatomy, characteristics of HA fillers, proper injection technique, and management of complications is required for safe injection and to achieve optimal aesthetic outcomes.

## DECLARATIONS

### Authors' contributions

Study concept and design; drafting, critical revision of the manuscript for important intellectual content; collected the data; administrative, technical, or material support: Ziai K, Lighthall JG

Study supervision: Lighthall JG

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

The informed consent was obtained for publication of patient images used in this manuscript.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Liew S, Nguyen DQ. Nonsurgical volumetric upper periorbital rejuvenation: a plastic surgeon's perspective. *Aesthetic Plast Surg* 2011;35:319-25.
2. De Biasio F, Miotti G, Zingaretti N, Castriotta L, Parodi PC. Study on the aging dynamics of the periorbital region: from observation to knowledge of physiopathology. *Ophthalmic Plast Reconstr Surg* 2019;35:333-41.
3. Mendelson B, Wong CH. Changes in the facial skeleton with aging: implications and clinical applications in facial rejuvenation. *Aesthetic Plast Surg* 2012;36:753-60.
4. Goldberg RA, Fiaschetti D. Filling the periorbital hollows with hyaluronic acid gel: initial experience with 244 injections. *Ophthalmic Plast Reconstr Surg* 2006;22:335-41; discussion 341-3.
5. Kane MA. Treatment of tear trough deformity and lower lid bowing with injectable hyaluronic acid. *Aesthetic Plast Surg* 2005;29:363-7.
6. Mustak H, Fiaschetti D, Goldberg RA. Filling the periorbital hollows with hyaluronic acid gel: Long-term review of outcomes and complications. *J Cosmet Dermatol* 2018;17:611-6.
7. Lighthall JG. Rejuvenation of the upper face and brow: neuromodulators and fillers. *Facial Plast Surg* 2018;34:119-27.
8. Cotozana S, Fratila AA, Schenck TL, Redka-Swoboda W, Zilinsky I, et al. The anatomy of the aging face: a review. *Facial Plast Surg* 2016;32:253-60.
9. Bartlett SP, Grossman R, Whitaker LA. Age-related changes of the craniofacial skeleton: an anthropometric and histologic analysis. *Plast Reconstr Surg* 1992;90:592-600.
10. Clemons RA. Rejuvenation of the male brow. *Facial Plast Surg Clin North Am* 2008;16:299-312, vi.
11. Maas CS. Botulinum neurotoxins and injectable fillers: minimally invasive management of the aging upper face. *Otolaryngol Clin North Am* 2007;40:283-90.
12. Briceño CA, Zhang-Nunes SX, Massry GG. Minimally invasive options for the brow and upper lid. *Facial Plast Surg Clin North Am* 2015;23:153-66.
13. Knize DM. An anatomically based study of the mechanism of eyebrow ptosis. *Plast Reconstr Surg* 1996;97:1321-33.
14. Kashkouli MB, Abdolalizadeh P, Abolfathzadeh N, Sianati H, Sharepour M, et al. Periorbital facial rejuvenation; applied anatomy and pre-operative assessment. *J Curr Ophthalmol* 2017;29:154-68.
15. Mendelson BC, Muzaffar AR, Adams WP Jr. Surgical anatomy of the midcheek and malar mounds. *Plast Reconstr Surg* 2002;110:885-96; discussion 897-911.
16. Moon HS, Ahn B, Lee JH, Rah DK, Park TH. Rejuvenation of the deep superior sulcus in the eyelid. *J Cosmet Dermatol* 2016;15:458-68.
17. Sand JP, Zhu BZ, Desai SC. Surgical anatomy of the eyelids. *Facial Plast Surg Clin North Am* 2016;24:89-95.
18. De Boule K, Heydenrych I. Patient factors influencing dermal filler complications: prevention, assessment, and treatment. *Clin Cosmet*

- Investig Dermatol 2015;8:205-14.
19. Lee S, Yen MT. Nonsurgical rejuvenation of the eyelids with hyaluronic acid gel injections. *Semin Plast Surg* 2017;31:17-21.
  20. Attenello NH, Maas CS. Injectable fillers: review of material and properties. *Facial Plast Surg* 2015;31:29-34.
  21. Lee JC, Lorenc ZP. Synthetic fillers for facial rejuvenation. *Clin Plast Surg* 2016;43:497-503.
  22. Greene JJ, Sidle DM. The hyaluronic acid fillers: current understanding of the tissue device interface. *Facial Plast Surg Clin North Am* 2015;23:423-32.
  23. Rzany B, DeLorenzi C. Understanding, avoiding, and managing severe filler complications. *Plast Reconstr Surg* 2015;136:196-203S.
  24. Murthy R, Roos JCP, Goldberg RA. Periocular hyaluronic acid fillers: applications, implications, complications. *Curr Opin Ophthalmol* 2019;30:395-400.
  25. Chesnut C. Restoration of visual loss with retrobulbar hyaluronidase injection after hyaluronic acid filler. *Dermatol Surg* 2018;44:435-7.
  26. Lee W, Oh W, Ko HS, Lee SY, Kim KW, et al. Effectiveness of retrobulbar hyaluronidase injection in an iatrogenic blindness rabbit model using hyaluronic acid filler injection. *Plast Reconstr Surg* 2019;144:137-43.
  27. Paap MK, Milman T, Ugradar S, Silkiss RZ. Assessing retrobulbar hyaluronidase as a treatment for filler-induced blindness in a cadaver model. *Plast Reconstr Surg* 2019;144:315-20.
  28. Paap MK, Milman T, Ugradar S, Goldberg R, Silkiss RZ. Examining the role of retrobulbar hyaluronidase in reversing filler-induced blindness: a systematic review. *Ophthalmic Plast Reconstr Surg* 2020;36:231-8.
  29. Rootman DB, Lin JL, Goldberg R. Does the Tyndall effect describe the blue hue periodically observed in subdermal hyaluronic acid gel placement? *Ophthalmic Plast Reconstr Surg* 2014;30:524-7.
  30. Beleznyay K, Carruthers JD, Carruthers A, Mummert ME, Humphrey S. Delayed-onset nodules secondary to a smooth cohesive 20 mg/mL hyaluronic acid filler: cause and management. *Dermatol Surg* 2015;41:929-39.
  31. Looi AL, Yong KL. "Walk the Rim, Feel the Bone" technique in superior sulcus filling. *Plast Reconstr Surg Glob Open* 2015;3:e592.
  32. Cho SY, Park JW, An H, Ko HJ, Kim H, et al. Physical properties of a novel small-particle hyaluronic acid filler: In vitro, in vivo, and clinical studies. *J Cosmet Dermatol* 2018;17:347-54.

Review

Open Access



# Inflammation as an orchestrator of cutaneous scar formation: a review of the literature

Traci A. Wilgus

Department of Pathology, Ohio State University, Columbus, OH 43210, USA.

**Correspondence to:** Dr. Traci A. Wilgus, Department of Pathology, Ohio State University, 1645 Neil Avenue, 129 Hamilton Hall, Columbus, OH 43210, USA. E-mail: traci.wilgus@osumc.edu

**How to cite this article:** Wilgus TA. Inflammation as an orchestrator of cutaneous scar formation: a review of the literature. *Plast Aesthet Res* 2020;7:54. <http://dx.doi.org/10.20517/2347-9264.2020.150>

**Received:** 15 Jul 2020 **First Decision:** 1 Sep 2020 **Revised:** 14 Sep 2020 **Accepted:** 17 Sep 2020 **Published:** 16 Oct 2020

**Academic Editor:** Alexis Desmoulière **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Inflammation is a key phase in the cutaneous wound repair process. The activation of inflammatory cells is critical for preventing infection in contaminated wounds and results in the release of an array of mediators, some of which stimulate the activity of keratinocytes, endothelial cells, and fibroblasts to aid in the repair process. However, there is an abundance of data suggesting that the strength of the inflammatory response early in the healing process correlates directly with the amount of scar tissue that will eventually form. This review will summarize the literature related to inflammation and cutaneous scar formation, highlight recent discoveries, and discuss potential treatment modalities that target inflammation to minimize scarring.

**Keywords:** Inflammation, scar, skin, wound healing, macrophage, mast cell, neutrophil

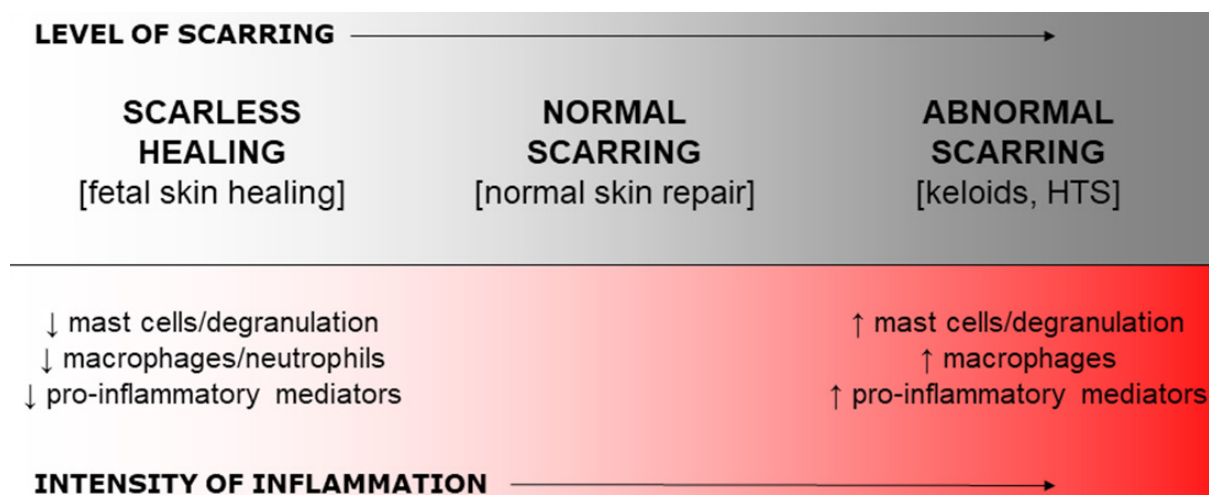
## INTRODUCTION

The repair of wounds in mature skin has been studied extensively. This process is highly complex and interactive, and it is made up of a series of well-defined stages that include inflammation, proliferation, and remodeling/scar formation<sup>[1-3]</sup>. Scar tissue is generated from activated fibroblasts, which produce excess levels of irregularly organized collagen. Clinically significant scars can develop from surgical, traumatic, or thermal (e.g., burn) injury<sup>[4]</sup>. Scars essentially function as a quick patch for damaged dermal tissue, but they can be problematic in many ways. Compared to normal skin, scar tissue is weaker in terms of tensile



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** Summary of the relationship between scarring and inflammation. Most studies published to date indicate that the robustness of the inflammatory response resulting from skin injury correlates with the amount of scar tissue that will be produced, with low levels of inflammation in scarless wounds and high levels of inflammation in cases of abnormal or excessive scarring

strength and it is also more rigid<sup>[5,6]</sup>, which adversely affects the biomechanical properties of the skin. The replacement of normal tissue with scar tissue reduces the functional capacity of the skin in that area as well, and this can cause problems such as limiting joint motion and impairing normal tissue growth. Scars can also have significant psychosocial implications and can negatively impact a patient's quality of life<sup>[7]</sup>.

Although inflammation occurs relatively early after injury, it can impact later stages of repair such as scar formation. In the early stages of repair, the primary functions of inflammation are to clean the wound site, clear debris, and prevent infection<sup>[8]</sup>. However, many of the mediators produced by activated inflammatory cells can stimulate fibroblasts, which drives the production of scar tissue and consequently shapes the final outcome of repair. Several innate immune cells have been linked to scar formation, including neutrophils, mast cells, and macrophages. In addition, a variety of inflammatory mediators have been shown to influence scar formation. This review will summarize what is known about the role of inflammation in scar formation and discuss some potentially useful approaches to reduce scar formation by modulating inflammation.

## CORRELATIVE DATA LINKING INFLAMMATION AND SCARRING

A large number of studies suggest that the level of inflammation in injured skin correlates directly with scar formation. A direct association between the extent of injury (and hence the levels of inflammation) and the amount of scarring that will ultimately result from the wound healing process has been established. For example, larger/deeper wounds or injuries that cause more severe damage are associated with higher levels of inflammation and heal with more scar tissue<sup>[9-13]</sup>. Furthermore, a wide range of studies have shown that high levels of inflammation are associated with excessive scarring or abnormal scars such as keloids and hypertrophic scars (HTS), whereas inflammation is significantly blunted in wounds that heal without scars [Figure 1]. Evidence supporting this concept will be discussed throughout the remainder of this section.

### Abnormal scars

#### Keloids

Keloids are abnormal, raised scars that develop after injury. These scars display some similarities with tumors, as they tend to invade the adjacent skin and extend beyond the initial site of injury. There are no widely accepted experimental animal models of keloid disease, so studies on keloid pathogenesis are



generally limited to experiments on tissue from human patients. A number of studies have examined inflammation in keloid tissue and the vast majority have indicated that keloids are associated with an increase in pro-inflammatory mediators and inflammatory cells.

Several studies have suggested an increase in pro-inflammatory mediator expression in keloids. A study by Zhang *et al.*<sup>[14]</sup> suggested that a pro-inflammatory niche exists in keloids, based on observations that interleukin (IL)-6 and IL-17 were increased in keloid tissue compared to normal skin. Another group reported an increase in chemokine-like factor 1 and other pro-inflammatory cytokines, such as IL-6, IL-8, and IL-18, in keloid tissue compared to normal skin and normal scars<sup>[15]</sup>. Jumper *et al.*<sup>[16]</sup> performed a site-specific gene expression profiling study and found that keloid tissue was enriched for pro-inflammatory genes and pro-inflammatory signaling pathway members, including IL-1 $\beta$ , IL-8, and IL-17, among others. Interestingly, the data pointed to a possible role for the overlying epidermal cells in keloid lesions in the regulation of inflammation.

In addition to pro-inflammatory mediators, several inflammatory cell types are prominent in keloids, particularly mast cells and macrophages. A study by Dong *et al.*<sup>[17]</sup> suggested that the total number of mast cells and the number of mast cells expressing chymase, a serine protease found primarily in mast cells, are higher in keloid tissue compared to normal skin. An increase in chymase activity in keloids was also observed, and chymase was shown to stimulate collagen production in cultured fibroblasts<sup>[17]</sup>. Another study found a low number of mast cells expressing tryptase, another mast cell-related serine protease, in keloids compared to tissue from several other organs; however, the sample size was small ( $n = 3$  keloids) and there was no direct analysis of or comparison to normal skin<sup>[18]</sup>. Several studies have also shown an increase in macrophages in keloids compared to normal skin<sup>[19,20]</sup>. A study by Shaker *et al.*<sup>[21]</sup> showed that both macrophages and mast cells are frequently found in close proximity to fibroblasts in keloid tissue, and another study by Arbi *et al.*<sup>[22]</sup> showed a close association between mast cells and fibroblasts in keloid samples by transmission electron microscopy. Although these studies were limited in scope, they do suggest the possibility that direct cell-cell interactions between inflammatory cells and fibroblasts could be important for fibrosis.

A more comprehensive analysis of inflammatory and immune cells was performed by Bagabir *et al.*<sup>[23]</sup> In this study, the number of mast cells and degranulated (activated) mast cells were found to be increased in keloids compared to normal skin and normal scar tissue, as were the number of M1 and M2 macrophages. M1 (classically activated; pro-inflammatory) and M2 (alternatively activated; anti-inflammatory/pro-fibrotic) macrophages express different biomarkers, and this general classification system is commonly used to delineate different macrophage phenotypes<sup>[24]</sup>. Interestingly, inflammatory cell enrichment was more closely associated with intralesional and perilesional keloid sites as opposed to extralesional sites.

While most published studies indicate an increase in mast cells and macrophages in keloid scars, one study comparing human keloids and equine exuberant granulation tissue (a fibrotic condition suggested to have some similarities to human keloids) reported minimal mast cells and macrophages in both fibrotic conditions<sup>[25]</sup>. Two other studies suggested either minimal differences or fewer mast cells in keloids<sup>[26,27]</sup>. While the reasons for the discrepancies between the studies are not entirely clear, the observations by Bagabir *et al.*<sup>[23]</sup> described earlier showed that the number of inflammatory cells varied across different sites within a keloid lesion. Also, in some of the studies, granule markers were the sole staining method used, which could underestimate the number of mast cells if they have degranulated. Therefore, the specific locations at which the cells were quantified, in addition to differences in staining methods, sample sizes, and patient populations, could contribute to the inconsistent results.

## HTS

HTS, which are raised red scars, also fall into the category of abnormal scars. Unlike keloids, HTS are confined to the borders of the original wound. A wide range of studies in both small and large animal models as well as human clinical samples have examined inflammation in hypertrophic scarring.

Several rodent models of HTS have been described. One such model uses the application of a mechanical loading device to apply tension to wounds, inducing a HTS-like phenotype reminiscent of human HTS<sup>[28]</sup>. In this model, the scars were shown to have a high number of mast cells, similar to human HTS<sup>[28]</sup>. The HTS-like lesions were also shown to have higher numbers of macrophages compared to control wounds with no mechanical loading<sup>[29]</sup>. Several other studies have been published using models of HTS formation in which human skin is grafted onto nude mice. In these studies, higher numbers of mast cells<sup>[30]</sup> and macrophages<sup>[30,31]</sup> were observed in the HTS-like lesions compared to control tissue. Upon further examination of macrophage subtypes, M2 macrophages were found to be elevated, and higher levels of pro-inflammatory cytokines were noted in HTS-like samples<sup>[31]</sup>. An increase in both mast cells and macrophages has also been reported in a mouse model of hypertrophic scar contracture<sup>[32]</sup>.

Large animal models, such as pigs, are considered by many to be an ideal wound healing model based on similarities in skin anatomy between pig and human. A study by Harunari *et al.*<sup>[33]</sup> examined mast cells in human HTS samples and samples from a large animal model of HTS, the red Duroc pig. The authors found an increase in mast cells in HTS from both humans and red Duroc pigs compared to the corresponding normal skin controls.

In human HTS, there are conflicting results regarding whether inflammation is enhanced or reduced in HTS. Early studies by Kischer *et al.*<sup>[34]</sup> reported higher numbers of mast cells in HTS compared to granulation tissue or mature scar tissue samples using toluidine blue, a metachromatic stain that binds mast cell granules. A study by Beer *et al.*<sup>[35]</sup> did not see differences in tryptase-positive mast cells when comparing among keloids, HTS, and surgical scars; however, normal skin samples were not included for comparison. Another study by Niessen *et al.*<sup>[36]</sup> found no significant differences in tryptase-positive mast cells in HTS compared to normal scars, although they did note a trend toward increased subepidermal mast cells in HTS. There are several possible explanations for the contradictory results between the studies. The use of different techniques to identify mast cells could have played a role, as toluidine blue should indiscriminately stain all mast cell subtypes (including chymase- and tryptase-positive mast cells), whereas tryptase staining may not easily identify mast cells if they predominately express chymase. In addition, the age of the scars examined may play a role. The study by Beer *et al.*<sup>[35]</sup> showed a direct correlation between mast cell density and scar age in surgical scar samples and the study by Niessen *et al.*<sup>[36]</sup> noted an overall increase in mast cells between 3 and 12 month scar samples, so standardization of scar age may be needed to properly compare studies from different investigators.

There are only a few reports examining macrophages in human HTS. In one study comparing normal scars and HTS from breast surgeries, no differences were found in macrophages between scar types<sup>[36]</sup>. In another study looking at scars from cardiothoracic surgery patients, an increase in macrophages was observed in normal scars compared to HTS at earlier time points, but total macrophage and M2 macrophage numbers were increased in HTS at later time points and remained elevated for a longer period of time<sup>[37]</sup>.

The data on pro-inflammatory cytokine expression in human HTS also appears to be somewhat mixed. One study compared inflammatory gene expression in a small prospective study comparing patients that developed normal scars or HTS<sup>[37]</sup>. The authors reported reduced expression of inflammatory genes (including TNF $\alpha$ , IL-1 $\alpha$ , IL-1RN, several chemokines, and IL-10) in HTS, but the downregulated genes contained both pro- and anti-inflammatory genes. Similarly, another prospective study compared various

parameters of inflammation at a very early time point (3 h post-injury) in patients that healed with normal scarring or HTS<sup>[38]</sup>. The authors reported reduced protein levels of IL-6, IL-8, and CCL2 in wounds from HTS patients compared to normal scar patients and suggested that reduced inflammation is associated with HTS formation. However, increases in P-selectin mRNA were found in HTS compared to normal scar samples. In addition, TNF- $\alpha$ , CXCL4, VCAM, and TLR4 mRNA were significantly increased after surgery only in HTS and there were more M2 macrophages in pre-operative HTS samples.

Taken together, the data suggest that more detailed analysis of inflammatory cells and inflammatory mediators is needed to definitively show whether inflammation is associated with hypertrophic scarring in humans.

## Scarless healing

### *Fetal skin wounds*

It is well established that developing fetal skin is capable of healing wounds in a scarless, regenerative manner at certain gestational stages. Fetal skin heals scarlessly at early stages of development (until about the third trimester), but as the skin matures at later stages of development it heals through a fibrotic repair process that produces a permanent scar<sup>[39-41]</sup>. One of the key differences between scarless and fibrotic fetal wound healing is the level of inflammation. Many studies have shown that there are few, if any, of the traditional features of inflammation in scarless fetal wounds, but fibrotic fetal wounds that occur later in gestation heal with a strong inflammatory response<sup>[42]</sup>. In addition, artificially inducing inflammation in fetal wounds that would normally heal scarlessly causes them to heal with a scar<sup>[43]</sup>.

Several studies have been performed to identify specific differences in the inflammatory response between scarless and fibrotic wounds. Levels of pro-inflammatory mediators, such as lipids (PGE<sub>2</sub>)<sup>[44]</sup>, cytokines (IL-6, IL-8, IL-33)<sup>[45-47]</sup>, and alarmins (HMGB-1)<sup>[48]</sup> have been shown to be lower while anti-inflammatory mediators (IL-10)<sup>[49]</sup> has been shown to be higher in fetal skin or scarless wounds. Fetal fibroblasts produce less IL-6 and IL-8 compared to adult fibroblasts<sup>[45,46]</sup> and microarray studies showed that expression of inflammatory genes is reduced in fibroblasts from less developed fetal skin (scarless) compared to more developed fetal skin (fibrotic)<sup>[50]</sup>.

In addition to reduced pro-inflammatory mediators, fewer inflammatory cells or reduced inflammatory cell activation have been described in scarless fetal wounds. Fetal platelets do not aggregate to the same extent and release lower levels of cytokines compared to adult platelets<sup>[51,52]</sup>. Mast cells are present in lower numbers and are less mature in fetal skin at earlier gestational ages, and mast cells do not become activated or degranulate in response to injury in scarless wounds<sup>[53,54]</sup>. Macrophages are fewer in number and persist for a shorter period of time in early embryonic wounds, and the macrophages that are present do not appear to be activated<sup>[55,56]</sup>. In addition, very few, if any, neutrophils are recruited to fetal wounds when the skin is injured at ages corresponding to scarless healing<sup>[42,57]</sup>. It is possible that differences in fetal endothelial cells could play a role in minimizing the number of circulatory inflammatory cells recruited to fetal wounds. Studies have shown that neutrophils adhere less to fetal endothelial cells, which is likely due to lower expression of P-selectin<sup>[58,59]</sup>. This adhesion molecule mediates leukocyte-endothelial cell interactions that are required for effective recruitment of leukocytes from the circulation and into damaged tissues.

Studies examining human fetal skin also support the idea that less developed skin has a suppressed inflammatory response. An early study by Rowlatt described a lack of an acute inflammatory reaction and granulation tissue formation at the site of limb amputations caused by amniotic constriction bands in a 20 week human fetus<sup>[60]</sup>. In another study, Walraven *et al.*<sup>[61]</sup> reported that mid-gestation fetal skin (18-22 weeks) has fewer macrophages, mast cells, dendritic cells, and other immune cell types compared to adult skin.

Because there was an adequate number of immune cells in the lymph nodes, the authors speculated that the reduced immune cell numbers present in the skin was due to a deficiency in homing signals. Indeed, they found reduced levels of chemokines such as CCL17, CCL21, and CCL27 in fetal skin.

#### *Other types of scarless wounds*

Besides fetal skin, scarless healing has also been described in mucosal tissues, such as the oral mucosa<sup>[62]</sup>. There is also evidence that oral mucosal wounds have a blunted inflammatory response compared to other tissues that heal with scarring. In a mouse model, Szpadarska *et al.*<sup>[63]</sup> showed fewer macrophages, less myeloperoxidase (a marker of neutrophil presence), and lower pro-inflammatory cytokine production in oral mucosal wounds (scarless) compared to cutaneous wounds (scar-forming). Similarly, in a porcine model, Mak *et al.*<sup>[64]</sup> compared oral and skin wounds in red Duroc pigs and found reduced macrophage and mast cell numbers in oral wounds, which also displayed accelerated resolution of inflammation. In healthy human oral and skin tissue (uninjured), fewer neutrophils and macrophages have been reported<sup>[65]</sup>. Global transcriptome analysis by multiple groups suggests that several pro-inflammatory genes and regulatory pathways are suppressed or are less persistent in oral mucosal wounds compared to skin wounds<sup>[66,67]</sup>.

Scarless healing has also been documented in a unique mouse species, the African spiny mouse (*Acomys*). *Acomys* was shown to completely regenerate and heal without scarring in response to large dorsal skin wounds<sup>[68]</sup>. Follow up studies compared transcription profiles of scar-forming wounds from standard laboratory mice (*Mus*) and regenerative wounds from *Acomys*, and showed *Acomys* wounds had a diminished cytokine/chemokine response<sup>[69]</sup>. *Acomys* has also been reported to be neutropenic (reduced blood neutrophils) and have less pronounced macrophage recruitment to wounds compared to *Mus* strains<sup>[70]</sup>. These results fit the general theme observed with scarless oral mucosal and fetal wounds, which have a dampened inflammatory response compared to wounds that heal with scars.

## **FUNCTIONAL DATA LINKING INFLAMMATION AND SCARRING**

In addition to correlative data, which generally link higher inflammatory mediator levels and/or elevated inflammatory cell numbers in a wound to more abundant scar formation, there are also functional data supporting a role for inflammation in promoting scar formation. These include studies showing that inflammatory cells or inflammatory mediators stimulate scar tissue/collagen production as well as studies showing that depletion or knockdown of inflammatory components reduces scar formation.

### **Inflammatory cells**

#### *Mast cells*

Two general approaches have been used to study the function of mast cells in wound healing and scar formation: treatment with mast cell stabilizing drugs and examination of mast cell-deficient mouse strains.

Drugs that act as mast cell stabilizers, which prevent mast cell degranulation, have been used in animal models of wound healing to study the importance of mast cells in scar formation. By preventing mast cell degranulation, these drugs inhibit the release of pre-stored mediators present within the granules. Disodium cromoglycate (also known as cromolyn) has been shown to reduce collagen content in a rat model when injected directly into wounds<sup>[71]</sup>. Systemic treatment with disodium cromoglycate has also been shown in a mouse excisional wound model to reduce pro-inflammatory cytokine levels and myeloperoxidase levels (commonly used to estimate the presence of neutrophils) at early time points post-injury, while reducing scar size and normalizing collagen architecture/collagen fibril density at later time points<sup>[72]</sup>. Another study showed that oral administration of the mast cell stabilizer ketotifen reduced wound contraction and collagen deposition, causing thinner, less dense collagen fibrils to be produced in a red Duroc pig model<sup>[73]</sup>.

The role of mast cells in scar formation has also been examined in mast cell-deficient mouse strains (either naturally occurring mutants or genetically modified mice), and the results seem to differ depending on the mouse strain and wound model used. Kit<sup>W/W-v</sup> mice are mutant mice that lack mast cells due to functional mutations in the tyrosine kinase receptor c-kit, which binds an important growth factor for mast cells (stem cell factor). Younan *et al.*<sup>[74]</sup> used these mice in a study looking at the effects of microdeformation in wounds using a negative pressure wound therapy device. They showed that microdeformation induced higher levels of mast cell degranulation, which correlated with an increase in granulation tissue thickness and collagen deposition. These changes were normalized in Kit<sup>W/W-v</sup> mice, suggesting that the increases in granulation tissue and collagen production from the microdeformation device were mast cell-dependent. Kit<sup>W/W-v</sup> mice were also shown to heal with smaller scars in late-gestation fetal skin wounds compared to control mice<sup>[53]</sup> and less fibrosis at the wound edge in adult scald wounds<sup>[75]</sup>, supporting the idea that mast cells promote scar formation and fibrosis. In contrast to these studies, several other mast cell-deficient mouse strains have been shown to heal with similar levels of scar tissue and collagen deposition compared to control mice that have normal mast cell numbers<sup>[76-78]</sup>. Many of the published studies discussed above used different wound models and different mast cell-deficient strains, which could partially explain the variable results. In addition, most mast cell-deficient strains also have defects in one or more other immune cell types and non-specifically deplete the entire mast cell population, so it will be important to revisit these ideas once we understand more about mast cell heterogeneity and have more precise mouse models to specifically target mast cells and possibly different functional mast cell subtypes<sup>[79]</sup>.

#### *Neutrophils and macrophages*

Several mutant mouse strains have been used to examine the importance of neutrophils and macrophages in wound-induced scar formation. One of the first studies of this kind explored wound healing in mice lacking the transcription factor PU.1<sup>[80]</sup>. PU.1 null mice, which lack macrophages and functional neutrophils, were shown to heal quickly and with minimal scarring. However, the relative importance of macrophages versus neutrophils is unclear since both cell types are absent in wounds from PU.1 null mice. While some studies suggest that the early neutrophil response may be important for scar formation and the presence of neutrophil extracellular traps have been reported in skin scars and other fibrotic conditions<sup>[81]</sup>, there do not appear to be published studies examining scar formation using animal models in which neutrophils have been specifically depleted.

More specific studies examining the role of macrophages in collagen deposition and scar formation have been performed using several approaches. Studies from two groups have used slightly different genetically modified mouse strains in combination with diphtheria toxin to deplete macrophages. Mirza *et al.*<sup>[82]</sup> showed reduced collagen density in wounds from macrophage-depleted mice and Lucas *et al.*<sup>[83]</sup> showed that depletion of macrophages at early stages of wound healing reduced the amount of granulation tissue and the size of scars that formed. Several studies have also used clodronate liposomes to deplete macrophages. One study showed that macrophage depletion reduced collagen expression and deposition in wounds<sup>[84]</sup> and another showed that macrophage depletion reduced scar formation in a xenograft model of HTS<sup>[85]</sup>. While it is generally accepted that macrophages release mediators that can stimulate collagen production by fibroblasts, several recent studies have suggested the possibility of novel mechanisms. For example, macrophages were shown to support adipocyte-derived myofibroblasts through insulin-like growth factor and platelet-derived growth factor C<sup>[86]</sup>. A recent report also highlighted the plasticity of myeloid cells, which may be converted directly to collagen-producing fibroblasts in healing wounds<sup>[87]</sup>. More work will have to be done to understand exactly how macrophages contribute to scar formation.

#### **Inflammatory mediators**

Aside from specific inflammatory cell types, a wide range of inflammatory mediators, including both pro- and anti-inflammatory mediators, have been shown to play a functional role in cutaneous scar formation.



### *Pro-inflammatory mediators*

Both fetal and adult wound healing studies in animal models have implicated specific pro-inflammatory mediators in the stimulation of scar formation. Fetal wound healing models have been useful for identifying inflammatory mediators with fibrogenic potential since a specific mediator can be injected into fetal wounds to determine whether it can convert the scarless healing process into a fibrotic repair process. With this approach, the formation of a scar in a wound that would otherwise heal scarlessly can be used as a readout of pro-fibrotic activity. A number of pro-inflammatory mediators have been shown to promote scar formation in fetal wounds using this system, including cytokines such as IL-6 and IL-33<sup>[45,47]</sup>, pro-inflammatory lipids like PGE<sub>2</sub><sup>[44]</sup>, and alarmins such as HMGB-1<sup>[48]</sup>. Some of these same mediators have also been studied in adult wound healing models. For example, HMGB-1 has been linked to scar formation<sup>[88]</sup> and blocking PGE<sub>2</sub> production with drugs that inhibit cyclooxygenase-2 activity has been shown to reduce scar formation in adult incisional wound models<sup>[89,90]</sup>. Other pro-inflammatory cytokines that have been linked to cutaneous scar formation and/or collagen production in adult scar/fibrosis models include IL-17<sup>[91]</sup> and monocyte chemoattractant protein-1<sup>[92,93]</sup>. Additionally, osteopontin (OPN), which has pro-inflammatory cytokine-like properties and is associated with wound-induced inflammation<sup>[94]</sup>, has been linked to scar formation. Studies in a mouse model showed that osteopontin knockdown in the skin resulted in less inflammation (reduced neutrophil, macrophage, and mast cell numbers) as well as reduced scar formation compared to control wounds<sup>[95]</sup>.

### *Anti-inflammatory mediators*

In contrast to pro-inflammatory mediators, anti-inflammatory mediators have been shown to limit scar formation in fetal and adult wound healing models. The most well-documented example of this is the anti-inflammatory cytokine IL-10. Studies have shown that IL-10 levels are higher in scarless fetal wounds compared to scar-forming wounds<sup>[49]</sup>. Furthermore, scar formation is amplified in IL-10 knockout mice<sup>[96]</sup> and scar formation is reduced when IL-10 levels are artificially enhanced<sup>[11,49,97-99]</sup>. In addition to IL-10 having anti-inflammatory effects, studies have suggested that IL-10 signaling enhances the production of hyaluronic acid<sup>[100-103]</sup>, an extracellular matrix molecule associated with scarless healing and regeneration<sup>[104-110]</sup>. Other anti-inflammatory and pro-resolution mediators have also been linked to scar formation. Mice lacking the chemokine receptor CXCR3, which has been implicated in wound resolution, heal with abnormal scarring<sup>[111]</sup>, and the pro-resolution mediator chemerin15 has been shown to reduce inflammation and scar formation<sup>[112]</sup>.

## **THERAPEUTIC STRATEGIES TO PREVENT SCAR FORMATION**

Given the evidence supporting the idea that inflammation promotes scar formation, it seems logical that targeting inflammation might be a viable therapeutic strategy for restricting scar tissue production and enhancing the cosmetic and functional clinical outcomes resulting from skin injury. There are data supporting various anti-inflammatory approaches that could be effective for minimizing the appearance of scars and several other proposed scar therapies may reduce scarring in part by altering inflammation. These will be discussed below.

### **Traditional anti-inflammatory strategies**

Steroids and nonsteroidal anti-inflammatory drugs (NSAIDs) are traditional anti-inflammatory drugs that may be beneficial for preventing or treating scars. Corticosteroids are anti-inflammatory drugs commonly used in clinical settings to treat raised scars such as HTS and keloids. Steroid therapy can be used in an attempt to induce scar regression, but may be more effective when used to prevent recurrence after scar revision surgery<sup>[113]</sup>. The use of steroids for scar therapy has been reviewed previously<sup>[114-116]</sup>.

NSAIDs are another class of anti-inflammatory drugs that block the production of inflammatory lipid mediators, such as PGE<sub>2</sub>, by inhibiting the function of one or more cyclooxygenase enzymes. These drugs

are commonly used to treat pain, fever, and inflammation. Some of these drugs have been suggested to reduce collagen deposition and scar formation in animal models of wound healing. In an incisional murine wound model, topical application of celecoxib resulted in decreased PGE<sub>2</sub> production, inhibition of neutrophil recruitment, and significantly reduced scar size<sup>[89]</sup>. In a rabbit ear model of HTS formation, celecoxib treatment was shown to reduce scarring (measured by scar elevation index score)<sup>[90]</sup>, and a combination of celecoxib and the angiotensin-converting enzyme inhibitor captopril reduced inflammation and scar height<sup>[117]</sup>. Studies have also suggested that dressings incorporated with anti-inflammatory drugs, such as electrospun fibrous scaffolds loaded with ibuprofen<sup>[118]</sup> and emulgel dressings containing the anti-inflammatory drug acetylsalicylic acid along with stratifin<sup>[119]</sup> (a protein produced by keratinocytes that has been shown to suppress scar formation<sup>[120,121]</sup>), may lead to reduced scarring.

## Other anti-inflammatory strategies

### *TLR4 inhibitors*

Drugs that target innate immune receptors have the potential to be used to limit scar formation based on their anti-inflammatory mechanism of action. TLR4 inhibitors are one example. TLR4 is a pattern recognition receptor that binds to many different pathogen-associated molecular patterns as well as damage-associated molecular patterns (also known as alarmins) that stimulate inflammation in response to microbes or tissue damage, respectively<sup>[122]</sup>. High TLR4 expression has been documented in human HTS tissue and HTS fibroblasts<sup>[123]</sup>. TLR4 has also been suggested to play a role in HTS development in a mouse model, where treatment with the TLR4 inhibitor TAK-242 (restorvid) was found to reduce scar formation<sup>[124]</sup>. The data were very limited in this study, with only 3 mice per group and no quantitative scar data; however, TLR4 has been linked to fibrosis previously and TAK-242 has been shown to reduce fibrosis in several organs, including the skin, in other animal studies<sup>[125-127]</sup>. Together, these studies suggest TLR4 inhibitors may be a promising strategy to minimize scarring. In addition, the fact that this drug has been used topically to reduce ultraviolet light-induced inflammation and skin carcinogenesis<sup>[128,129]</sup> and has been used in clinical trials for other diseases<sup>[130]</sup>, suggests that TLR4 inhibitors may be a worthwhile pursuit.

### *CXCR4 antagonists*

Another potential target is the chemokine receptor CXCR4, which binds to stromal-derived factor-1 (SDF-1), also known as CXCL12. Upregulation of SDF-1/CXCR4 signaling has been reported in human burn patients and in HTS tissue<sup>[131,132]</sup>. An increase in SDF-1 is believed to stimulate recruitment of CXCR4-positive leukocytes, and possibly collagen-producing fibrocytes, from the circulation, thereby promoting HTS formation<sup>[131,132]</sup>. Similar results have been reported in keloid tissue, with an increase in SDF-1 expression and CXCR4-positive cells in keloids compared to normal tissue<sup>[133]</sup>. In a small animal HTS model, a CXCR4 antagonist was found to reduce the number of macrophages and myofibroblasts, inhibit contraction, and reduce scar formation<sup>[131]</sup>. Although more work is needed, the results thus far suggest potential for CXCR4 antagonists in preventing scar tissue deposition.

## Additional scar reducing treatments affecting inflammation

### *Pirfenidone*

Pirfenidone is an anti-fibrotic drug with anti-inflammatory properties used to treat idiopathic pulmonary fibrosis. Although the ability of pirfenidone to treat or prevent skin scarring has not been thoroughly investigated, several studies suggest that it may be a promising option. Multiple studies have shown that pirfenidone can inhibit the pro-fibrotic behavior of cultured dermal fibroblasts. For example, pirfenidone can reduce TGF- $\beta$ -induced fibroblast proliferation, migration, collagen expression, and myofibroblast formation<sup>[134]</sup>, as well as fibroblast contraction<sup>[135,136]</sup>. Pirfenidone has also been shown to reduce proliferation and inhibit epithelial-mesenchymal transition in keloid keratinocytes<sup>[137]</sup>. In wound healing studies, pirfenidone has been shown to reduce pro-inflammatory cytokine production, neutrophil infiltration, and collagen synthesis<sup>[138,139]</sup>, and in a clinical trial topical application of an 8% pirfenidone gel

induced scar regression to a greater degree than control pressure therapy in burn-induced HTS in pediatric patients<sup>[140]</sup>.

#### *Epigallocatechin-3-gallate*

Epigallocatechin-3-gallate (EGCG) is a green tea polyphenol known to have antioxidant, anti-inflammatory, and anti-tumor effects. The ability of EGCG to be photoprotective and inhibit cutaneous inflammation in response to ultraviolet light-induced skin damage is well documented<sup>[141-144]</sup>. More recently, the potential for EGCG to inhibit inflammation and scar formation in skin wounds has been described. EGCG has been shown to inhibit mast cell-stimulated collagen protein expression by keloid fibroblasts, likely through alterations in PI3K/Akt signaling<sup>[145]</sup>. EGCG also inhibited proliferation, migration, and collagen expression in keloid fibroblasts via STAT3 inhibition<sup>[146]</sup>. In a study using a model in which human keloid fibroblasts were injected into nude mice, EGCG treatment reduced keloid nodule formation and inhibited collagen production<sup>[146]</sup>. Another study tested the effects of EGCG in long-term keloid organ cultures and showed that EGCG reduced mast cell numbers in the keloid tissue and reduced keloid volume<sup>[147]</sup>. Recently, a clinical trial examining the effects of a topical formulation of EGCG in human skin wounds showed that EGCG treatment reduced mast cell numbers and improved scar outcomes, as indicated by a reduction in scar thickness and increases in hydration and elasticity<sup>[148]</sup>. Together, the data suggest that EGCG could be a promising therapeutic to prevent excessive scarring.

#### *Fibromodulin*

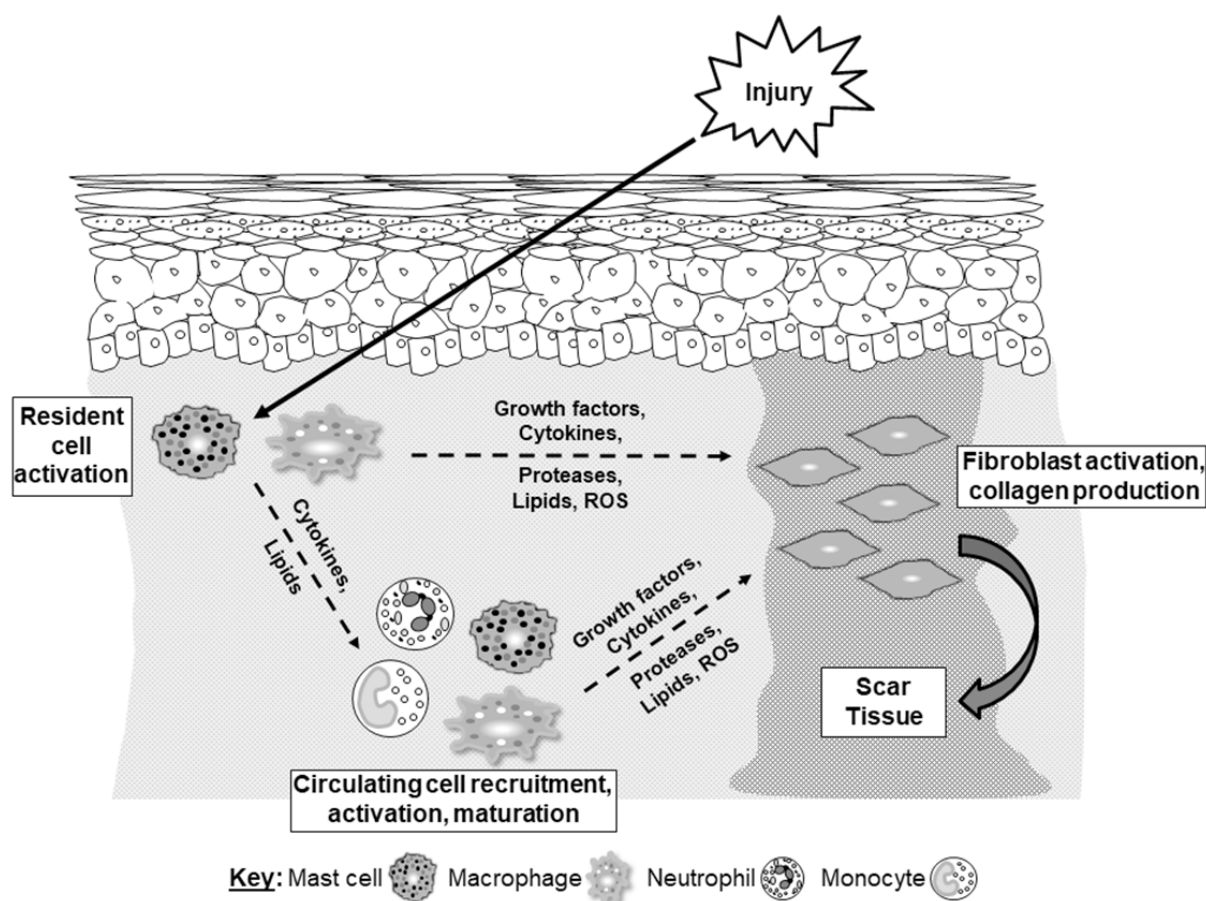
Fibromodulin is a member of the small leucine-rich proteoglycan family that has been shown to have an inverse relationship with scar formation. Fibromodulin levels are initially high in fetal skin at stages associated with scarless healing, then levels decrease during development as the skin starts to heal with a scar<sup>[149]</sup>. Additionally, fibromodulin levels increase during healing in scarless fetal wounds, which have minimal inflammation, but remain low in scar-forming wounds which display a typical inflammatory response<sup>[149]</sup>. Lower fibromodulin levels have been reported in HTS from human subjects and animal models compared to normal tissue<sup>[150,151]</sup>, and mice lacking fibromodulin heal with more inflammation<sup>[152]</sup> and more scar formation<sup>[153,154]</sup>. Increasing fibromodulin levels via adenoviral overexpression or treating with recombinant fibromodulin has been shown to reduce scar formation in rabbit, mouse, and red Duroc pig wounds<sup>[153-156]</sup>. Based on these studies, a fibromodulin-based peptide has been developed and is currently being tested in clinical trials<sup>[157]</sup>.

#### *Hydration*

Studies have shown that maintaining hydration and allowing healing to occur in a moist environment can be beneficial for healing and reducing scar formation. The underlying mechanisms appear to be related to reduced inflammation. Saline-filled polyurethane or vinyl chambers used to maintain a moist healing environment caused wounds to heal with less inflammation and less scarring compared to dry, air-exposed wounds<sup>[158-160]</sup>. Additionally, occlusive dressings, which maintain hydration, reduce both inflammation and scarring<sup>[161]</sup>. Occlusion reduces the production pro-inflammatory cytokines and lipids by epidermal keratinocytes, the release of pro-inflammatory alarmin molecules such as S100A8, S100A9, and S100A12, and the infiltration of inflammatory cells<sup>[90,162-165]</sup>. It is likely, then, that maintaining hydration mitigates scar formation in part by diminishing inflammation.

#### *Mechanoregulation*

Fibroblast behavior is known to be influenced by mechanical signals, and wounds that heal under tension typically heal with more scar tissue. Data from animal models have illustrated this concept, as devices which increase mechanical loading on a wound increase scar formation<sup>[28,166]</sup>. Mechanically-loaded wounds have also been shown to have a stronger inflammatory response<sup>[28,29,93]</sup>. In addition, it has been suggested that higher levels of mechanical strain in keloid tissue is associated with inflammation and may contribute



**Figure 2.** Summary of cellular interactions leading to cutaneous scar formation. When the skin is damaged, an inflammatory response is induced. Initially, resident inflammatory cells (e.g., mast cells and macrophages) in the dermis are activated. These activated cells secrete molecules that stimulate fibroblast activity and promote collagen production and scar tissue deposition. The resident cell-derived mediators, particularly cytokines (and chemokines) as well as lipids, stimulate the recruitment of circulating inflammatory cells (e.g., neutrophils, monocytes, and mast cell precursors) into the tissue. These cells become activated, and in some cases mature (monocytes become macrophages and mast cell precursors become mature mast cells), leading to even higher local levels of mediators that stimulate fibroblast activity and perpetuate scar tissue production. Several types of mediators produced by inflammatory cells have been linked to scar formation, including growth factors (TGF- $\beta$ 1), cytokines/chemokines [interleukin (IL)-6, IL-17, IL-33, MCP-1, SDF-1, OPN], proteases (mast cell chymase/tryptase, neutrophil elastase), lipids (PGE<sub>2</sub>), and reactive oxygen species or ROS (H<sub>2</sub>O<sub>2</sub>)

to keloid progression<sup>[167]</sup>. Focal adhesion kinase (FAK) signaling, which controls mechanosignaling in fibroblasts, has been suggested to mediate the enhanced inflammatory reaction associated with mechanical strain<sup>[93,167]</sup>. Mechanosensing has also been shown in recent studies to regulate inflammatory cell migration using sophisticated *in vitro* approaches<sup>[168]</sup>. Here, the authors demonstrated that deformations in collagen matrices caused by contractile activity of fibroblasts provides a strong signal for macrophage migration.

Several approaches have been tested to target mechanosignaling as a way to prevent or reduce scarring. A stress-shielding device, which reduces the tension on a healing wound, has been shown to improve scarring in large animal pre-clinical models as well as human clinical trials<sup>[166,169,170]</sup>. Microarray studies have shown that this device reduces the transcription of genes related to inflammatory pathways<sup>[171]</sup>. Another strategy to reduce scar formation by affecting mechanotransduction is to inhibit FAK signaling with a small molecule inhibitor. Several small animal studies have suggested that FAK inhibitors can reduce scarring<sup>[93,172]</sup>, and treatment with FAK inhibitors and genetic ablation of FAK in fibroblasts have been reported to reduce inflammatory mediator production and inflammatory cell recruitment<sup>[93]</sup>. Collectively, the data suggest that altering mechanical signaling pathways may reduce scar formation at least in part by influencing inflammation.

## CONCLUSION

There is a large body of evidence suggesting that the magnitude of the inflammatory response influences the amount of scar tissue that will result from the healing process [Figure 2]. This likely results, at least in part, from the array of mediators released by inflammatory cells capable of stimulating fibroblast activity. Studies examining human tissue and samples from animal models suggest that pro-inflammatory cytokines and the number of inflammatory cells (e.g., mast cells and macrophages) are elevated in problematic scars such as HTS and keloids whereas the inflammatory response is muted in scarless wounds. Functional data support these correlative results as depleting inflammatory cells or reducing pro-inflammatory mediators generally reduces scar formation. Collectively, these data support the idea that targeting inflammation could be useful for limiting scar formation. Indeed, there are several studies, mainly using animal models, that show anti-inflammatory compounds reduce scar formation, and several other potential anti-scar treatment modalities have documented anti-inflammatory effects.

Despite the large number of studies on inflammation and scar formation, more research is needed to fully understand this relationship. Conflicting data from some studies demonstrates the complexity of the relationship between inflammation and scar formation and highlights the lack of standardized approaches for studying this relationship experimentally. In particular, variability in experimental models can make it difficult to draw broad conclusions; these models include the use of human tissue, large animal models (e.g., pigs, which have similar skin anatomy to human), and small animal models (e.g., mice, which have loose skin, but can be used for advanced functional studies). More precise characterization of inflammation that includes an expanded view of different inflammatory cell types as well as the specific subtypes of each cell and how changes in these cells over time affect scar formation are needed, especially in human specimens. In addition, further investigation is needed to determine how beneficial various anti-inflammatory approaches could be for minimizing scar formation in human skin.

## DECLARATIONS

### Authors' contributions

The author contributed solely to the article.

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

The author has received research support from NIH (AR071115).

### Conflicts of interest

The author declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.



## REFERENCES

1. Eming SA, Martin P, Tomic-Canic M. Wound repair and regeneration: mechanisms, signaling, and translation. *Sci Transl Med* 2014;6:265sr6.
2. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. *Nature* 2008;453:314-21.
3. Martin P. Wound healing--aiming for perfect skin regeneration. *Science* 1997;276:75-81.
4. Aarabi S, Longaker MT, Gurtner GC. Hypertrophic scar formation following burns and trauma: new approaches to treatment. *PLoS Med* 2007;4:e234.
5. Corr DT, Gallant-Behm CL, Shrive NG, Hart DA. Biomechanical behavior of scar tissue and uninjured skin in a porcine model. *Wound Repair Regen* 2009;17:250-9.
6. Dunn MG, Silver FH, Swann DA. Mechanical analysis of hypertrophic scar tissue: structural basis for apparent increased rigidity. *J Invest Dermatol* 1985;84:9-13.
7. Brown BC, McKenna SP, Siddhi K, McGrouther DA, Bayat A. The hidden cost of skin scars: quality of life after skin scarring. *J Plast Reconstr Aesthet Surg* 2008;61:1049-58.
8. Wilgus TA. Immune cells in the healing skin wound: influential players at each stage of repair. *Pharmacol Res* 2008;58:112-6.
9. Carlsson AH, Rose LF, Fletcher JL, Wu JC, Leung KP, et al. Antecedent thermal injury worsens split-thickness skin graft quality: a clinically relevant porcine model of full-thickness burn, excision and grafting. *Burns* 2017;43:223-31.
10. Jabeen S, Clough ECS, Thomlinson AM, Chadwick SL, Ferguson MWJ, et al. Partial thickness wound: does mechanism of injury influence healing? *Burns* 2019;45:531-42.
11. Morris MW Jr, Allukian M 3rd, Herdrich BJ, Caskey RC, Zgheib C, et al. Modulation of the inflammatory response by increasing fetal wound size or interleukin-10 overexpression determines wound phenotype and scar formation. *Wound Repair Regen* 2014;22:406-14.
12. Dunkin CS, Pleat JM, Gillespie PH, Tyler MP, Roberts AH, et al. Scarring occurs at a critical depth of skin injury: precise measurement in a graduated dermal scratch in human volunteers. *Plast Reconstr Surg* 2007;119:1722-32; discussion 1733-4.
13. Qian LW, Fourcaudot AB, Yamane K, You T, Chan RK, et al. Exacerbated and prolonged inflammation impairs wound healing and increases scarring. *Wound Repair Regen* 2016;24:26-34.
14. Zhang Q, Yamaza T, Kelly AP, Shi S, Wang S, et al. Tumor-like stem cells derived from human keloid are governed by the inflammatory niche driven by IL-17/IL-6 axis. *PLoS One* 2009;4:e7798.
15. Zhang M, Xu Y, Liu Y, Cheng Y, Zhao P, et al. Chemokine-like factor 1 (CKLF-1) is overexpressed in keloid patients: a potential indicating factor for keloid-predisposed individuals. *Medicine (Baltimore)* 2016;95:e3082.
16. Jumper N, Hodgkinson T, Paus R, Bayat A. Site-specific gene expression profiling as a novel strategy for unravelling keloid disease pathobiology. *PLoS One* 2017;12:e0172955.
17. Dong X, Zhang C, Ma S, Wen H. Mast cell chymase in keloid induces profibrotic response via transforming growth factor-beta1/Smad activation in keloid fibroblasts. *Int J Clin Exp Pathol* 2014;7:3596-607.
18. Craig SS, DeBlois G, Schwartz LB. Mast cells in human keloid, small intestine, and lung by an immunoperoxidase technique using a murine monoclonal antibody against tryptase. *Am J Pathol* 1986;124:427-35.
19. Boyce DE, Ciampolini J, Ruge F, Murison MS, Harding KG. Inflammatory-cell subpopulations in keloid scars. *Br J Plast Surg* 2001;54:511-6.
20. Jiao H, Fan J, Cai J, Pan B, Yan L, et al. Analysis of characteristics similar to autoimmune disease in keloid patients. *Aesthetic Plast Surg* 2015;39:818-25.
21. Shaker SA, Ayuob NN, Hajrah NH. Cell talk: a phenomenon observed in the keloid scar by immunohistochemical study. *Appl Immunohistochem Mol Morphol* 2011;19:153-9.
22. Arbi S, Eksteen EC, Oberholzer HM, Taute H, Bester MJ. Premature collagen fibril formation, fibroblast-mast cell interactions and mast cell-mediated phagocytosis of collagen in keloids. *Ultrastruct Pathol* 2015;39:95-103.
23. Bagabir R, Byers RJ, Chaudhry IH, Müller W, Paus R, et al. Site-specific immunophenotyping of keloid disease demonstrates immune upregulation and the presence of lymphoid aggregates. *Br J Dermatol* 2012;167:1053-66.
24. Novak ML, Koh TJ. Macrophage phenotypes during tissue repair. *J Leukoc Biol* 2013;93:875-81.
25. Theoret CL, Olutoye OO, Parnell LK, Hicks J. Equine exuberant granulation tissue and human keloids: a comparative histopathologic study. *Vet Surg* 2013;42:783-9.
26. Gaber MA, Seliet IA, Ehsan NA, Megahed MA. Mast cells and angiogenesis in wound healing. *Anal Quant Cytopathol Histopathol* 2014;36:32-40.
27. Hellström M, Hellström S, Engström-Laurent A, Bertheim U. The structure of the basement membrane zone differs between keloids, hypertrophic scars and normal skin: a possible background to an impaired function. *J Plast Reconstr Aesthet Surg* 2014;67:1564-72.
28. Aarabi S, Bhatt KA, Shi Y, Paterno J, Chang EI, et al. Mechanical load initiates hypertrophic scar formation through decreased cellular apoptosis. *FASEB J* 2007;21:3250-61.
29. Wong VW, Paterno J, Sorkin M, Glotzbach JP, Levi K, et al. Mechanical force prolongs acute inflammation via T-cell-dependent pathways during scar formation. *FASEB J* 2011;25:4498-510.
30. Wang J, Ding J, Jiao H, Honardoust D, Momtazi M, et al. Human hypertrophic scar-like nude mouse model: characterization of the molecular and cellular biology of the scar process. *Wound Repair Regen* 2011;19:274-85.
31. Zhu Z, Ding J, Ma Z, Iwashina T, Tredget EE. The natural behavior of mononuclear phagocytes in HTS formation. *Wound Repair Regen* 2016;24:14-25.
32. Ibrahim MM, Bond J, Bergeron A, Miller KJ, Ehanire T, et al. A novel immune competent murine hypertrophic scar contracture model: a

- tool to elucidate disease mechanism and develop new therapies. *Wound Repair Regen* 2014;22:755-64.
33. Harunari N, Zhu KQ, Armendariz RT, Deubner H, Muangman P, et al. Histology of the thick scar on the female, red Duroc pig: final similarities to human hypertrophic scar. *Burns* 2006;32:669-77.
  34. Kischer CW, Bunce H 3rd, Shetlah MR. Mast cell analyses in hypertrophic scars, hypertrophic scars treated with pressure and mature scars. *J Invest Dermatol* 1978;70:355-7.
  35. Beer TW, Baldwin H, West L, Gallagher PJ, Wright DH. Mast cells in pathological and surgical scars. *Br J Ophthalmol* 1998;82:691-4.
  36. Niessen FB, Schalkwijk J, Vos H, Timens W. Hypertrophic scar formation is associated with an increased number of epidermal Langerhans cells. *J Pathol* 2004;202:121-9.
  37. van den Broek LJ, van der Veer WM, de Jong EH, Gibbs S, Niessen FB. Suppressed inflammatory gene expression during human hypertrophic scar compared to normotrophic scar formation. *Exp Dermatol* 2015;24:623-9.
  38. Butzelaar L, Schooneman DP, Soykan EA, Talhout W, Ulrich MM, et al. Inhibited early immunologic response is associated with hypertrophic scarring. *Exp Dermatol* 2016;25:797-804.
  39. Moore AL, Marshall CD, Barnes LA, Murphy MP, Ransom RC, et al. Scarless wound healing: transitioning from fetal research to regenerative healing. *Wiley Interdiscip Rev Dev Biol* 2018;7:e309.
  40. Wilgus TA. Regenerative healing in fetal skin: a review of the literature. *Ostomy Wound Manage* 2007;53:16-31.
  41. Wilgus TA. Fetal wound healing. In: Bagchi D, Das A, Roy S, editors. *Wound healing, tissue repair, and regeneration in diabetes*. Cambridge, MA: Academic Press; 2020. pp. 579-91.
  42. Armstrong JR, Ferguson MW. Ontogeny of the skin and the transition from scar-free to scarring phenotype during wound healing in the pouch young of a marsupial, *Monodelphis domestica*. *Dev Biol* 1995;169:242-60.
  43. Kumta S, Ritz M, Hurley JV, Crowe D, Romeo R, et al. Acute inflammation in foetal and adult sheep: the response to subcutaneous injection of turpentine and carrageenan. *Br J Plast Surg* 1994;47:360-8.
  44. Wilgus TA, Bergdall VK, Tober KL, Hill KJ, Mitra S, et al. The impact of cyclooxygenase-2 mediated inflammation on scarless fetal wound healing. *Am J Pathol* 2004;165:753-61.
  45. Liechty KW, Adzick NS, Crombleholme TM. Diminished interleukin 6 (IL-6) production during scarless human fetal wound repair. *Cytokine* 2000;12:671-6.
  46. Liechty KW, Crombleholme TM, Cass DL, Martin B, Adzick NS. Diminished interleukin-8 (IL-8) production in the fetal wound healing response. *J Surg Res* 1998;77:80-4.
  47. Wulff BC, Pappa NK, Wilgus TA. Interleukin-33 encourages scar formation in murine fetal skin wounds. *Wound Repair Regen* 2019;27:19-28.
  48. Dardenne AD, Wulff BC, Wilgus TA. The alarmin HMGB-1 influences healing outcomes in fetal skin wounds. *Wound Repair Regen* 2013;21:282-91.
  49. Gordon A, Kozin ED, Keswani SG, Vaikunth SS, Katz AB, et al. Permissive environment in postnatal wounds induced by adenoviral-mediated overexpression of the anti-inflammatory cytokine interleukin-10 prevents scar formation. *Wound Repair Regen* 2008;16:70-9.
  50. Wulff BC, Yu L, Parent AE, Wilgus TA. Novel differences in the expression of inflammation-associated genes between mid- and late-gestational dermal fibroblasts. *Wound Repair Regen* 2013;21:103-12.
  51. Olutoye OO, Alaish SM, Carr ME, Paik M, Yager DR, et al. Aggregatory characteristics and expression of the collagen adhesion receptor in fetal porcine platelets. *J Pediatr Surg* 1995;30:1649-53.
  52. Olutoye O, Yager D, Cohen I, Diegelmann R. Lower cytokine release by fetal porcine platelets: a possible explanation for reduced inflammation after fetal wounding. *J Pediatr Surg* 1996;31:91-5.
  53. Wulff BC, Parent AE, Meleski MA, DiPietro LA, Schrementi ME, et al. Mast cells contribute to scar formation during fetal wound healing. *J Invest Dermatol* 2012;132:458-65.
  54. Wulff BC, Wilgus TA. Examining the role of mast cells in fetal wound healing using cultured cells *in vitro*. In: Gourdie RG, Myers TA, editors. *Wound regeneration and repair*. Totowa: Humana Press; 2013. pp. 495-506.
  55. Cowin A, Brosnan M, Holmes T, Ferguson M. Endogenous inflammatory response to dermal wound healing in the fetal and adult mouse. *Dev Dyn* 1998;212:385-93.
  56. Hopkinson-Woolley J, Hughes D, Gordon S, Martin P. Macrophage recruitment during limb development and wound healing in the embryonic and foetal mouse. *J Cell Sci* 1994;107 (Pt 5):1159-67.
  57. Burrington JD. Wound healing in the fetal lamb. *J Pediatr Surg* 1971;6:523-8.
  58. Naik-Mathuria B, Gay AN, Yu L, Hsu JE, Smith CW, et al. Fetal wound healing using a genetically modified murine model: the contribution of P-selectin. *J Pediatr Surg* 2008;43:675-82.
  59. Olutoye OO, Zhu X, Cass DL, Smith CW. Neutrophil recruitment by fetal porcine endothelial cells: implications in scarless fetal wound healing. *Pediatr Res* 2005;58:1290-4.
  60. Rowlatt U. Intrauterine wound healing in a 20 week human fetus. *Virchows Arch A Pathol Anat Histol* 1979;381:353-61.
  61. Walraven M, Talhout W, Beelen RH, van Egmond M, Ulrich MM. Healthy human second-trimester fetal skin is deficient in leukocytes and associated homing chemokines. *Wound Repair Regen* 2016;24:533-41.
  62. Wong JW, Gallant-Behm C, Wiebe C, Mak K, Hart DA, et al. Wound healing in oral mucosa results in reduced scar formation as compared with skin: evidence from the red Duroc pig model and humans. *Wound Repair Regen* 2009;17:717-29.
  63. Szpaderska AM, Zuckerman JD, DiPietro LA. Differential injury responses in oral mucosal and cutaneous wounds. *J Dent Res* 2003;82:621-6.
  64. Mak K, Manji A, Gallant-Behm C, Wiebe C, Hart DA, et al. Scarless healing of oral mucosa is characterized by faster resolution of

- inflammation and control of myofibroblast action compared to skin wounds in the red Duroc pig model. *J Dermatol Sci* 2009;56:168-80.
65. Glim JE, Beelen RH, Niessen FB, Everts V, Ulrich MM. The number of immune cells is lower in healthy oral mucosa compared to skin and does not increase after scarring. *Arch Oral Biol* 2015;60:272-81.
66. Chen L, Arbueva ZH, Guo S, Marucha PT, Mustoe TA, et al. Positional differences in the wound transcriptome of skin and oral mucosa. *BMC Genomics* 2010;11:471.
67. Iglesias-Bartolome R, Uchiyama A, Molinolo AA, Abusleme L, Brooks SR, et al. Transcriptional signature primes human oral mucosa for rapid wound healing. *Sci Transl Med* 2018;10:eaap8798.
68. Seifert AW, Kiama SG, Seifert MG, Goheen JR, Palmer TM, et al. Skin shedding and tissue regeneration in African spiny mice (*Acomys*). *Nature* 2012;489:561-5.
69. Brant JO, Lopez MC, Baker HV, Barbazuk WB, Maden M. A comparative analysis of gene expression profiles during skin regeneration in *Mus* and *Acomys*. *PLoS One* 2015;10:e0142931.
70. Brant JO, Yoon JH, Polvadore T, Barbazuk WB, Maden M. Cellular events during scar-free skin regeneration in the spiny mouse, *Acomys*. *Wound Repair Regen* 2016;24:75-88.
71. Dabrowski R, Drobnik J. The effect of disodium cromoglycate on the skin wound healing and collagen content in the wounds of rats. *Acta Physiol Pol* 1990;41:195-8.
72. Chen L, Schrementi ME, Ranzer MJ, Wilgus TA, DiPietro LA. Blockade of mast cell activation reduces cutaneous scar formation. *PLoS One* 2014;9:e85226.
73. Gallant-Behm CL, Hildebrand KA, Hart DA. The mast cell stabilizer ketotifen prevents development of excessive skin wound contraction and fibrosis in red Duroc pigs. *Wound Repair Regen* 2008;16:226-33.
74. Younan GJ, Heit YI, Dastouri P, Kekhia H, Xing W, et al. Mast cells are required in the proliferation and remodeling phases of microdeformational wound therapy. *Plast Reconstr Surg* 2011;128:649e-58.
75. Shiota N, Nishikori Y, Kakizoe E, Shimoura K, Niibayashi T, et al. Pathophysiological role of skin mast cells in wound healing after scald injury: study with mast cell-deficient W/W(V) mice. *Int Arch Allergy Immunol* 2010;151:80-8.
76. Antsiferova M, Martin C, Huber M, Feyerabend TB, Förster A, et al. Mast cells are dispensable for normal and activin-promoted wound healing and skin carcinogenesis. *J Immunol* 2013;191:6147-55.
77. Nauta AC, Grova M, Montoro DT, Zimmermann A, Tsai M, et al. Evidence that mast cells are not required for healing of splinted cutaneous excisional wounds in mice. *PLoS One* 2013;8:e59167.
78. Willenborg S, Eckes B, Brinckmann J, Krieg T, Waisman A, et al. Genetic ablation of mast cells redefines the role of mast cells in skin wound healing and bleomycin-induced fibrosis. *J Invest Dermatol* 2014;134:2005-15.
79. Wulff BC, Wilgus TA. Mast cell activity in the healing wound: more than meets the eye? *Exp Dermatol* 2013;22:507-10.
80. Martin P, D'souza D, Martin J, Grose R, Cooper L, et al. Wound healing in the PU.1 null mouse-tissue repair is not dependent on inflammatory cells. *Curr Biol* 2003;13:1122-8.
81. Chrysanthopoulou A, Mitroulis I, Apostolidou E, Arelaki S, Mikroulis D, et al. Neutrophil extracellular traps promote differentiation and function of fibroblasts. *J Pathol* 2014;233:294-307.
82. Mirza R, DiPietro LA, Koh TJ. Selective and specific macrophage ablation is detrimental to wound healing in mice. *Am J Pathol* 2009;175:2454-62.
83. Lucas T, Waisman A, Ranjan R, Roes J, Krieg T, et al. Differential roles of macrophages in diverse phases of skin repair. *J Immunol* 2010;184:3964-77.
84. Rodero MP, Legrand JM, Bou-Gharios G, Khosrotehrani K. Wound-associated macrophages control collagen 1 $\alpha$ 2 transcription during the early stages of skin wound healing. *Exp Dermatol* 2013;22:143-5.
85. Zhu Z, Ding J, Ma Z, Iwashina T, Tredget EE. Systemic depletion of macrophages in the subacute phase of wound healing reduces hypertrophic scar formation. *Wound Repair Regen* 2016;24:644-56.
86. Shook BA, Wasko RR, Rivera-Gonzalez GC, Salazar-Gatzimas E, López-Giráldez F, et al. Myofibroblast proliferation and heterogeneity are supported by macrophages during skin repair. *Science* 2018;362:eaar2971.
87. Sinha M, Sen CK, Singh K, Das A, Ghatak S, et al. Direct conversion of injury-site myeloid cells to fibroblast-like cells of granulation tissue. *Nat Commun* 2018;9:936.
88. Jeong W, Yang CE, Roh TS, Kim JH, Lee JH, et al. Scar prevention and enhanced wound healing induced by polydeoxyribonucleotide in a rat incisional wound-healing model. *Int J Mol Sci* 2017;18:1698.
89. Wilgus TA, Vodovotz Y, Vittadini E, Clubbs EA, Oberyshyn TM. Reduction of scar formation in full-thickness wounds with topical celecoxib treatment. *Wound Repair Regen* 2003;11:25-34.
90. Xu W, Hong SJ, Zeitchek M, Cooper G, Jia S, et al. Hydration status regulates sodium flux and inflammatory pathways through epithelial sodium channel (ENaC) in the skin. *J Invest Dermatol* 2015;135:796-806.
91. Zhang J, Qiao Q, Liu M, He T, Shi J, et al. IL-17 promotes scar formation by inducing macrophage infiltration. *Am J Pathol* 2018;188:1693-702.
92. Ferreira AM, Takagawa S, Fresco R, Zhu X, Varga J, et al. Diminished induction of skin fibrosis in mice with MCP-1 deficiency. *J Invest Dermatol* 2006;126:1900-8.
93. Wong VW, Rustad KC, Akaishi S, Sorkin M, Glotzbach JP, et al. Focal adhesion kinase links mechanical force to skin fibrosis via inflammatory signaling. *Nat Med* 2011;18:148-52.
94. Cooper L, Johnson C, Burslem F, Martin P. Wound healing and inflammation genes revealed by array analysis of 'macrophageless' PU.1 null mice. *Genome Biol* 2005;6:R5.

95. Mori R, Shaw TJ, Martin P. Molecular mechanisms linking wound inflammation and fibrosis: knockdown of osteopontin leads to rapid repair and reduced scarring. *J Exp Med* 2008;205:43-51.
96. Liechty KW, Kim HB, Adzick NS, Crombleholme TM. Fetal wound repair results in scar formation in interleukin-10-deficient mice in a syngeneic murine model of scarless fetal wound repair. *J Pediatr Surg* 2000;35:866-72; discussion 872-3.
97. Kieran I, Knock A, Bush J, So K, Metcalfe A, et al. Interleukin-10 reduces scar formation in both animal and human cutaneous wounds: results of two preclinical and phase II randomized control studies. *Wound Repair Regen* 2013;21:428-36.
98. Wise LM, Stuart GS, Jones NC, Fleming SB, Mercer AA. Orf virus IL-10 and VEGF-E act synergistically to enhance healing of cutaneous wounds in mice. *J Clin Med* 2020;9:1085.
99. Wise LM, Stuart GS, Real NC, Fleming SB, Mercer AA. Orf virus IL-10 accelerates wound healing while limiting inflammation and scarring. *Wound Repair Regen* 2014;22:356-67.
100. Balaji S, King A, Marsh E, LeSaint M, Bhattacharya SS, et al. The role of interleukin-10 and hyaluronan in murine fetal fibroblast function in vitro: implications for recapitulating fetal regenerative wound healing. *PLoS One* 2015;10:e0124302.
101. Balaji S, Wang X, King A, Le LD, Bhattacharya SS, et al. Interleukin-10-mediated regenerative postnatal tissue repair is dependent on regulation of hyaluronan metabolism via fibroblast-specific STAT3 signaling. *FASEB J* 2017;31:868-81.
102. King A, Balaji S, Le LD, Marsh E, Crombleholme TM, et al. Interleukin-10 regulates fetal extracellular matrix hyaluronan production. *J Pediatr Surg* 2013;48:1211-7.
103. King A, Balaji S, Marsh E, Le LD, Shaaban AF, et al. Interleukin-10 regulates the fetal hyaluronan-rich extracellular matrix via a STAT3-dependent mechanism. *J Surg Res* 2013;184:671-7.
104. Caskey RC, Allukian M, Lind RC, Herdrich BJ, Xu J, et al. Lentiviral-mediated over-expression of hyaluronan synthase-1 (HAS-1) decreases the cellular inflammatory response and results in regenerative wound repair. *Cell Tissue Res* 2013;351:117-25.
105. Estes JM, Scott Adzick N, Harrison MR, Longaker MT, Stern R. Hyaluronate metabolism undergoes and ontogenic transition during fetal development: Implications for Scar-free wound healing. *J Pediatr Surg* 1993;28:1227-31.
106. Longaker MT, Scott Adzick N, Hall JL, Stair SE, Crombleholme TM, et al. Studies in fetal wound healing. VII. Fetal wound healing may be modulated by hyaluronic acid stimulating activity in amniotic fluid. *J Pediatr Surg* 1990;25:430-3.
107. Longaker MT, Chiu ES, Adzick NS, Stern M, Harrison MR, et al. Studies in fetal wound healing. V. A prolonged presence of hyaluronic acid characterizes fetal wound fluid. *Ann Surg* 1991;213:292-6.
108. Longaker MT, Chiu ES, Harrison MR, Crombleholme TM, Langer JC, et al. Studies in fetal wound healing. IV. Hyaluronic acid-stimulating activity distinguishes fetal wound fluid from adult wound fluid. *Ann Surg* 1989;210:667-72.
109. Longaker MT, Harrison MR, Crombleholme TM, Langer JC, Decker M, et al. Studies in fetal wound healing: I. A factor in fetal serum that stimulates deposition of hyaluronic acid. *J Pediatr Surg* 1989;24:789-92.
110. West DC, Shaw DM, Lorenz P, Adzick NS, Longaker MT. Fibrotic healing of adult and late gestation fetal wounds correlates with increased hyaluronidase activity and removal of hyaluronan. *Int J Biochem Cell Biol* 1997;29:201-10.
111. Yates CC, Krishna P, Whaley D, Bodnar R, Turner T, et al. Lack of CXC chemokine receptor 3 signaling leads to hypertrophic and hypercellular scarring. *Am J Pathol* 2010;176:1743-55.
112. Cash JL, Bass MD, Campbell J, Barnes M, Kubes P, et al. Resolution mediator chemerin15 reprograms the wound microenvironment to promote repair and reduce scarring. *Curr Biol* 2014;24:1406-14.
113. Sidgwick GP, McGeorge D, Bayat A. A comprehensive evidence-based review on the role of topicals and dressings in the management of skin scarring. *Arch Dermatol Res* 2015;307:461-77.
114. Amini-Nik S, Yousuf Y, Jeschke MG. Scar management in burn injuries using drug delivery and molecular signaling: Current treatments and future directions. *Adv Drug Deliv Rev* 2018;123:135-54.
115. Ogawa R, Akita S, Akaishi S, Aramaki-Hattori N, Dohi T, et al. Diagnosis and treatment of keloids and hypertrophic scars-japan scar workshop consensus document 2018. *Burns Trauma* 2019;7:39.
116. Roques C, Téot L. The use of corticosteroids to treat keloids: a review. *Int J Low Extrem Wounds* 2008;7:137-45.
117. Kim DY, Han YS, Kim SR, Chun BK, Park JH. Effects of a topical angiotensin-converting enzyme inhibitor and a selective COX-2 inhibitor on the prevention of hypertrophic scarring in the skin of a rabbit ear. *Wounds* 2012;24:356-64.
118. Yuan Z, Zhao J, Chen Y, Yang Z, Cui W, et al. Regulating inflammation using acid-responsive electrospun fibrous scaffolds for skin scarless healing. *Mediators Inflamm* 2014;2014:858045.
119. Rahmani-Neishaboore E, Jallili R, Hartwell R, Leung V, Carr N, et al. Topical application of a film-forming emulgel dressing that controls the release of stratifin and acetylsalicylic acid and improves/prevents hypertrophic scarring. *Wound Repair Regen* 2013;21:55-65.
120. Medina A, Ghaffari A, Kilani RT, Ghahary A. The role of stratifin in fibroblast-keratinocyte interaction. *Mol Cell Biochem* 2007;305:255-64.
121. Rahmani-Neishaboore E, Yau FM, Jallili R, Kilani RT, Ghahary A. Improvement of hypertrophic scarring by using topical anti-fibrogenic/anti-inflammatory factors in a rabbit ear model. *Wound Repair Regen* 2010;18:401-8.
122. Wilgus TA. Alerting the body to tissue injury: the role of alarmins and DAMPs in cutaneous wound healing. *Curr Pathobiol Rep* 2018;6:55-60.
123. Wang J, Hori K, Ding J, Huang Y, Kwan P, et al. Toll-like receptors expressed by dermal fibroblasts contribute to hypertrophic scarring. *J Cell Physiol* 2011;226:1265-73.
124. Li XP, Liu P, Li YF, Zhang GL, Zeng DS, et al. LPS induces activation of the TLR4 pathway in fibroblasts and promotes skin scar formation through collagen I and TGF-beta in skin lesions. *Int J Clin Exp Pathol* 2019;12:2121-9.
125. Bhattacharyya S, Tamaki Z, Wang W, Hinchcliff M, Hoover P, et al. Fibronectin/EDA promotes chronic cutaneous fibrosis through Toll-



- like receptor signaling. *Sci Transl Med* 2014;6:232ra50.
126. Bhattacharyya S, Wang W, Qin W, Cheng K, Coulup S, et al. TLR4-dependent fibroblast activation drives persistent organ fibrosis in skin and lung. *JCI Insight* 2018;3:98850.
127. Bhattacharyya S, Wang W, Tamaki Z, Shi B, Yeldandi A, et al. Pharmacological inhibition of Toll-like receptor-4 signaling by TAK242 prevents and induces regression of experimental organ fibrosis. *Front Immunol* 2018;9:2434.
128. Blohm-Mangone K, Burkett NB, Tahsin S, Myrdal PB, Aodah A, et al. Pharmacological TLR4 antagonism using topical resatorvid blocks solar UV-induced skin tumorigenesis in SKH-1 mice. *Cancer Prev Res (Phila)* 2018;11:265-78.
129. Janda J, Burkett NB, Blohm-Mangone K, Huang V, Curiel-Lewandrowski C, et al. Resatorvid-based pharmacological antagonism of cutaneous TLR4 blocks UV-induced NF- $\kappa$ B and AP-1 signaling in keratinocytes and mouse skin. *Photochem Photobiol* 2016;92:816-25.
130. Rice TW, Wheeler AP, Bernard GR, Vincent JL, Angus DC, et al. A randomized, double-blind, placebo-controlled trial of TAK-242 for the treatment of severe sepsis. *Crit Care Med* 2010;38:1685-94.
131. Ding J, Hori K, Zhang R, Marcoux Y, Honardoust D, et al. Stromal cell-derived factor 1 (SDF-1) and its receptor CXCR4 in the formation of postburn hypertrophic scar (HTS). *Wound Repair Regen* 2011;19:568-78.
132. Liu H, Ding J, Ma Z, Zhu Z, Shankowsky HA, et al. A novel subpopulation of peripheral blood mononuclear cells presents in major burn patients. *Burns* 2015;41:998-1007.
133. Shin JU, Kim SH, Kim H, Noh JY, Jin S, et al. TSLP is a potential initiator of collagen synthesis and an activator of CXCR4/SDF-1 axis in keloid pathogenesis. *J Invest Dermatol* 2016;136:507-15.
134. Hall CL, Wells AR, Leung KP. Pirfenidone reduces profibrotic responses in human dermal myofibroblasts, in vitro. *Lab Invest* 2018;98:640-55.
135. Saito M, Yamazaki M, Maeda T, Matsumura H, Setoguchi Y, et al. Pirfenidone suppresses keloid fibroblast-embedded collagen gel contraction. *Arch Dermatol Res* 2012;304:217-22.
136. Wells AR, Leung KP. Pirfenidone attenuates the profibrotic contractile phenotype of differentiated human dermal myofibroblasts. *Biochem Biophys Res Commun* 2020;521:646-51.
137. Satish L, Evdokiou A, Geletu E, Hahn JM, Supp DM. Pirfenidone inhibits epithelial-mesenchymal transition in keloid keratinocytes. *Burns Trauma* 2020;8:tkz007.
138. Dorati R, Medina JL, DeLuca PP, Leung KP. Development of a topical 48-H release formulation as an anti-scarring treatment for deep partial-thickness burns. *AAPS PharmSciTech* 2018;19:2264-75.
139. Medina JL, Sebastian EA, Fourcaudot AB, Dorati R, Leung KP. Pirfenidone ointment modulates the burn wound bed in c57bl/6 mice by suppressing inflammatory responses. *Inflammation* 2019;42:45-53.
140. Armendariz-Borunda J, Lyra-Gonzalez I, Medina-Preciado D, Gonzalez-García I, Martinez-Fong D, et al. A controlled clinical trial with pirfenidone in the treatment of pathological skin scarring caused by burns in pediatric patients. *Ann Plast Surg* 2012;68:22-8.
141. Afaq F, Adhami VM, Ahmad N, Mukhtar H. Inhibition of ultraviolet B-mediated activation of nuclear factor kappaB in normal human epidermal keratinocytes by green tea Constituent (-)-epigallocatechin-3-gallate. *Oncogene* 2003;22:1035-44.
142. Elmets CA, Singh D, Tubesing K, Matsui M, Katiyar S, et al. Cutaneous photoprotection from ultraviolet injury by green tea polyphenols. *J Am Acad Dermatol* 2001;44:425-32.
143. Gensler HL, Timmermann BN, Valcic S, Wächter GA, Dorr R, et al. Prevention of photocarcinogenesis by topical administration of pure epigallocatechin gallate isolated from green tea. *Nutr Cancer* 1996;26:325-35.
144. Katiyar SK, Mukhtar H. Green tea polyphenol (-)-epigallocatechin-3-gallate treatment to mouse skin prevents UVB-induced infiltration of leukocytes, depletion of antigen-presenting cells, and oxidative stress. *J Leukoc Biol* 2001;69:719-26.
145. Zhang Q, Kelly AP, Wang L, French SW, Tang X, et al. Green tea extract and (-)-epigallocatechin-3-gallate inhibit mast cell-stimulated type I collagen expression in keloid fibroblasts via blocking PI-3K/Akt signaling pathways. *J Invest Dermatol* 2006;126:2607-13.
146. Park G, Yoon BS, Moon JH, Kim B, Jun EK, et al. Green tea polyphenol epigallocatechin-3-gallate suppresses collagen production and proliferation in keloid fibroblasts via inhibition of the STAT3-signaling pathway. *J Invest Dermatol* 2008;128:2429-41.
147. Syed F, Bagabir RA, Paus R, Bayat A. Ex vivo evaluation of antifibrotic compounds in skin scarring: EGCG and silencing of PAI-1 independently inhibit growth and induce keloid shrinkage. *Lab Invest* 2013;93:946-60.
148. Ud-Din S, Foden P, Mazhari M, Al-Habba S, Baguneid M, et al. A double-blind, randomized trial shows the role of zonal priming and direct topical application of epigallocatechin-3-gallate in the modulation of cutaneous scarring in human skin. *J Invest Dermatol* 2019;139:1680-90.e16.
149. Soo C, Hu F, Zhang X, Wang Y, Beanes SR, et al. Differential expression of fibromodulin, a transforming growth factor- $\beta$  modulator, in fetal skin development and scarless repair. *The American Journal of Pathology* 2000;157:423-33.
150. Honardoust D, Varkey M, Hori K, Ding J, Shankowsky HA, et al. Small leucine-rich proteoglycans, decorin and fibromodulin, are reduced in postburn hypertrophic scar. *Wound Repair Regen* 2011;19:368-78.
151. Honardoust D, Varkey M, Marcoux Y, Shankowsky HA, Tredget EE. Reduced decorin, fibromodulin, and transforming growth factor- $\beta$ 3 in deep dermis leads to hypertrophic scarring. *J Burn Care Res* 2012;33:218-27.
152. Zheng Z, Lee KS, Zhang X, Nguyen C, Hsu C, et al. Fibromodulin-deficiency alters temporospatial expression patterns of transforming growth factor- $\beta$  ligands and receptors during adult mouse skin wound healing. *PLoS One* 2014;9:e90817.
153. Zheng Z, Zhang X, Dang C, Beanes S, Chang GX, et al. Fibromodulin is essential for fetal-type scarless cutaneous wound healing. *Am J Pathol* 2016;186:2824-32.
154. Zheng Z, Nguyen C, Zhang X, Khorasani H, Wang JZ, et al. Delayed wound closure in fibromodulin-deficient mice is associated with increased TGF- $\beta$ 3 signaling. *J Invest Dermatol* 2011;131:769-78.



155. Jiang W, Ting K, Lee S, Zara JN, Song R, et al. Fibromodulin reduces scar size and increases scar tensile strength in normal and excessive-mechanical-loading porcine cutaneous wounds. *J Cell Mol Med* 2018;22:2510-3.
156. Stoff A, Rivera AA, Mathis JM, Moore ST, Banerjee NS, et al. Effect of adenoviral mediated overexpression of fibromodulin on human dermal fibroblasts and scar formation in full-thickness incisional wounds. *J Mol Med (Berl)* 2007;85:481-96.
157. Pang X, Dong N, Zheng Z. Small leucine-rich proteoglycans in skin wound healing. *Front Pharmacol* 2019;10:1649.
158. Breuing K, Eriksson E, Liu P, Miller DR. Healing of partial thickness porcine skin wounds in a liquid environment. *J Surg Res* 1992;52:50-8.
159. Junker JP, Kamel RA, Caterson EJ, Eriksson E. Clinical impact upon wound healing and inflammation in moist, wet, and dry environments. *Adv Wound Care (New Rochelle)* 2013;2:348-56.
160. Reish RG, Zuhaili B, Bergmann J, Aflaki P, Koyama T, et al. Modulation of scarring in a liquid environment in the Yorkshire pig. *Wound Repair Regen* 2009;17:806-16.
161. Mustoe TA, Gurjala A. The role of the epidermis and the mechanism of action of occlusive dressings in scarring. *Wound Repair Regen* 2011;19 Suppl 1:s16-21.
162. Gallant-Behm CL, Mustoe TA. Occlusion regulates epidermal cytokine production and inhibits scar formation. *Wound Repair Regen* 2010;18:235-44.
163. Zhao J, Zhong A, Friedrich EE, Jia S, Xie P, et al. S100A12 induced in the epidermis by reduced hydration activates dermal fibroblasts and causes dermal fibrosis. *J Invest Dermatol* 2017;137:650-9.
164. Zhong A, Xu W, Zhao J, Xie P, Jia S, et al. S100A8 and S100A9 are induced by decreased hydration in the epidermis and promote fibroblast activation and fibrosis in the dermis. *Am J Pathol* 2016;186:109-22.
165. O'Shaughnessy KD, De La Garza M, Roy NK, Mustoe TA. Homeostasis of the epidermal barrier layer: a theory of how occlusion reduces hypertrophic scarring. *Wound Repair Regen* 2009;17:700-8.
166. Gurtner GC, Dauskardt RH, Wong VW, Bhatt KA, Wu K, et al. Improving cutaneous scar formation by controlling the mechanical environment: large animal and phase I studies. *Ann Surg* 2011;254:217-25.
167. Dohi T, Padmanabhan J, Akaishi S, Than PA, Terashima M, et al. The interplay of mechanical stress, strain, and stiffness at the keloid periphery correlates with increased caveolin-1/ROCK signaling and scar progression. *Plast Reconstr Surg* 2019;144:58e-67.
168. Pakshir P, Alizadehgiashi M, Wong B, Coelho NM, Chen X, et al. Dynamic fibroblast contractions attract remote macrophages in fibrillar collagen matrix. *Nat Commun* 2019;10:1850.
169. Lim AF, Weintraub J, Kaplan EN, Januszyk M, Cowley C, et al. The embrace device significantly decreases scarring following scar revision surgery in a randomized controlled trial. *Plast Reconstr Surg* 2014;133:398-405.
170. Longaker MT, Rohrich RJ, Greenberg L, Furnas H, Wald R, et al. A randomized controlled trial of the embrace advanced scar therapy device to reduce incisional scar formation. *Plast Reconstr Surg* 2014;134:536-46.
171. Januszyk M, Wong VW, Bhatt KA, Vial IN, Paterno J, et al. Mechanical offloading of incisional wounds is associated with transcriptional downregulation of inflammatory pathways in a large animal model. *Organogenesis* 2014;10:186-93.
172. Ma K, Kwon SH, Padmanabhan J, Duscher D, Trotsyuk AA, et al. Controlled delivery of a focal adhesion kinase inhibitor results in accelerated wound closure with decreased scar formation. *J Invest Dermatol* 2018;138:2452-60.

Case Report

Open Access



# Wolfring dacryops: a case of acquired ptosis in a child

Sammie E. Fung<sup>1</sup>, Clara J. Men<sup>2</sup>, Bobby S. Korn<sup>2,3</sup>, Don O. Kikkawa<sup>2,3</sup>, Catherine Y. Liu<sup>2</sup>

<sup>1</sup>University of California San Diego School of Medicine, La Jolla, CA 92037, USA.

<sup>2</sup>Division of Oculofacial Plastic and Reconstructive Surgery, UC San Diego Department of Ophthalmology, La Jolla, CA 92037, USA.

<sup>3</sup>Division of Plastic Surgery, UC San Diego Department of Surgery, La Jolla, CA 92037, USA.

**Correspondence to:** Dr. Catherine Y. Liu, Division of Oculofacial Plastic and Reconstructive Surgery, UC San Diego Department of Ophthalmology, La Jolla, CA 92037, USA. E-mail: yul107@health.ucsd.edu

**How to cite this article:** Fung SE, Men CJ, Korn BS, Kikkawa DO, Liu CY. Wolfring dacryops: a case of acquired ptosis in a child. *Plast Aesthet Res* 2020;7:55. <http://dx.doi.org/10.20517/2347-9264.2020.60>

**Received:** 31 Mar 2020 **First Decision:** 2 Sep 2020 **Revised:** 9 Sep 2020 **Accepted:** 29 Sep 2020 **Published:** 21 Oct 2020

**Academic Editor:** Raúl González-García **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

A healthy, nine-year-old boy presented to the oculofacial plastic service with left upper eyelid ptosis progressively worsening for the past two years. On eyelid eversion, a cystic mass was found on the medial palpebral conjunctiva. Magnetic resonance imaging confirmed a fluid-filled cystic structure without posterior orbital extension. Based on imaging and clinical findings, the patient was diagnosed with dacryops of the accessory lacrimal duct of the Wolfring gland. Although prior literature suggests that the risk of Wolfring dacryops may be associated with conjunctival scarring, this report presents a case of spontaneous Wolfring dacryops without history of ocular manipulation or inflammation. Small, asymptomatic cases of dacryops can be safely monitored with serial eye exams.

**Keywords:** Accessory gland, ductal cyst, lacrimal gland, Wolfring gland, dacryops

## INTRODUCTION

Blepharoptosis (or ptosis) is a common presenting complaint in the oculoplastics clinic and can be found amongst all age groups. In the adult population, acquired aponeurotic ptosis is the most common type, making up about 60% of cases in one study<sup>[1]</sup>. In children, congenital ptosis, a type of myogenic ptosis,



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** External front view of the patient, who presents with ptosis of the left upper eyelid



**Figure 2.** Eversion of the left upper eyelid revealed a bluish cystic mass

represents the majority of cases, making up about 79%-90% of pediatric cases<sup>[2-4]</sup>. Pediatric ptosis associated with amblyopia, refractive error, and strabismus warrants surgery<sup>[5,6]</sup>.

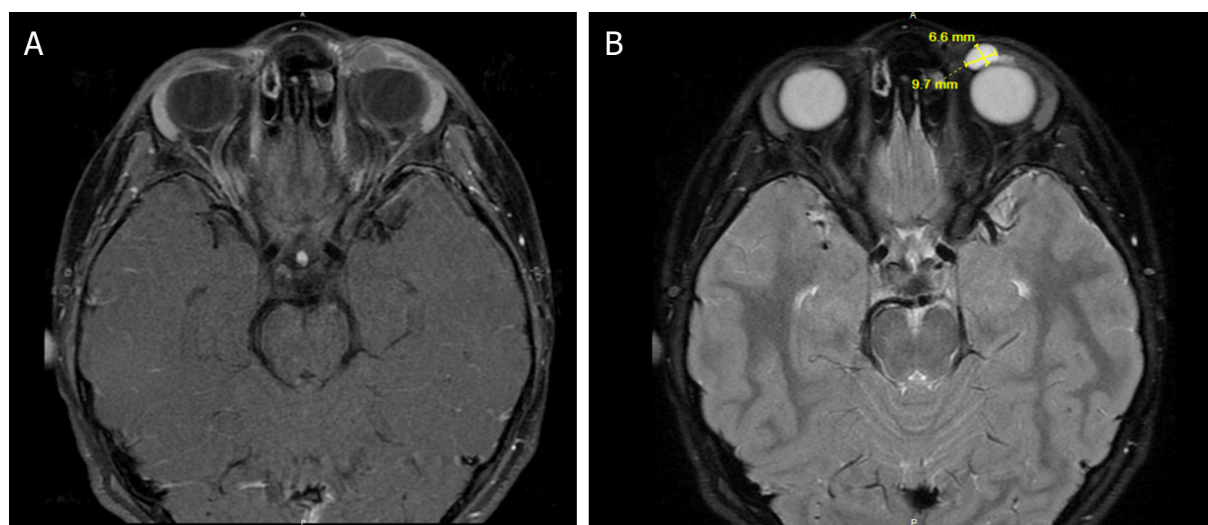
Acquired childhood ptosis is less common, making up the remainder of pediatric cases (10%-21%)<sup>[2,3]</sup>. Mechanical ptosis may result from masses, such as neurofibroma, capillary hemangioma, infiltration, inflammation, and foreign body, and may represent up to 2% of all pediatric cases<sup>[3]</sup>. Dacryops are cysts arising from the lacrimal ductules and can rarely present as an acquired mechanical ptosis<sup>[7]</sup>. Here, we report a patient with newly acquired mechanical ptosis due to a Wolfring dacryops (WD) with no known history of neurological, myogenic, or congenital deficits.

## CASE REPORT

A healthy nine-year old Asian male presented with new onset left upper eyelid ptosis and swelling gradually worsening over the last two years [Figure 1]. He denied pain, vision loss, diplopia, or discharge. He had no previous history of trauma, periocular inflammation, or prior surgeries.

On exam, his vision was 20/20 corrected OU. He had a margin reflex distance (MRD1) of 4.5 mm OD, 2 mm OS, a margin fold distance of 3 mm OD, 4.5 mm OS, a levator function of 15 mm OD and 13 mm OS, and no lagophthalmos. He had no proptosis or globe dystopia. Extraocular motility was full OU. There was no relative afferent pupillary defect. On palpation, there was a soft, non-tender, mobile mass of the medial left upper eyelid. Eversion of the upper lid revealed a cystic mass with a bluish hue [Figure 2]. The remainder of his exam was unremarkable.

To better characterize the mass, magnetic resonance imaging (MRI) with and without gadolinium enhancement was performed, which revealed an ovoid-shaped cystic lesion located in the left medial upper eyelid, measuring 10 mm × 7 mm × 8 mm [Figure 3]. The cyst was confined to the eyelid without posterior, orbital extension. The diagnosis of a ductal cyst of the accessory lacrimal gland (i.e., Wolfring dacryops) was made. Surgical excision was discussed but the patient and family opted to observe given the benign nature of the lesion.



**Figure 3.** Magnetic resonance imaging scan (axial view) reveals a T1 hypointense (A) and T2 hyperintense (B) fluid filled cystic structure in the left upper eyelid consistent with Wolfring dacryops

**Table 1. Summary table of previously reported cases of dacryops of the accessory lacrimal gland**<sup>[8,10,11,15-22]</sup>

Unilateral	48 out of 49 (98%)
Bilateral	1 out of 49 (2%)
Upper eyelid	35 out of 49 (71%)
Lower eyelid	14 out of 49 (29%)

## DISCUSSION

Dacryops is a closed ductal cyst arising from lacrimal gland tissue. It presents as a painless, well-circumscribed, translucent lesion often with a bluish tint, and it may be derived from the main or accessory lacrimal glands<sup>[8]</sup>. These cysts are commonly classified by their location, with palpebral lobe cysts of the main lacrimal gland (simple dacryops) being the most common and cysts of the accessory lacrimal glands of Krause and Wolfring being quite rare<sup>[9-11]</sup>. However, there are limited data on its incidence and prevalence. The majority of cases have been reported from Saudi Arabia, where the prevalence of cysts of the accessory lacrimal glands has been found to be between 1 in 6,800<sup>[10]</sup> and 1 in 7000<sup>[11]</sup>. The estimates in these reports are based on studies of patients seen in an oculoplastic clinic and may overestimate the prevalence of these lesions due to ascertainment bias.

Dacryops usually occur unilaterally<sup>[12,13]</sup>, but they have rarely been shown to occur bilaterally<sup>[14,15]</sup>. The upper eyelid is more commonly involved (70.8%) than the lower eyelid, likely due to the fact that there are more ducts in the upper eyelid<sup>[8]</sup>. In previously reported cases of dacryops of the accessory glands, the mean age of occurrence was 30.5 years (ranging from ages 2 to 81), without male/female predominance<sup>[8,10,11,15-22]</sup> [Table 1].

The etiology of WD is unclear, and it has been hypothesized that conjunctival scarring (from previous trauma, infection, or chronic inflammation) or IgA hypersecretion may contribute to its formation<sup>[10-12,21]</sup>. A review of the literature by Galindo-Ferreiro estimates that up to 85% of dacryops have been linked to previous conjunctival scarring<sup>[11]</sup>. In our patient's case, no predisposing cause was identified.

While congenital ptosis in children is more common, acquired ptosis is rare<sup>[2,23]</sup> and warrants a more detailed exam and history. Eversion of the eyelid made the clinical diagnosis of WD in this case, based on the mobile, non-tender mass located near the superior tarsal border. MRI findings can be supportive and

typically show a T2 hyperintense, T1 hypointense mass with enhancement of the wall after gadolinium consistent with a fluid filled cyst<sup>[8,24]</sup>. Histopathology of WD typically reveals a non-keratinizing, double layer of cuboidal to columnar epithelium<sup>[9,11,12,21]</sup>. Given the location of the cyst at the superior tarsal border, the cyst could potentially stretch or cause a dehiscence of the Muller's muscle underneath, which is a potential alternative mechanism for the ptosis<sup>[25]</sup>.

Complete surgical excision of the intact cysts is recommended for symptomatic lesions (large lesions, lesions that induce significant refractive error, symptomatic ptosis, disfigurement, or are potentially amblyogenic)<sup>[11,13,26]</sup>, but our patient's upper eyelid WD was asymptomatic, and his mild ptosis was not amblyogenic. However, ptosis in children can often cause amblyopia, and, as such, a referral to an ophthalmologist may be warranted. Dacryops is a benign entity, but complicated cases involving fistulization of the cyst, hemorrhage, and superimposed infection have been reported<sup>[9]</sup>. After a discussion of options, the patient and his family decided to monitor the lesion with serial eye exams.

This report describes a rare cause of mechanical ptosis in a child and illustrates the importance of a thorough eye exam in cases of acquired ptosis in childhood.

## **DECLARATIONS**

### **Acknowledgments**

The authors thank Research to Prevent Blindness (RPB) for their support of the UC San Diego Shiley Eye Center.

### **Authors' contributions**

Made substantial contributions to the conception and design of this case, contributed to the drafting and editing of the manuscript, and approved the final version to be published: Fung SE, Liu CY

Made substantial contributions to the drafting and editing of the manuscript, and approved the final version to be published: Men CJ, Korn BS, Kikkawa DO

### **Availability of data and materials**

Not applicable.

### **Financial support and sponsorship**

None.

### **Conflict of interest**

All authors declared that there are no conflicts of interest.

### **Ethical approval and consent to participate**

The case report adheres to the ethical principles outlined in the Declaration of Helsinki and is HIPAA compliant. The patient consented to participation of the clinic visit and consultation in accordance with the institution policy.

### **Consent for publication**

Consent was obtained from the patient for publication of images and details of the clinical case discussed.

### **Copyright**

© The Author(s) 2020.



## REFERENCES

1. Lim JM, Hou JH, Singa RM, Aakalu VK, Setabutr P. Relative incidence of blepharoptosis subtypes in an oculoplastics practice at a tertiary care center. *Orbit* 2013;32:231-4.
2. Griepentrog GJ, Diehl NN, Mohney BG. Incidence and demographics of childhood ptosis. *Ophthalmology* 2011;118:1180-3.
3. Lee V, Konrad H, Bunce C, Nelson C, Collin JR. Aetiology and surgical treatment of childhood blepharoptosis. *Br J Ophthalmol* 2002;86:1282-6.
4. Harrad RA, Graham CM, Collin JR. Amblyopia and strabismus in congenital ptosis. *Eye (Lond)* 1988;2:625-7.
5. Gillum WN, Anderson RL. Dominantly inherited blepharoptosis, high myopia, and ectopia lentis. *Arch Ophthalmol* 1982;100:282-4.
6. Oral Y, Ozgur OR, Akcay L, Ozbas M, Dogan OK. Congenital ptosis and amblyopia. *J Pediatr Ophthalmol Strabismus* 2010;47:101-4.
7. Salam A, Barrett AW, Malhotra R, Olver J. Marsupialization for lacrimal ductular cysts (dacryops): a case series. *Ophthalmic Plast Reconstr Surg* 2012;28:57-62.
8. Alsulaiman HM, Fatani DR, Al Sheikh O, Elkhamary S, Maktabi A, et al. Krause's accessory lacrimal gland dacryops - case report and literature review. *Orbit* 2020;1-5.
9. Bullock JD, Fleishman JA, Rosset JS. Lacrimal ductal cysts. *Ophthalmology* 1986;93:1355-60.
10. Weatherhead RG. Wolfring Dacryops. *Ophthalmology* 1992;99:1575-81.
11. Galindo-Ferreiro A, Alkatan HM, Muinos-Diaz Y, Akaishi PM, Galvez-Ruiz A, et al. Accessory lacrimal gland duct cyst: 23 years of experience in the Saudi population. *Ann Saudi Med* 2015;35:394-9.
12. Tanaboonyawat S, Idowu OO, Copperman TS, Vagefi MR, Kersten RC. Dacryops - a review. *Orbit* 2020;39:128-34.
13. Ozgonul C, Uysal Y, Ayyildiz O, Kucukevcilioglu M. Clinical features and management of dacryops. *Orbit* 2018;37:262-5.
14. Tsiouris AJ, Deshmukh M, Sanelli PC, Brazzo BG. Bilateral dacryops: correlation of clinical, radiologic, and histopathologic features. *AJR Am J Roentgenol* 2005;184:321-3.
15. Feijó ED, Alencastro Landim G, de Melo Dias M, Alves de Souza BA, Murillo Limongi R, et al. Giant bilateral cysts of the accessory lacrimal glands of Wolfring in a child. *Ophthalmic Plast Reconstr Surg* 2020;36:e4-6.
16. Durán JA, Cuevas J. Cyst of accessory lacrimal gland. *Br J Ophthalmol* 1983;67:485-6.
17. Remulla HD, Rubin PA. Giant dacryops in a patient with ocular cicatricial pemphigoid. *Br J Ophthalmol* 1995;79:1052-3.
18. Woo KI, Kim YD. Cyst of accessory lacrimal gland. *Korean J Ophthalmol* 1995;9:117-21.
19. O'Duffy D, Watts P. Wolfring dacryops and needling. *Acta Ophthalmol Scand* 1997;75:319.
20. Jastrzebski A, Brownstein S, Jordan DR, de Nanassy J. Dacryops of Krause gland in the inferior fornix in a child. *Arch Ophthalmol* 2012;130:252-4.
21. Lam K, Brownstein S, Jordan DR, Jastrzebski A. Dacryops: a series of 5 cases and a proposed pathogenesis. *JAMA Ophthalmol* 2013;131:929-32.
22. Khoury NJ, Haddad MC, Tawil AN, Ma'luf RN. Ductal cysts of the accessory lacrimal glands: CT findings. *Am J Neuroradiol* 1999;20:1140-2.
23. Rasiah S, Hardy TG, Elder JE, Ng CY, McNab A. Etiology of pediatric acquired blepharoptosis. *J AAPOS* 2017;21:485-7.
24. Jakobiec FA, Zakka FR, Perry LP. The cytologic composition of dacryops: an immunohistochemical investigation of 15 lesions compared to the normal lacrimal gland. *Am J Ophthalmol* 2013;155:380-96.e1.
25. Nakauchi K, Katori N, Imagawa Y, Yamada T. A case report on lacrimal ductal cyst causing unilateral blepharoptosis. *Br J Ophthalmol* 2009;93:1143-5.
26. Men CJ, Yang P, Gur Z, Paik JS, Kikkawa DO, et al. Complete excision of a simple dacryops using fibrin sealant and trypan blue mixture. *Ophthalmic Plast Reconstr Surg* 2019;35:e16-8.

Review

Open Access



# Current techniques in adult-acquired buried penis repair: where are we now

Katherine M. Theisen<sup>1</sup>, Ashley V. Alford<sup>1</sup>, Nicholas Kim<sup>2</sup>, Joseph J. Pariser<sup>1</sup>

<sup>1</sup>Department of Urology, University of Minnesota, Minneapolis, MN 55455, USA.

<sup>2</sup>Department of Plastic Surgery, University of Minnesota, Minneapolis, MN 55455, USA.

**Correspondence to:** Dr. Joseph J. Pariser, Department of Urology, University of Minnesota, Minneapolis, MN 55455, USA.  
E-mail: jpariser@umn.edu

**How to cite this article:** Theisen KM, Alford AV, Kim N, Pariser JJ. Current techniques in adult acquired buried penis repair: where are we now. *Plast Aesthet Res* 2020;7:56. <http://dx.doi.org/10.20517/2347-9264.2020.83>

**Received:** 16 Apr 2020 **First Decision:** 15 May 2020 **Revised:** 19 May 2020 **Accepted:** 19 Jun 2020 **Published:** 21 Oct 2020

**Academic Editor:** Marlon E. Buncamper, Stan J. Monstrey **Copy Editor:** Jing-Wen Zhang **Production Editor:** Jing Yu

## Abstract

Adult-acquired buried penis (AABP) is a condition associated with penile entrapment, penile shaft skin loss, and an enlarged pannus which engulfs the penis. The increased prevalence, awareness, and availability of surgical repair have led to a relative standardization in repairs. The surgical approach to AABP has evolved from a lengthy procedure with extended inpatient stay to one that may be done in an outpatient setting. The critical steps for surgical management of AABP have remained largely consistent over time, including: release of the penis with removal of diseased skin, suprapubic and/or abdominal panniculectomy, and skin coverage (usually with grafts). In contrast, the finer points of the procedure and perioperative care have undergone evolution. The aim of our approach was to optimize postoperative aesthetic and functional outcomes. Our perioperative management was modeled after enhanced recovery after surgery principles to minimize morbidity and expedite recovery. There remains room for improvement in the care of individuals with AABP, specifically multi-institutional collaboration, development of disease-specific outcome measures, and standardization of treatment algorithms.

**Keywords:** Buried penis, morbidity, surgical algorithms, postoperative period, quality of life

## INTRODUCTION

Individuals with adult-acquired buried penis (AABP) are increasingly seeking care due to the increasing rates of obesity in the United States<sup>[1]</sup> and to an increase in the number of centers that offer surgical



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** The condition of adult-acquired buried penis presents on a spectrum of severity

treatment for this condition. Early series describing surgical approaches in the management of AABP involved a relatively long procedure with prolonged postoperative bed rest and lengthy hospital stays<sup>[2-4]</sup>. “Success” of an operation was predominantly defined by surgeon-specific objective measures of success, while patient-related quality of life measures were not considered until more recently<sup>[5-7]</sup>. Much like many other operations in the field of urology (e.g., transurethral resection of the prostate, radical prostatectomy, and urethroplasty), the surgical approach to AABP has undergone a rapid evolution to minimize morbidity and expedite discharge from the hospital. However, some variation in treatment and postoperative care still exists. The purpose of this article is to describe the maturation of surgical approaches for this condition, describe our enhanced recovery after surgery (ERAS)-inspired approach for postoperative care, and make a plea for improved collaboration amongst surgeons to reduce the variability in care for this population.

## EVOLUTION OF DISEASE MANAGEMENT

The early approach to AABP management was modeled after the treatment of concealed penis in pediatric patients. This quickly evolved as surgeons began to realize that the etiology and anatomy of buried penis in adulthood differs from that seen in childhood<sup>[2,8]</sup>. It became increasingly apparent that aggressive surgical resection of diseased penile skin with suprapubic/abdominal lipectomy was needed to prevent re-burying<sup>[3,9,10]</sup>. Therefore, the surgical approach to AABP was modified to more closely resemble the surgical approach utilized for genital lymphedema that had been described as early as the 1800s and refined between the 1930s-1980s<sup>[11,12]</sup>.

With more experience treating this condition, surgeons have come to appreciate that AABP falls on a spectrum of severity [Figure 1] and that precise surgical techniques must be tailored to each individual patient. Several classification systems and treatment algorithms have been devised to promote a protocolized and stepwise approach to surgical intervention<sup>[13-15]</sup>. Despite variations across the different classification systems, the critical factors utilized to properly plan a surgical approach include: assessment of penile skin viability after it is released, determining the degree to which the escutcheon and/or pannus contribute to penile burying, and evaluation for concomitant scrotal lymphedema, which may need to be resected.

### Surgical steps - release of the buried penis

One feature that is commonly ascribed to the pathophysiology of AABP is abnormal attachments of the penile dartos fascia to Buck’s fascia such that penile skin - under downward pressure from abdominal adiposity - slides over the head of the penis. Therefore, early series focused on re-establishing the penopubic and penoscrotal angles after simply removing the cicatrix that trapped the penis; the rest of the penile skin was not resected. Angles were re-established using tacking sutures placed between the subdermis of the proximal penile skin and the tunica albuginea dorsally and ventrally<sup>[3,8]</sup>. However, there was some concern that leaving most of the penile skin behind was leading to high re-bury rates<sup>[3,14]</sup>. Therefore, it became



**Figure 2.** Remnant of healthy penile skin at the base of the penis with full-thickness graft covering the skin defect on the distal penile shaft

commonplace to remove all penile skin with the hope of achieving better long-term success<sup>[16,17]</sup>, similar to the approach for genital lymphedema<sup>[12]</sup>.

Penile skin defects were then covered with local skin flaps or grafts. Local skin flaps fell out of favor as scrotal rugae and hair bearing skin tended to result in poorer cosmetic outcomes<sup>[18]</sup>. Free skin grafts have long been a common adjunct to surgical correction of AABP. An early series in the plastic surgery literature utilized full thickness skin grafts from the abdominal pannus<sup>[10]</sup>. However, split-thickness skin grafts have become the modality of choice in the urologic literature<sup>[5,6,13,16,17,19-22]</sup>. Split-thickness skin grafts have the advantage of maximizing graft take rates, while being universally available (usually from the thigh). More recently, there has been renewed interest in full-thickness grafts because of the benefits of decreasing secondary graft contracture and improving cosmesis (both from a graft perspective and the ability to avoid a thigh scar by harvesting from the escutcheon)<sup>[23]</sup>.

Our approach has become a hybrid of multiple techniques. In our experience, we have found that preservation of some healthy penile skin can improve cosmetic outcomes. We save as much viable penile skin as possible and aggressively re-establish the penopubic and penoscrotal angles with suprapubic lipectomy and tacking sutures between the skin and base of the penis. We leave a “skin bridge” at the superior base of the penis which is tacked to the fascia overlying the pubic symphysis. Remaining penile skin defects are preferentially covered with full-thickness skin grafts from an area of the escutcheon or pannus specimen relatively devoid of hair [Figure 2].

We utilize split-thickness grafts when graft take is a concern or when there is too little skin available from the escutcheon/pannus specimen to obtain a full-thickness graft. We use fibrin sealant to aid graft take<sup>[24]</sup>. We cover penile skin grafts with a bolster dressing composed of a layer of Vaseline gauze, a thick layer of Kling gauze, and a thin final layer of Coban. Bolster dressings are commonly utilized after penile skin grafting, but some groups prefer to use negative pressure wound therapy (VAC)<sup>[6,25]</sup>. No randomized trials

**Table 1. Variability in the intraoperative, perioperative, and postoperative management of patients undergoing surgical repair of adult-acquired buried penis**

Series	Graft preference	Graft management	Post-operative bedrest after skin graft	VTE prophylaxis on discharge	Antibiotics on discharge
Donatucci and Ritter 1998	STSG	Bolster dressing	6 days	No	No
Tang <i>et al.</i> <sup>[3]</sup> 2008	STSG	Bolster dressing	3 days	No	14 days
Rybak <i>et al.</i> <sup>[5]</sup> 2014	STSG	Bolster dressing	Not specified	No	No
Voznesensky <i>et al.</i> <sup>[6]</sup> 2017	STSG	Wound VAC	2 days	No	No
Pariser <i>et al.</i> <sup>[13]</sup> 2018	STSG	Bolster dressing	2 days	Apixiban 2.5 mg PO BID, 30 days	No
Monn <i>et al.</i> <sup>[23]</sup> 2019	FTSG	Bolster dressing	Not specified	No	No
Cocci <i>et al.</i> <sup>[39]</sup> 2019	STSG	Bolster dressing	Not specified	No	No
Theisen <i>et al.</i> <sup>[7]</sup> 2018	STSG	Bolster dressing	2 days	No	14 days
Strother <i>et al.</i> <sup>[26]</sup> 2018	STSG	Wound VAC	3-5 days (up to chair, no ambulation)	No	No
Hampson <i>et al.</i> <sup>[22]</sup> 2017	STSG	Bolster dressing	5 days	No	No
Hesse <i>et al.</i> <sup>[15]</sup> 2019	STSG	Bolster dressing	Not specified, mean LOS 8 days so suspect some bedrest	No	No
Erpelding <i>et al.</i> <sup>[34]</sup> 2019	STSG	Bolster dressing	None	No	No
Our practice	FTSG	Bolster dressing	None	Apixiban 2.5 mg PO BID, 30 days	No

STSG: split thickness skin graft; FTSG: full thickness skin graft; LOS: length of stay; VTE: venous thromboembolism; PO: "per os" or by mouth; BID: twice daily

have directly compared the two modalities. Of note, rates of graft take across series utilizing either bolster or VAC dressings have been excellent.

The need for penile skin grafting largely drives the length of hospital stay. Postoperative bed rest is often prescribed to prevent movement and sheer forces on the graft, which could disturb the earliest steps of graft take. The use and timing of bed rest after penile skin grafting in AABP has evolved greatly over time. Historically, bed rest was mandatory for up to 6 days postoperatively<sup>[2]</sup>, while more modern series have generally limited this time of immobility to 48-72 h<sup>[3,4,6,16,17]</sup>. Some groups still advocate 5 days of bed rest<sup>[22]</sup>. Table 1 shows the variability across series for intraoperative and postoperative care. We have adopted a "fast-track" approach to postoperative management of patients with AABP who require penile skin grafting. With a bolster dressing sutured in place, our patients do not require any bed rest. Patients generally ambulate early and discharge home on postoperative day 1. Patients return to the office 4-6 days later for dressing, catheter, and drain removal. They continue daily dressing changes for 2 additional weeks at home. This practice has not resulted in noticeable detrimental effects on our graft outcomes.

### Surgical steps - escutcheonectomy/panniculectomy

The importance of surgical resection of the mons fat pad, or escutcheon, for adequate treatment of AABP was first described by Horton *et al.*<sup>[9]</sup> in 1987. Shortly thereafter, Donatucci and Ritter<sup>[2]</sup> published the first algorithm for the management of buried penis in adults that included resection of any impinging fat from the escutcheon and abdomen. Multiple variations in the approach to escutcheonectomy have been described. Some surgeons resect an ellipsoid<sup>[5]</sup> or trapezoidal<sup>[22]</sup> shaped specimen from the suprapubic fat pad, while others prefer to resect all penile and mons tissue and then bring the abdominal skin flap down to the base of the penis<sup>[3,16,26]</sup> [Figure 3]. In our experience, this latter approach obfuscates the penopubic junction with an inferior edge of the panniculectomy skin flap meeting the proximal edge of the skin graft. This can sometimes lead to a divot particularly in the midline [Figure 4].

We favor leaving a 4- to 6- cm skin bridge at the superior base of the penis with as much viable proximal penile skin as possible, as suggested by other authors [Figure 5]<sup>[6,14,22]</sup>. This bridge allows us to tack the skin at the base of the penis to the periosteum of the pubic symphysis, which re-creates the penopubic angle





**Figure 3.** Intraoperative photograph showing abdominal and/or escutcheon skin flap approximated directly to the base of the penis



**Figure 4.** Post-operative images showing a divot or "moat" at the base of the penis

while also eliminating the need to approximate skin flaps to the base of the penis. Variations of the skin-bridge have been previously described to improve contour and limit dog-ears in the mons region<sup>[27]</sup>. A critical step is to prevent a "moat", which is in the invagination of the proximal edge of the skin graft at



**Figure 5.** Skin bridge at the base of the penis that can be tacked upwards to the superior skin flap to help reduce the possibility of divot formation by having an anastomosis at the base of the penis

the base of the penis [Figures 3 and 4]. This tends to occur when the skin graft is sutured in place with the penis on maximum stretch. The misconception is that grafting a stretched penis will result in improved unbursed length post-operatively. On the contrary, this can lead to wound issues postoperatively due to retraction of the proximal graft into the surrounding skin, resulting in a persistently moist environment similar to that in the preoperative state [Figure 6].

One proposed solution was to create a “turtle-neck”<sup>[17]</sup> by tacking the abdominal skin flap or remaining proximal penile skin a few centimeters distally on the penile shaft, which partially “re-buries” the penis, but prevents an invaginated moist edge. This invagination is especially prominent postoperatively when the patient is sitting or standing without an erection. A drain is placed underneath the escutcheonectomy skin flaps prior to closure to limit seroma and hematoma formation.

Abdominal panniculectomy is an adjunctive procedure during AABP repair in certain settings, and this should be done in conjunction with a plastic surgeon. This is typically necessary when the abdominal adipose tissue impinges on the penis directly. Thus, there is no delineation between the abdominal pannus and the (suprapubic) escutcheon (i.e., “single-bubble”) [Figure 7]. Some prefer to obtain preoperative axial imaging to ensure that there is no concomitant abdominal wall hernia prior to panniculectomy in the setting of a massive pannus. Preoperative skin marking in the standing and sitting position can facilitate symmetry at the time of wound closure. It is our preference to perform panniculectomy in a wedge-shaped fashion taking care to avoid disruption of the lymphatics that run superficial to the abdominal wall fascia as much as possible; this can facilitate wound healing. During resection of the abdominal pannus, the umbilicus may need to be sacrificed but this does not negatively impact surgical outcomes. However, the patient must be counseled about this potential cosmetic alteration before surgery to ensure satisfaction with the postsurgical outcome.





**Figure 6.** Chronic wound at the proximal aspect of the graft, where it was anastomosed to the native skin at the base of the penis. Must have penis on maximum stretch in supine position just to see entire graft and wound



**Figure 7.** The first image is a patient with a separate abdominal pannus and escutcheon. The second shows a "single-bubble", no separation and direct impingement of the abdominal pannus onto the penis. The latter benefit is from plastic surgery involvement and panniculectomy

Drains are essential when patients undergo panniculectomy, as the increased potential space that is created during elevation of the abdominal wall flap puts the patient at a higher risk for seroma or hematoma formation. We generally place two large drains (19 Fr). Drains are removed once they have less than 30 milliliters per day output for multiple consecutive days. For abdominal panniculectomy patients, drains are sometimes required for weeks. We also require that patients who undergo panniculectomy wear an abdominal binder at all times for 4 weeks after surgery.



**Figure 8.** Large specimen of escutcheon adipose tissue. Skin can be harvested for penile skin grafting if needed

### **Surgical steps - scrotoectomy and scrotoplasty**

Buried penis for most patients in Western society can be ultimately attributed to obesity rather than genital lymphedema. Therefore, most patients have minimal scrotal involvement, which allows surgery to be performed in the supine position. This reduces the risk of positioning injury in a population already at high risk due to body habitus. For patients with scrotal lymphedema, we do perform surgery in the lithotomy position, so we can resect all diseased tissue. The posterior scrotal skin is usually uninvolved<sup>[28]</sup> and can be used to help reconstruct the scrotum with the additional benefit of sparing the perirectal lymphatic supply. Another option is to place the testes in thigh pouches and close the perineum with local tissue flaps<sup>[15]</sup>, but we have preferred the cosmetic appearance of the reconfigured scrotum.

### **Further sentiments regarding skin coverage**

While this was covered to some extent in the section “Release of the Buried Penis”, we wanted to add a few more thoughts regarding penile skin coverage during AABP repair. We prefer to leave skin graft sizing and placement to the end of the procedure as the penile skin defect is only fully realized once the escutcheonectomy with or without panniculectomy incision has been closed, the penopubic angle re-established, and the turtle-neck created.

There are a few choices to make when obtaining a skin graft during AABP repair. In most series, the graft is taken from the anterior thigh, which almost always has healthy skin. However, there is significant pain and scarring associated with a thigh graft harvest. Skin grafts can also be taken from a healthy portion of the escutcheonectomy specimen to eliminate donor site morbidity. We usually find that there is ample skin to allow for this [Figure 8].

If using the anterior thigh, split-thickness grafts are preferred given the inability to perform primary closure of the skin in this location. Our split-thickness grafts are usually 0.016 inch thick. If obtaining a split-thickness graft from an escutcheon specimen, anecdotally, it is easier to harvest prior to excision as retraction on a back table is very challenging.

### **Our approach summarized - fast track AABP**

All patients are seen in the clinic and undergo a thorough history and physical examination. Moderate to severe obstructive voiding symptoms warrant preoperative cystoscopy or urethrogram to rule out urethral stricture disease, which is found in up to 47% of patients with AABP<sup>[13,29,30]</sup>. Management of urethral strictures in this population has been detailed previously<sup>[17,29]</sup>. Patients who are active smokers must quit at least 4 weeks prior to surgery. We check preoperative hemoglobin A1c on all diabetic patients to ensure adequate glycemic control. We note the duration of burying and whether or not the penis can be exhumed for examination. There is some concern about a potential association between buried penis and penile cancer<sup>[31,32]</sup>, so this should be considered and evaluated in the clinic. In the setting of carcinoma, one may be able to palpate a firm, indurated mass along the penile shaft or glans. Preoperative imaging and biopsy are warranted in these cases. Biopsy can be performed cystoscopically (through the cicatrix) and/or using a core biopsy needle. Both are generally done in an operative setting. Lastly, we obtain preoperative CT scans for patients who require concomitant panniculectomy to rule out abdominal wall hernias. This also allows us to rule out any asymptomatic inguinal hernias that are difficult to palpate on examination due to obesity.

On the day of surgery, patients receive preoperative subcutaneous heparin prior to induction of anesthesia. They receive broad spectrum antimicrobials. Patients are placed in the supine position whenever feasible to reduce the risk of positioning injury associated with lithotomy. We use the LigaSure vessel sealing device for our abdominal panniculectomy resections, which decreases operative time and blood loss<sup>[33]</sup>. We leave as much healthy penile skin as possible as well as a skin bridge at the base of the penis which is tacked to the abdominal wall fascia. This re-establishes the penopubic angle and reduces issues with a “moat” as mentioned previously. The tacking of the skin bridge at the base of the penis is done using five to seven 0 PDS sutures. Some surgeons use permanent suture, but we do not find this necessary. We generally tack the dermis to the periosteum of the pubic symphysis. Great care should be taken to ensure a physiologic contour while avoiding the spermatic cords. Pitfalls of this tacking include: (1) sutures not adequately spread out (leading to a divot at the midline); (2) sutures tied too tight (also leads to a divot); and (3) sutures placed too close to the skin edge (prevents eversion of the inferior skin edge to meet the superior skin flap, resulting in wound healing issues). The abdominoplasty incisions are approximated over 1-2 closed suction drains with several layers of running absorbable sutures and skin glue.

A full-thickness skin graft is harvested from the escutcheon/pannus specimen to cover any penile skin defects. If the escutcheon skin is diseased, a split-thickness graft is taken from the anterior thigh. We prefer to use a sutured penile bolster dressing with a Foley catheter rather than wound VAC therapy [Figure 9]. Patients are instructed to ambulate and are discharged home on postoperative day 1 with their catheter, dressing, and drains in place. Subcutaneous heparin is continued in the hospital and transitioned to oral apixaban 2.5 mg twice daily upon discharge for 1 month after surgery, since this population is at high risk for venous thromboembolism<sup>[13]</sup>. The dressings and drains are removed in the clinic 4-6 days after surgery if the drain output is less than 30 milliliters per day. Patients are taught to perform twice-daily dressing changes at home for an additional two weeks with Vaseline gauze and dry gauze. As we have transitioned to fast-tracking these patients, we have not noticed any worsening in our surgical outcomes or patient satisfaction. Another group recently published their positive experience utilizing outpatient (i.e., discharge on or before postoperative day 1) management of patients with AABP who underwent penile skin grafting<sup>[34]</sup>. We suspect this will become the standard of care in the future.





**Figure 9.** Bolster dressing protecting and immobilization of skin graft

### Surgical and quality of life outcomes

Most series on surgical management of AABP to date have published outcomes that are focused on surgeons' objective measurements (penile length, graft take, successful unburying, *etc.*). The outcomes are highly favorable and have been described in detail in prior review articles<sup>[35-37]</sup>. These also highlight a relatively high rate of postoperative wound issues; however, it has been our experience that most of these can be managed conservatively with local wound care. Unfortunately, due to the complexity and comorbidities of the patient population, some of these wound complications can be devastating [Figure 10] and require surgical intervention or revision. When faced with a patient with a wound complication, recurrent burying, or aesthetic complaint, we strive to utilize conservative measures upfront and avoid re-operation until the tissues have completely healed (anecdotally, this can range from 4-8 months). Of note, when reviewing the literature on surgical outcomes after AABP repair, one must be aware that the surgical techniques, perioperative care, and the outcome measures deemed important vary largely from institution to institution, making comparisons across studies challenging.

Patient-reported quality of life (QOL) outcomes have been of interest more recently and show similar positive results. The first paper to suggest a potential detrimental effect of buried penis on QOL was a small case series from Detroit, Michigan, where all 5 patients treated for AABP had moderate to severe depression and 1 reported suicidal ideation<sup>[3]</sup>. Six years later, the first series focused on mental health and QOL outcomes was published<sup>[5]</sup>, which showed improvements in depression scores, health-related QOL and erectile function after surgical correction of AABP. Similar improvements in psychological well-being, urinary and sexual function, and overall QOL after surgery have since been reported by other groups<sup>[6,7,22,38,39]</sup>.

It is important to note that there is no validated QOL questionnaire dedicated to this condition, and that these series used various instruments including: modified post-bariatric surgery questionnaire<sup>[6]</sup>, the



**Figure 10.** Significant wound complications including infection and breakdown

European Organization for Research and Treatment of Cancer 15 questionnaire<sup>[5]</sup>, the Expanded Prostate Cancer Index Composite questionnaire<sup>[7]</sup>, and the Changes in Sexual Functioning Questionnaire short-form<sup>[38]</sup>. Furthermore, specific to sexual function and QOL, patients are often counseled that skin grafts should undergo innervation within approximately one year after surgery<sup>[19]</sup>, yet no studies to date have documented time to return of sensation of penile skin grafts

### Room for improvement

There are many opportunities for improvement in the management of AABP. Efforts have been made to develop classification systems and surgical algorithms, as well as methods to ensure that our treatments are resulting in improvements in patient-specific QOL. However, there are several different classification systems published to date<sup>[13-15,40]</sup> and thus, despite their similarities, fall short of allowing us to compare outcomes across series. Most series in the literature have fewer than 50 patients, highlighting the need for collaboration between institutions and centers of excellence to develop a universal classification system and treatment algorithm<sup>[41]</sup>. This would allow for comparison of results across studies and promote multi-institutional research.

In addition, we need disease-specific QOL questionnaires and outcome measures to determine which surgical techniques result in the best functional and cosmetic outcomes. Understanding how surgery impacts long-term sexual function, penile sensory function with split- thickness and full-thickness skin grafts, and urinary function is critical to assist with honest preoperative counseling. We should evaluate the role of adjunctive measures such as phosphodiesterase inhibitors at improving graft take and limiting graft contracture<sup>[39]</sup>. Head-to-head trials comparing different techniques would be ideal. Lastly, understanding the relationship between buried penis and penile cancer is necessary since many patients with this condition do not seek care for many years (if at all) because of embarrassment of their condition and/or unawareness of surgical options for repair.

### CONCLUSION

AABP is a condition that is becoming more commonly seen by surgeons due to increased community awareness and overall prevalence. The main surgical steps to AABP repair include: release of the trapped penis and resection of diseased penile skin, escutcheonectomy/panniculectomy, scrotoplasty if needed, and

penile coverage. The intricacies of the surgical technique are not universally agreed upon, and variation is widespread. It is difficult to optimize and generalize surgical approaches and postoperative care for this condition, as published reports often include few patients and fail to take into account patient satisfaction and QOL. The development of disease-specific QOL questionnaires and outcome measures will assist with future studies and improve the counseling of and care for patients with AABP.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design and composition of the manuscript: Theisen KM, Pariser JJ

Contributed by data and photo gathering, and editing manuscript: Alford AV

Contributed by helping with revision of the manuscript and ensuring quality content and structure: Kim N

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Informed consent for use of perioperative images was obtained from the participants.

### Copyright

© The Author(s) 2020.

## REFERENCE

- 1 Hales CM, Carroll MD, Fryar CD, Ogden CL. Prevalence of obesity and severe obesity among adults: United States, 2017-2018. NCHS Data Brief, no 360. Hyattsville, MD: National Center for Health Statistics; 2020.
- 2 Donatucci CF, Ritter EF. Management of the buried penis in adults. *J Urology* 1998;159:420-4.
- 3 Tang SH, Kamat D, Santucci RA. Modern management of adult-acquired buried Penis. *J Urology* 2008;72:124-7.
- 4 Pestana IA, Greenfield JM, Walsh M, Donatucci CF, Erdmann D. Management of "Buried" penis in adulthood: an overview. *Plast ReConstr Surg* 2009;124:1186-95.
- 5 Rybak J, Larsen S, Yu M, Levine LA. Single center outcomes after reconstructive surgical correction of adult acquired buried penis: measurements of erectile function, depression, and quality of life. *J Sex Med* 2014;11:1086-91.
- 6 Voznesensky MA, Lawrence WT, Keith JN, Erickson BA. Patient-reported social, psychological, and urologic outcomes after adult buried penis repair. *J Urology* 2017;103:240-4.
- 7 Theisen KM, Fuller TW, Rusilko P. Surgical management of adult-acquired buried penis: impact on urinary and sexual quality of life outcomes. *J Urology* 2018;116:180-4.
- 8 Alter GJ, Ehrlich RM. A new technique for correction of the hidden penis in children and adults. *J Urol* 1999;161:455-9.
- 9 Horton CE, Vorstman B, Teasley D, Winslow B. Hidden penis release: adjunctive suprapubic lipectomy. *Ann Plast Surg* 1987;19:131-4.
- 10 Chopra CW, Ayoub NT, Bromfield C, Witt PD. Surgical management of acquired (cicatricial) buried penis in an adult patient. *Ann Plast Surg* 2002;49:545-9.
- 11 Dandapat MC, Mohapatro SK, Patro SK. Elephantiasis of the penis and scrotum. A review of 350 cases. *Am J Surg* 1985;149:686-90.
- 12 Malloy TR, Wein AJ, Gross P. Scrotal and penile lymphedema: surgical considerations and management. *J Urol* 1983;130:263-5.
- 13 Pariser JJ, Soto-Aviles OE, Miller B, Husainat M, Santucci RA. A simplified adult acquired buried penis repair classification system with an analysis of perioperative complications and urethral stricture disease. *J Urology* 2018;120:248-52.

- 14 Tausch TJ, Tachibana I, Siegel JA, Hoxworth R, Scott JM, et al. Classification system for individualized treatment of adult buried penis syndrome. *Plast Reconstr Surg* 2016;138:703-11.
- 15 Hesse MA, Israel JS, Shulzhenko NO, Sanchez RJ, Garland CB, et al. The surgical treatment of adult acquired buried penis syndrome: a new classification system. *Aesthet Surg J* 2019;39:979-88.
- 16 Fuller TW, Theisen K, Rusilko P. Surgical management of adult acquired buried penis: escutcheonectomy, scrotoectomy, and penile split-thickness skin graft. *J Urology* 2017;108:237-8.
- 17 Jun MS, Gallegos MA, Santucci RA. Contemporary management of adult-acquired buried penis. *BJU Int* 2018;122:713-5.
- 18 Zhao YQ, Zhang J, Yu MS, Long DC. Functional restoration of penis with partial defect by scrotal skin flap. *J Urol* 2009;182:2358-61.
- 19 Thakar HJ, Dugi DD. Skin grafting of the penis. *Urol Clin North Am* 2013;40:439-48.
- 20 King IC, Tahir A, Ramanathan C, Siddiqui H. Buried penis: evaluation of outcomes in children and adults, modification of a unified treatment algorithm, and review of the literature. *ISRN Urol* 2013;2013:109349.
- 21 Boonjindasup A, Pinsky M, Stewart C, Trost L, Chaffin A, et al. Management of adult concealed penis using a meshed, split-thickness skin graft. *Can Urol Assoc J* 2016;10:E407-11.
- 22 Hampson LA, Muncey W, Chung PH, Ma CC, Friedrich J, et al. Surgical and functional outcomes following buried penis repair with limited panniculectomy and split-thickness skin graft. *J Urology* 2017;110:234-8.
- 23 Monn MF, Socas J, Mellon MJ. The use of full thickness skin graft phalloplasty during adult acquired buried penis repair. *J Urology* 2019;129:223-7.
- 24 Morris MS, Morey AF, Stackhouse DA, Santucci RA. Fibrin sealant as tissue glue: preliminary experience in complex genital reconstructive surgery. *J Urology* 2006;67:688-91.
- 25 Weinfeld AB, Kelley P, Yuksel E, Tiwari P, Hsu P, et al. Circumferential negative-pressure dressing (VAC) to bolster skin grafts in the reconstruction of the penile shaft and scrotum. *Ann Plast Surg* 2005;54:178-83.
- 26 Strother MC, Skokan AJ, Sterling ME, Butler PD, Kovell RC. Adult buried penis repair with escutcheonectomy and split-thickness skin grafting. *J Sex Med* 2018;15:1198-204.
- 27 Blanton MW, Pestana IA, Donatucci CF, Erdmann D. A unique abdominoplasty approach in management of “buried” penis in adulthood. *Plast Reconstr Surg* 2010;125:1579-80.
- 28 Muller GP, Jordan CG. Elephantiasis Nostra. *Ann Surg* 1933;97:226-36.
- 29 Fuller TW, Pekala K, Theisen KM, Tapper A, Burks F, et al. Prevalence and surgical management of concurrent adult acquired buried penis and urethral stricture disease. *World J Urol* 2019;37:1409-13.
- 30 Liaw A, Rickborn L, McClung C. Incidence of urethral stricture in patients with adult acquired buried penis. *Adv Urol* 2017;2017:7056173.
- 31 Pekala KR, Pelzman D, Theisen KM, Rogers D, Maganty A, et al. The prevalence of penile cancer in patients with adult acquired buried penis. *J Urology* 2019;133:229-33.
- 32 Abdulla A, Daya D, Pinthus J, Davies T. Buried penis: an unrecognized risk factor in the development of invasive penile cancer. *Can Urol Assoc J* 2012;6:E199-202.
- 33 Siegel JA, Zhao L, Tachibana I, Carlson S, Tausch TJ, et al. Rapid excision of massive localized lymphedema of the male genitalia with vessel sealing device. *Can J Urol* 2016;23:8291-5.
- 34 Erpelding SG, Hopkins M, Dugan A, Liao JY, Gupta S. Outpatient surgical management for acquired buried penis. *J Urology* 2019;123:247-51.
- 35 Shaer O, Shaer K. Revealing the buried penis in adults. *J Sex Med* 2009;6:876-85.
- 36 Fuller TW, Theisen KM, Shah A, Rusilko PJ. Surgical management of adult acquired buried penis. *Curr Urol Rep* 2018;19:22.
- 37 Smith-Harrison LI, Piotrowski J, Machen GL, Guise A. Acquired buried penis in adults: a review of surgical management. *Sex Med Rev* 2020;8:150-7.
- 38 Hughes DB, Perez E, Garcia RM, Aragon OR, Erdmann D. Sexual and overall quality of life improvements after surgical correction of “buried penis”. *Ann Plast Surg* 2016;76:532-5.
- 39 Cocci A, Cito G, Falcone M, Capece M, Maida FD, et al. Subjective and objective results in surgical correction of adult acquired buried penis: a single-centre observational study. *Arch Ital Urol Androl* 2019;91:25-9.
- 40 Mirastschijski U. Classification and treatment of the adult buried penis. *Ann Plast Surg* 2018;80:653-9.
- 41 Poore SO. The classification and surgical treatment of adult acquired buried penis syndrome: a call for data and collaboration. *Aesthet Surg J* 2020;40:NP83-4.

Review

Open Access



# Botanicals for photoprotection

Angeli E. Torres, Kevin M. Luk, Henry W. Lim

Photomedicine and Photobiology Unit, Department of Dermatology, Henry Ford Health System, Detroit, Michigan 48202, USA.

**Correspondence to:** Dr. Henry W. Lim, Department of Dermatology, Henry Ford Hospital, 3031 West Grand Boulevard, Detroit, Michigan 48202, USA. E-mail: hlim1@hfhs.org

**How to cite this article:** Torres AE, Luk KM, Lim HW. Botanicals for photoprotection. *Plast Aesthet Res* 2020;7:57.  
<http://dx.doi.org/10.20517/2347-9264.2020.87>

**Received:** 20 Apr 2020 **First Decision:** 24 Aug 2020 **Revised:** 13 Sep 2020 **Accepted:** 29 Sep 2020 **Published:** 21 Oct 2020

**Academic Editor:** Salvador Gonzalez **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

The importance of photoprotection against the deleterious effects of excessive and chronic exposure to sunlight is now well established. Photoprotective measures include behavioral modifications such as seeking shade, wearing photoprotective clothing, wide-brimmed hat and sunglasses, and applying sunscreen to exposed areas. Data on botanical topical and oral preparations have demonstrated photoprotective potential in *in vitro*, animal, and human studies. This review will focus on botanicals that have been most extensively studied, namely, *Polypodium leucotomos* extract, green tea, pomegranate, resveratrol, curcumin, and silymarin. These agents have shown promise in mitigating ultraviolet-induced acute changes on the skin, chronic photodamage, and even skin cancer prevention. However, it must be emphasized that current evidence indicates that these agents should be used as adjunctive measures rather than as a replacement of the photoprotective behavioral modifications described above.

**Keywords:** Botanical photoprotection, *Polypodium leucotomos* extract, green tea, pomegranate, resveratrol, curcumin, silymarin

## INTRODUCTION

Electromagnetic radiation, including infrared, visible, and ultraviolet (UV) radiation (UVR), have both beneficial and harmful effects on the health of human skin. In particular, UVR exposure plays a significant role in the development of sunburns, photoaging, photoimmunosuppression, keratinocyte carcinomas, and cutaneous melanoma. They can also induce and exacerbate photosensitive dermatoses. Following



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





exposure to UVR, reactive oxygen species (ROS) are generated in the skin, which result in oxidative stress. This contributes to acute UV-induced erythema (i.e., photoinflammation) and tanning through upregulation of cyclooxygenase-2 (COX-2), which is involved in early inflammation<sup>[1]</sup>. With chronic UVR exposure, the formation of photoproducts and ROS can lead to DNA damage, while at the same time cause downregulation of tumor suppressor genes. This allows cells to continue replicating while genetic mutations go unrepaired resulting in cancer formation (i.e., photocarcinogenesis)<sup>[1]</sup>. ROS in the skin can also activate proteins which play an important role in photoaging through blockage of collagen gene transcription, inhibition of collagen synthesis, and overexpression of enzymes that break down collagen<sup>[1]</sup>.

Photoprotection against UV and visible light is one preventative health strategy to reduce the negative effects of electromagnetic radiation. Historically, photoprotection has been achieved through topical routes. Photoprotection strategies include behavioral modifications such as seeking shade while outdoors, wearing photoprotective clothing including hats and sunglasses, as well as applying sunscreen on otherwise exposed skin sites<sup>[2,3]</sup>.

Sunscreens are the most widely recognized means of photoprotection by the public; however, they do have several limitations. These include the need for regular reapplication and lack of efficacy due to under-application (i.e., not applying sufficient amounts). More recently, the ecological safety and potential human toxicity of organic sunscreens, such as oxybenzone and octinoxate, have raised concerns among dermatologists and the general public<sup>[3,4]</sup>. However, there is limited evidence of direct toxic effects of organic sunscreens in humans and coral reef species. Nevertheless, inorganic-based sunscreens - namely, zinc oxide and titanium oxide-based sunscreens - have been recommended as an alternative for those concerned about the potential health and environmental impact of organic sunscreens<sup>[4,5]</sup>. In addition, alternative photoprotective methods have gained increased interest as adjunct protection against UVR and visible light exposure.

Systemic photoprotection has been used in conjunction with topical photoprotection. It may be administered either subcutaneously<sup>[6]</sup> or orally. Examples include vitamins, minerals, polyphenols, carotenoids, and  $\alpha$ -melanocyte stimulating hormone analog, as well as various plant-based agents that have been reported to yield photoprotective and anti-photocarcinogenic properties. These agents act through their antioxidant, anti-inflammatory, and immunomodulatory effects<sup>[7]</sup>. The focus of this article will be to review evidence-based systemic and topical botanicals as photoprotective agents.

## BOTANICALS WITH PHOTOPROTECTIVE PROPERTIES

### *Polypodium leucotomos* extract

*Polypodium leucotomos* is a tropical fern belonging to the family *Polypodiaceae*. It is native to Central and South America where it has been used traditionally to treat various skin diseases including psoriasis and atopic dermatitis<sup>[1,7,8]</sup>. At this time, *Polypodium leucotomos* extract (PLE) is the most well studied botanical photoprotective agent. It is commercially available worldwide as an over-the-counter oral supplement<sup>[9]</sup>. While there are many different preparations of PLE, most of the studies reported in peer-reviewed literature have been done with Fernblock® (Cantabria Labs, Madrid, Spain). PLE has antioxidant, anti-inflammatory, immunomodulatory, tumor suppressive, and anti-aging properties<sup>[1,7]</sup>. These qualities are mainly attributed to the fern's high polyphenol content, which is obtained from the leaves. Polyphenols are the most abundant class of antioxidants present in plant-based food and beverages. The polyphenols present in PLE are *p*-coumaric acid, chlorogenic acid, vanillic acid, caffeic acid, and ferulic acid. Of these, the most powerful antioxidants are ferulic and caffeic acids<sup>[1]</sup>. It must be emphasized that the concentration of these constituents can vary depending on the PLE preparation. Accordingly, the different PLE preparations may vary in term of their photoprotective ability. This was demonstrated in a study by Gonzalez et al.<sup>[10]</sup>, where the photoprotective activity of six different PLE preparations (including Fernblock®) were tested *in vitro*.

Results showed that Fernblock® was by far the most active (> 5x on fibroblasts and > 3x on keratinocytes) compared to the other five PLE preparations, of which two were found to have almost no activity<sup>[10]</sup>.

PLE augments the body's natural antioxidant system and minimizes the accumulation of ROS in the skin. It has the unique ability to scavenge superoxide anion in contrast to conventional antioxidants, which can neutralize singlet oxygen only<sup>[7]</sup>. As an anti-inflammatory compound, PLE has been shown to suppress UV-induced erythema and reduce cutaneous phototoxicity from photochemotherapy<sup>[7]</sup>. It can also increase the minimal erythema dose (MED), the UVR dose required for immediate pigment darkening, and the minimal phototoxic UVR dose<sup>[9]</sup>. One randomized controlled trial found that healthy human subjects who took oral PLE supplements 240 mg twice daily for 60 days were 6 times less likely to have a sunburn, 22 times more likely to have increased MED, and 15 times less likely to exhibit visible erythema post-UVB exposure<sup>[11]</sup>. These effects were quantified in a later study by Kohli *et al.*<sup>[12]</sup> where it was noted that the intensity of UVB-induced erythema decreased by an average of 8% post-PLE supplementation. Similarly, one study which did not utilize Fernblock®, noted increased MED among subjects with skin phototype I-III following once daily intake of an oral supplement containing PLE for 12 weeks<sup>[13]</sup>. The possible mechanism underlying PLE's anti-inflammatory properties include the inhibition of transcription factors and cytokines that mediate photoinflammation, namely, tumor necrosis factor (TNF), inducible nitric oxide synthase (iNOS), activator protein 1 (AP-1), and nuclear factor kappa B (NF-κB). In addition, PLE can also reduce the expression of COX-2 and prostaglandin E2, both of which are involved in the initial steps of the inflammatory pathway<sup>[1,7]</sup>.

In terms of immunomodulation, PLE has been found to preserve epidermal Langerhans cells that are otherwise depleted as a result of UVR<sup>[7]</sup>. Its anti-tumor effects were exhibited in mice that were given oral PLE daily as evidenced by increased expression of the tumor suppressor protein p53<sup>[14]</sup>. PLE has likewise been shown to accelerate extracellular matrix turnover and promote renewal of dermal collagen through inhibition of matrix metalloproteinases (MMPs) and upregulation of tissue inhibitor of metalloproteinase, thereby supporting its role in the prevention of photoaging<sup>[1,8]</sup>.

PLE can also provide photoprotection from wavelengths beyond UV. In a study by Mohammad *et al.*<sup>[15]</sup>, patients taking 480 mg of PLE daily demonstrated a substantial decrease in visible light-induced persistent pigment darkening and delayed tanning. Furthermore, an *in vitro* study by Zamarrón *et al.*<sup>[16]</sup> showed that PLE decreased cell death and collagen degradation in human dermal fibroblasts that were exposed to visible light and near infrared radiation. Additional benefits of PLE include suppression of photodermatoses such as polymorphous light eruption and solar urticaria. It can also be used as an adjunctive treatment for vitiligo and actinic keratosis in combination with phototherapy and photodynamic therapy, respectively<sup>[14]</sup>.

PLE has relatively minor side effects, including mild to moderate gastrointestinal symptoms and pruritus, which have been reported in a small percentage of patients receiving doses ranging from 120 mg to 1,080 mg daily<sup>[17]</sup>. No changes in physical examination, vital signs, and laboratory tests (complete metabolic panel and clotting studies) were observed from baseline among patients taking 480 mg daily of PLE for 2 months<sup>[11]</sup>.

### Green tea

Produced from the leaves and leaf buds of *Camellia sinensis*, green tea is one of the most widely consumed beverages in the world. Its many benefits are primarily due to its polyphenols - otherwise known as green tea catechins (GTC) - of which the most abundant (65%) is epigallocatechin-3-gallate<sup>[7,8,14]</sup>. Similar to other phenolic compounds, GTC has antioxidant, anti-inflammatory, immunomodulatory, and chemopreventive properties.

*In vitro* studies on human keratinocytes have shown that GTCs can inhibit activation of AP-1 and NF-κB, decrease UVB-induced apoptosis, and stimulate the production of interleukin (IL)-12. IL-12 is postulated

to play a role in photoprotection since photoinflammation, impaired DNA repair, and tumorigenesis are associated with an absence of IL-12. In mice, topical and oral GTC has been found to protect against photoinflammation and photocarcinogenesis through inhibition of the mitogen-activated protein kinase inflammatory pathway and upregulation of genes involved in DNA repair.

In humans, previous studies have reported that topical GTC reduces photodamage by decreasing production of UVR-induced cyclobutane pyrimidine dimers (CPDs) and visible sunburn<sup>[18,19]</sup>. However, GTCs have poor skin penetration when topically applied due to their poor lipid solubility<sup>[7]</sup>. They are also subject to photodegradation.<sup>[19]</sup> In contrast, orally administered GTC has been shown to have good skin bioavailability as evidenced by their presence in skin biopsy specimens and skin blister fluid. This was noted after GTC supplementation equivalent to 5 cups of green tea daily for 12 weeks<sup>[20]</sup>. However, there is conflicting evidence in the literature as to the efficacy of oral GTC as a photoprotectant<sup>[21,22]</sup>.

One study showed that women who consumed one liter of green tea daily for 3 months were found to have increased skin elasticity, improved skin texture, decreased transepidermal water loss, and increased cutaneous blood flow<sup>[22]</sup>. In contrast, a study of healthy, light-skinned (skin phototype I-II) adults revealed no significant differences in UVR-induced erythema, dermal leukocytic infiltration, and induction of cyclooxygenase and lipoxygenase inflammatory pathways among those taking daily GTC supplements (1,080 mg plus 100 mg vitamin C) compared to those in the placebo group<sup>[21]</sup>. Further studies are needed to reconcile these conflicting evidences.

### Pomegranate

The extract from *Punica granatum* or pomegranate is rich in phenolic compounds, specifically, anthocyanins, catechins, and tannins<sup>[7]</sup>. Significant amounts of these compounds are present in different parts of the fruit but are most concentrated in the peel and juice<sup>[23]</sup>. At present, pomegranate extract is widely available as an over-the-counter oral supplement or topical formulation, and is often incorporated in commercially sold skin care products.

Pomegranate extract has anti-inflammatory properties and a very potent antioxidant activity - even greater than that of green tea or red wine<sup>[7,8]</sup>. It confers photoprotection through inhibition of UV-induced production of free radicals, erythema and burning, DNA damage, cell proliferation, and apoptosis<sup>[8,24]</sup>. It can also decrease collagen breakdown<sup>[25]</sup>.

Murine *in vivo* studies have demonstrated that topical application of pomegranate extract can replenish antioxidants (including catalase, peroxidase, and superoxide dismutase), as well as reduce photoinflammation. This was evidenced by decreased skin edema, epidermal thickening, dermal neutrophilic infiltrates, ornithine decarboxylase, and COX-2. Additionally, topical pomegranate extract has been found to prolong the latency and lessen the multiplicity of skin tumors, thereby supporting its benefit against photocarcinogenesis<sup>[26]</sup>.

Oral administration of pomegranate extract was found to be protective against UVB-induced skin cancer formation in mice through downregulation of COX-2, iNOS, cyclin D2, and MMPs. Women who consumed 1000 mg of pomegranate extract or 8 ounces of pomegranate juice daily for 12 weeks were reported to have increased MED in a randomized controlled, open-label study<sup>[25]</sup>.

The anti-aging benefits of pomegranate extract were further investigated in a 2017 study by Kang *et al.*<sup>[27]</sup> using orally administered pomegranate juice concentrated powder (PCP) in hairless mice. In this 15-week, placebo-controlled trial, PCP was given at 100, 200, or 400 mg/kg once daily, and 1 hour prior to thrice weekly UVB exposure. The investigators reported dose-dependent decreases in wrinkle formation, skin

edema, expression of pro-inflammatory cytokines, apoptosis, and MMP activity. Moreover, PCP-treated mice showed increases in water, collagen type I, and hyaluronic acid contents in the skin, indicating a skin moisturizing effect.

### **Resveratrol (Grape seed, grape peel, and red wine)**

Resveratrol is a stilbenoid compound belonging to the non-flavonoid class of polyphenols<sup>[28]</sup>. It is present in grape seeds, grape peels, as well as red wine<sup>[7]</sup>. In terms of photoprotection, resveratrol has demonstrated anti-oxidant, anti-inflammatory, and anti-tumor effects in several *in vitro*, animal, and human studies<sup>[8]</sup>.

In an *in vitro* study by Zhou *et al.*<sup>[29]</sup>, pre-treatment of cultured human keratinocytes with different concentrations of resveratrol prior to UVB irradiation resulted in concentration-dependent increase in cell viability and a decreased rate of apoptosis. Following UVB irradiation, samples that were pre-treated with resveratrol had 15%-52% more viable cells and 15%-22% less apoptosis than non-treated samples. Furthermore, resveratrol pre-treated samples demonstrated a 1.4-fold increase in expression of Bcl-2 (anti-apoptotic protein), and decreased expression of Bax and caspase-3 (i.e., pro-apoptotic proteins) by 52% and 45%, respectively.

In mice, topical application of resveratrol prior to UVB radiation exposure was found to inhibit UVB-induced skin edema, inflammation, generation of ROS (e.g., hydrogen peroxide and lipoperoxides), and induction of COX and ornithine carboxylase<sup>[8]</sup>. Meanwhile, oral administration demonstrated anti-tumor effect through alteration of tumor growth factor beta and NF- $\kappa$ B, both of which are involved in cell proliferation and tumorigenesis<sup>[1,7]</sup>.

In humans, resveratrol has been found to afford partial protection against UVR-induced photodamage. Wu *et al.*<sup>[30]</sup> conducted a study on 15 healthy volunteers who were subject to repetitive UVR exposure from a solar simulator at a dose of 1.5 MED. Results showed that skin sites which were treated with topical resveratrol had less UV-induced erythema and sunburn cell formation compared to placebo (vehicle only) or negative control (no treatment).

### **Curcumin (Turmeric)**

Curcumin is the active constituent of turmeric (*Curcuma longa*), a rhizomatous plant native to South Asia that is now grown in many tropical and subtropical regions worldwide. Turmeric is commonly used as a spice, coloring agent, and for various indications in Ayurvedic and traditional Chinese medicine<sup>[8,14]</sup>.

Previous *in vitro* studies utilizing human keratinocytes and epidermoid carcinoma cells (i.e., squamous cell carcinoma cell line) have found that curcumin decreases UVB-induced apoptosis and inflammation through inhibition of the NF- $\kappa$ B and MAPK pathways<sup>[8,14]</sup>. In keratinocytes and fibroblasts, curcumin decreases the expression of MMP-1, which may help reduce the appearance of wrinkles in photoaged skin<sup>[8]</sup>. Moreover, curcumin has demonstrated ability to decrease squamous cell carcinoma tumor growth in mice<sup>[14]</sup>.

Curcumin may also confer protection against UVA-mediated photodamage. According to a study by Liu *et al.*<sup>[31]</sup> (2018), pre-treatment with curcumin 2 h prior to UVA exposure prevented accumulation of ROS and restores the innate antioxidant function of human fibroblasts *in vitro*. In addition, curcumin was shown to mitigate UVA-induced apoptosis, inflammation, and collagen degradation.

### **Silymarin (Milk thistle)**

Silymarin is an isoflavone derived from the seeds of the milk thistle plant (*Silybum marianum*), which is one of the oldest known medicinal herbs used in traditional European medicine. Nowadays, it is

**Table 1. Most extensively studied botanicals for photoprotection**

Botanical agent	Spectrum	Mechanism of photoprotection	Routes	Models
<i>Polypodium leucotomos</i> extract	UVB, VL + UVA1, IR-A	Antioxidant: Augments natural antioxidant system, can scavenge superoxide anion Anti-inflammatory: Increases MED, dose required for IPD, and minimal phototoxic dose; inhibits proinflammatory transcription factors, mediators, and cytokines; decreases VL + UVA1 induced PPD and DT Immunomodulatory: Preserves eLCs Anti-tumor: Increases expression of p53 tumor suppressor gene Anti-aging: Downregulates MMP; upregulates TIMP; prevents VL and IR-A induced cell death and collagen degradation Other: Suppresses photodermatoses	Oral	<i>In vitro</i> , mouse, human
Green tea	UVB	Increases skin elasticity and blood flow; stimulates IL-12; decreases apoptosis, CPDs, sunburn, and TEWL; inhibits AP-1, NF-κB, MAPK	Topical, oral	<i>In vitro</i> , mouse, human
Pomegranate	UVB	Increases MED, skin moisture, and tumor latency; decreases inflammation and multiplicity of tumors; inhibits ROS, erythema, burning, DNA damage, cell proliferation, apoptosis, and collagen breakdown	Topical, oral	Mouse, human
Resveratrol (Grape seed, grape peel, and red wine)	UVB	Increases cell viability; decreases apoptosis, erythema, and sunburn cell formation; inhibits COX, ornithine carboxylase, TGF beta, and NF-κB	Topical, oral	<i>In vitro</i> , mouse, human
Curcumin (Turmeric)	UVA, UVB	Prevents ROS accumulation; decreases apoptosis, inflammation, MMP-1 expression, and SCC tumor growth; inhibits NF-κB and MAPK	Topical, oral	<i>In vitro</i> , mouse, human
Silymarin (Milk Thistle)	UVA, UVB	Activates p53 tumor suppressor gene; decreases MMP-1 activation, inflammation, ROS, DNA damage, apoptosis, IL-10, and CPDs; inhibits collagenase, hyaluronidase, and elastase	NA	<i>In vitro</i>

UVB: ultraviolet B; UVA: ultraviolet A; VL + UVA1: visible light + ultraviolet A1; IR-A: infrared A; MED: minimal erythema dose; IPD: immediate pigment darkening; PPD: persistent pigment darkening; DT: delayed tanning; eLCs: epidermal Langerhans cells; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinase; IL: interleukin; CPD: cyclobutane pyrimidine dimers; TEWL: transepidermal water loss; AP-1: activator protein 1; NF-κB: nuclear factor kappa B; MAPK: mitogen-activated protein kinase; ROS: reactive oxygen species; DNA: deoxyribonucleic acid; COX: cyclooxygenase; TGF: tumor growth factor; SCC: squamous cell carcinoma; NA: not applicable

chiefly marketed as an oral hepatic supplement and has been used to treat hepatitis, alcoholic liver diseases, cirrhosis, and toxin-induced hepatotoxicity. The polyphenols present in silymarin, which is more appropriately termed flavonolignans, are silybin, silychristin, silydianin, isosilybin, and 2,3-dehydrosilybin<sup>[32-34]</sup>.

The antioxidant and anti-inflammatory effects of silymarin are similar to other phenolic compounds in that it downregulates the UVB-induced generation of ROS, expression of inflammatory transcription factors and cytokines (e.g., TNF, IL-1, and iNOS), and activation of inflammatory pathways including COX and lipoxygenase pathways. As an immunomodulatory compound, silymarin reverses UVB-induced immunosuppression through decreased production of IL-10, which has been found to be elevated in the presence of cancers and are thought to be responsible for the cancer's ability to evade the host's immune response. In addition, silymarin imparts protection against UVB photocarcinogenesis through activation of p53, and reduction of pyrimidine photoproduct formation<sup>[34]</sup>.

In terms of protection against UVA, one *in vitro* study on human dermal fibroblasts revealed that pre-treatment with silymarin one hour prior to UVA exposure decreased ROS production, DNA strand breaks, activation of MMP-1, and the pro-apoptotic protein caspase-3<sup>[35]</sup>. In another *in vitro* study, silymarin and its flavonolignans were found to inhibit the UVA-induced activity of collagenase, hyaluronidase, and elastase, which are respectively responsible for skin wrinkling, loss of hydration, and sagging, indicating an anti-photoaging effect<sup>[33]</sup>.

However, a study by Fidrus *et al.*<sup>[36]</sup> (2019) showed contradictory results wherein silymarin pre-treatment of human keratinocytes *in vitro* 30 min prior to UVA exposure enhanced UVA-induced cytotoxicity in a dose



**Table 2. Other botanicals with photoprotective effects**

Botanical agent	Spectrum	Mechanism of photoprotection	Routes	Models	Ref.
Cocoa extract	UVB	Increases MED; decreases erythema and skin wrinkling	Oral	Mouse, Human	[7]
Rosemary plus grapefruit extract	UVA, UVB	Increases MED, skin elasticity; decreases erythema, lipoperoxides, and skin wrinkling	Oral	Human	[37]
Strawberry extract	UVA	Increases cell viability	NA	<i>In vitro</i>	[38]
Blueberry extract	UVA, UVB and UVC	Increases cell viability	NA	<i>In vitro</i>	[39]
Melon concentrate	UVA, UVB	Increases MED and endogenous antioxidants; decreases sunburn cells	Topical, oral	Human, <i>In vitro</i>	[40]
<i>Sechium edule</i> (chayote) extract	UVA	Increases DNA repair; decreases apoptosis, ROS, DNA damage, and CPDs	NA	<i>In vitro</i>	[41]
<i>Oenanthe javanica</i> (water celery) extract	UVB	Increases collagen type I and III; decreases MMP-1, MMP-3, TNF, and COX-2 expression	Topical	Mouse	[42]

UVB: ultraviolet B; UVA: ultraviolet A; UVC: ultraviolet C; MMP: matrix metalloproteinase; TNF: tumor necrosis factor; COX-2: cyclooxygenase 2; MED: minimal erythema dose; DNA: deoxyribonucleic acid; ROS: reactive oxygen species; CPD: cyclobutane pyrimidine dimers; NA: Not applicable

dependent manner (i.e., less viable cells with higher silymarin doses). In addition, silymarin pre-treated keratinocytes produced higher amounts of CPDs following UVA exposure compared to non-pre-treated keratinocytes. The mechanism for this silymarin-induced phototoxicity is still poorly understood.

Table 1 summarizes the mechanism of photoprotection and spectrum coverage of the botanical agents discussed above.

## Others

Other botanicals that have been reported to have photoprotective effects, albeit have not been as rigorously studied, are summarized in Table 2.

## CONCLUSION

Botanical-based photoprotection is likely to increase in popularity as consumer trends worldwide continue to place an emphasis on naturally occurring compounds used solely or in conjunction with synthetic products. The botanicals reviewed above currently have the most evidence available and can serve as options for providers to recommend to patients. These oral and topical botanical products act through a variety of biologic mechanisms to confer protection against the adverse effects of UVR. However, unlike sunscreens, botanical products are not subject to FDA regulations and so rigorous efficacy and safety testing through large-scale controlled therapeutic trials are lacking for many of these agents. As such, their true photoprotective benefit compared to established measures like seeking shade, donning UV-blocking garments, or organic or inorganic topical sunscreens remains to be verified. In addition, the stability of botanical ingredients as well as the optimal concentration of their constituents is unregulated. Therefore, while evidence on their use as an adjunctive means of photoprotection appears favorable, they should be used in conjunction with, and not as a replacement of, pre-existing photoprotection recommendations. Finally, as the biologic effects of other wavelengths of electromagnetic radiation such as visible and infrared ranges continue to be elucidated, it will be critical for future research to evaluate the potential applicability of botanicals for protection in that realm as well.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to literature search and writing of initial manuscript: Torres AE, Luk KM  
Contributed to writing and editing of manuscript: Lim HW

**Availability of data and materials**

Not applicable.

**Financial support and sponsorship**

None.

**Conflicts of interest**

Torres AE and Luk KM declared that there are no conflicts of interest; Lim HW is an investigator for Incyte, L'Oréal, Pfizer, PCORI, has served as consultant for Pierre Fabre, ISDIN, Ferndale, and Galderma, and has participated as a speaker in general educational session for Johnson & Johnson, and Ra Medical System.

**Ethical approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Copyright**

© The Author(s) 2020.

**REFERENCES**

- Parrado C, Mascaraque M, Gilaberte Y, Juarranz A, Gonzalez S. Fernblock (Polypodium leucotomos extract): molecular mechanisms and pleiotropic effects in light-related skin conditions, photoaging and skin cancers, a review. *Int J Mol Sci* 2016;17:1026.
- Krutmann J, Passeron T, Gilaberte Y, Granger C, Leone G, et al. Photoprotection of the future: challenges and opportunities. *J Eur Acad Dermatol Venereol* 2020;34:447-54.
- Yeager DG, Lim HW. What's new in photoprotection: a review of new concepts and controversies. *Dermatol Clin* 2019;37:149-57.
- Schneider SL, Lim HW. Review of environmental effects of oxybenzone and other sunscreen active ingredients. *J Am Acad Dermatol* 2019;80:266-71.
- Schneider SL, Lim HW. A review of inorganic UV filters zinc oxide and titanium dioxide. *Photodermatol Photoimmunol Photomed* 2019;35:442-6.
- Langendonk JG, Balwani M, Anderson KE, Bonkovsky HL, Anstey AV, et al. Afamelanotide for erythropoietic protoporphyria. *N Engl J Med* 2015;373:48-59.
- Parrado C, Philips N, Gilaberte Y, Juarranz A, González S. Oral photoprotection: effective agents and potential candidates. *Front Med (Lausanne)* 2018;5:188.
- Saewan N, Jimtaisong A. Natural products as photoprotection. *J Cosmet Dermatol* 2015;14:47-63.
- Lim HW, Arellano-Mendoza MI, Stengel F. Current challenges in photoprotection. *J Am Acad Dermatol* 2017;76:S91-9.
- Gonzalez S, Lucena SR, Delgado P, Juarranz A. Comparison of several hydrophilic extracts of Polypodium leucotomos reveals different antioxidant moieties and photoprotective effects *in vitro*. *J Med Plants Res* 2018;12:336-45.
- Nestor MS, Berman B, Swenson N. Safety and efficacy of oral Polypodium leucotomos extract in healthy adult subjects. *J Clin Aesthet Dermatol* 2015;8:19-23.
- Kohli I, Shafi R, Isedeh P, Griffith JL, Al-Jamal MS, et al. The impact of oral Polypodium leucotomos extract on ultraviolet B response: a human clinical study. *J Am Acad Dermatol* 2017;77:33-41.e1.
- Granger C, Aladren S, Delgado J, Garre A, Trullas C, et al. Prospective evaluation of the efficacy of a food supplement in increasing photoprotection and improving selective markers related to skin photo-ageing. *Dermatol Ther (Heidelb)* 2020;10:163-78.
- Rabinovich L, Kazlouskaya V. Herbal sun protection agents: Human studies. *Clin Dermatol* 2018;36:369-75.
- Mohammad TF, Kohli I, Nicholson CL, Treyger G, Chaowattanapanit S, et al. Oral Polypodium leucotomos extract and its impact on visible light-induced pigmentation in human subjects. *J Drugs Dermatol* 2019;18:1198-203.
- Zamarrón A, Lorrio S, González S, Juarranz Á. Fernblock prevents dermal cell damage induced by visible and infrared A radiation. *Int J Mol Sci* 2018;19:2250.
- Winkelmann RR, Del Rosso J, Rigel DS. Polypodium leucotomos extract: a status report on clinical efficacy and safety. *J Drugs Dermatol* 2015;14:254-61.
- Farrar MD, Huq R, Mason S, Nicolaou A, Clarke KA, et al. Oral green tea catechins do not provide photoprotection from direct DNA damage induced by higher dose solar simulated radiation: a randomized controlled trial. *J Am Acad Dermatol* 2018;78:414-6.
- Nwanodi O. Skin protective nutraceuticals: the current evidence in brief. *Healthcare (Basel)* 2018;6:40.

20. Clarke KA, Dew TP, Watson RE, Farrar MD, Osman JE, et al. Green tea catechins and their metabolites in human skin before and after exposure to ultraviolet radiation. *J Nutr Biochem* 2016;27:203-10.
21. Farrar MD, Nicolaou A, Clarke KA, Mason S, Massey KA, et al. A randomized controlled trial of green tea catechins in protection against ultraviolet radiation-induced cutaneous inflammation. *Am J Clin Nutr* 2015;102:608-15.
22. Heinrich U, Moore CE, De Spirt S, Tronnier H, Stahl W. Green tea polyphenols provide photoprotection, increase microcirculation, and modulate skin properties of women. *J Nutr* 2011;141:1202-8.
23. Baccarin T, Mitjans M, Ramos D, Lemos-Senna E, Vinardell MP. Photoprotection by *Punica granatum* seed oil nanoemulsion entrapping polyphenol-rich ethyl acetate fraction against UVB-induced DNA damage in human keratinocyte (HaCaT) cell line. *J Photochem Photobiol B* 2015;153:127-36.
24. Baccarin T, Mitjans M, Lemos-Senna E, Vinardell MP. Protection against oxidative damage in human erythrocytes and preliminary photosafety assessment of *Punica granatum* seed oil nanoemulsions entrapping polyphenol-rich ethyl acetate fraction. *Toxicol In Vitro* 2015;30:421-8.
25. Henning SM, Yang J, Lee RP, Huang J, Hsu M, et al. Pomegranate juice and extract consumption increases the resistance to UVB-induced erythema and changes the skin microbiome in healthy women: a randomized controlled trial. *Sci Rep* 2019;9:14528.
26. Mintie CA, Singh CK, Ahmad N. Whole fruit phytochemicals combating skin damage and carcinogenesis. *Transl Oncol* 2020;13:146-56.
27. Kang SJ, Choi BR, Kim SH, Yi HY, Park HR, et al. Beneficial effects of dried pomegranate juice concentrated powder on ultraviolet B-induced skin photoaging in hairless mice. *Exp Ther Med* 2017;14:1023-36.
28. de Lima Cherubim DJ, Buzanello Martins CV, Oliveira Fariña L, da Silva de Lucca RA. Polyphenols as natural antioxidants in cosmetics applications. *J Cosmet Dermatol* 2020;19:33-7.
29. Zhou F, Huang X, Pan Y, Cao D, Liu C, et al. Resveratrol protects HaCaT cells from ultraviolet B-induced photoaging via upregulation of HSP27 and modulation of mitochondrial caspase-dependent apoptotic pathway. *Biochem Biophys Res Commun* 2018;499:662-8.
30. Wu Y, Jia LL, Zheng YN, Xu XG, Luo YJ, et al. Resveratrate protects human skin from damage due to repetitive ultraviolet irradiation. *J Eur Acad Dermatol Venereol* 2013;27:345-50.
31. Liu X, Zhang R, Shi H, Li X, Li Y, et al. Protective effect of curcumin against ultraviolet A irradiation-induced photoaging in human dermal fibroblasts. *Mol Med Rep* 2018;17:7227-37.
32. Skarupova D, Vostalova J, Rajnochova Svobodova A. Ultraviolet A protective potential of plant extracts and phytochemicals. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2020;164:1-22.
33. Vostálová J, Tinková E, Biedermann D, Kosina P, Ulrichová J, et al. Skin protective activity of silymarin and its flavonolignans. *Molecules* 2019;24:1022.
34. Dorjay K, Arif T, Adil M. Silymarin: an interesting modality in dermatological therapeutics. *Indian J Dermatol Venereol Leprol* 2018;84:238-43.
35. Rajnochová Svobodová A, Gabrielová E, Michaelides L, Kosina P, Ryšavá A, et al. UVA-photoprotective potential of silymarin and silybin. *Arch Dermatol Res* 2018;310:413-24.
36. Fidrus E, Ujhelyi Z, Fehér P, Hegedűs C, Janka EA, et al. Silymarin: friend or foe of UV exposed keratinocytes? *Molecules* 2019;24:1652.
37. Nobile V, Michelotti A, Cestone E, Caturla N, Castillo J, et al. Skin photoprotective and antiageing effects of a combination of rosemary (*Rosmarinus officinalis*) and grapefruit (*Citrus paradisi*) polyphenols. *Food Nutr Res* 2016;60:31871.
38. Gasparrini M, Forbes-Hernandez TY, Afrin S, Alvarez-Suarez JM, González-Paramàs AM, et al. A pilot study of the photoprotective effects of strawberry-based cosmetic formulations on human dermal fibroblasts. *Int J Mol Sci* 2015;16:17870-84.
39. Bucci P, Prieto MJ, Milla L, Calienni MN, Martinez L, et al. Skin penetration and UV-damage prevention by nanoberrries. *J Cosmet Dermatol* 2018;17:889-99.
40. Egoumenides L, Gauthier A, Barial S, Saby M, Orechenkoff C, et al. A specific melon concentrate exhibits photoprotective effects from antioxidant activity in healthy adults. *Nutrients* 2018;10:437.
41. Metral E, Rachidi W, Damour O, Demarne F, Bechetoille N. Long-term genoprotection effect of *Sechium edule* fruit extract against UVA irradiation in keratinocytes. *Photochem Photobiol* 2018;94:343-50.
42. Her Y, Shin BN, Lee YL, Park JH, Kim DW, et al. *Oenanthe javanica* extract protects mouse skin from UVB radiation via attenuating collagen disruption and inflammation. *Int J Mol Sci* 2019;20:1435.

Systematic Review

Open Access



# Partial flap loss in transgender phalloplasty using the anterolateral thigh or forearm - a systematic literature review

Isabel Cylinder<sup>1</sup>, Aaron Heston<sup>1</sup>, Breanna Jedrzejewski<sup>2</sup>, Zbigniew Sikora<sup>1</sup>, Blair Peters<sup>3</sup>, Jens Urs Berli<sup>2</sup>

<sup>1</sup>School of Medicine, Oregon Health & Science University, Portland, OR 97239, USA.

<sup>2</sup>Division of Plastic Surgery, Oregon Health & Science University, Portland, OR 97239, USA.

<sup>3</sup>Division of Plastic and Reconstructive Surgery, Washington University School of Medicine in St. Louis, MO 63110, USA.

**Correspondence to:** Dr. Jens Urs Berli, Division of Plastic Surgery, Oregon Health & Science University, 3303 SW Bond Avenue, Portland, OR 97239, United States. E-mail: berli@ohsu.edu

**How to cite this article:** Cylinder I, Heston A, Jedrzejewski B, Sikora Z, Peters B, Berli JU. Partial flap loss in transgender phalloplasty using the anterolateral thigh or forearm - a systematic literature review. *Plast Aesthet Res* 2020;7:58. <http://dx.doi.org/10.20517/2347-9264.2020.85>

**Received:** 20 Apr 2020 **First Decision:** 18 May 2020 **Revised:** 23 May 2020 **Accepted:** 19 Jun 2020 **Published:** 24 Oct 2020

**Academic Editor:** Marlon E. Buncamper, Stan J. Monstrey **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

**Aim:** In this study, we systematically review the current literature regarding partial flap loss (PFL) for the two most commonly performed types of phalloplasty, the radial forearm and the anterolateral thigh flaps. The primary purpose is to synthesize the available information to clarify anatomic location, etiology, extent of flap loss, and management thereof. Second, we utilize this information to inform strategies to mitigate the risk of PFL.

**Methods:** A systematic review of all abstracts published on phalloplasty on PubMed was performed. Abstracts were reviewed by two senior authors who included all studies discussing flap-related outcomes after radial forearm free flap (RFFF) phalloplasty or anterolateral thigh flap (ALT) phalloplasty for the treatment of gender dysphoria. Primary variables collected include: flap type, PFL rate, anatomic location, extent of and management of PFL.

**Results:** A total of 17 papers that reported on RFFF and/or ALT phalloplasty were included. A total of 780 RFFF and 182 ALT phalloplasties were identified. The PFL rate was 4.5% and 7.1% respectively. Only 4/17 papers commented on the anatomic location of PFL; none commented on the exact extent of PFL and only 4/17 commented on the management of PFL.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Conclusion:** The current literature suggests a higher rate of PFL in the ALT cohort (7.1% *vs.* 4.5%). The information available on PFL lacks detail as to the anatomic location, extent, and management of this complication. Future studies should strive to report on the above variables and include pertinent patient demographics and flap characteristics that may affect the rates and management of PFL. This information will assist in optimizing outcomes.

**Keywords:** Phalloplasty, transgender, flap loss, complications, gender dysphoria

## INTRODUCTION

Phalloplasty as a surgical treatment for gender dysphoria was pioneered by Sir Harold Gillies and has been performed since 1946<sup>[1]</sup>. Early techniques pre-dated the current era of mainstream microsurgery and relied on adjacent tissue transfer in the form of loco-regional pedicled flaps, typically tubed abdominal flaps<sup>[2]</sup>. As microsurgical techniques evolved and perforator flap dissection became clinically routine, tissue from more distant locations became available for the construction of a neophallus<sup>[3-5]</sup>. With better-suited skin and soft tissue quality and the ability for improved innervation, outcomes have improved significantly. This has ushered in the current era of phalloplasty for the treatment of gender dysphoria, where the focus is on achieving the best possible phallus reconstruction while minimizing overall morbidity. While many donor sites have been explored and are still currently utilized, the radial forearm and the anterolateral thigh are the most commonly used in phalloplasty surgery for gender dysphoria<sup>[6-9]</sup> [Figures 1 and 2]. Current techniques and surgical staging vary widely between institutions and individual surgeons, thus making it difficult to draw conclusions regarding the frequency and extent of complications. Furthermore, this makes it challenging to reach a consensus on best practice regarding the management of these complications and the sequelae of phalloplasty for the treatment of gender dysphoria.

The overall complication rate in phalloplasty for the treatment of gender dysphoria is extraordinarily high, with urologic complications being the most frequent<sup>[10,11]</sup>. While there are an abundance of publications regarding the management of urologic sequelae, the current body of literature suffers from a paucity of articles that report on flap-related complications in detail. Additionally, the majority of evidence available is in the form of case reports and retrospective case series. Currently, randomized controlled trials and cohort studies are exceedingly rare in this field of practice. Larger systematic reviews have been performed but due to the heterogenous nature of surgical techniques, staging, and extent of reporting in the current body of literature, they often lack granularity<sup>[12]</sup>.

One complication that suffers from the above shortcomings in the literature is that of partial flap loss (PFL). This is often only mentioned in a list of complications or as an overall percentage. With the size of flaps used for phalloplasty often extending beyond their traditional dimensions, it is no surprise that reported rates are relatively high. On anecdotal literature review, we noticed that beyond incidence, there is very little discussion regarding anatomic location, etiology, or extent of PFL, or the management of PFL when it occurs. We therefore felt it was timely to systematically analyze the current literature for the two most commonly performed types of phalloplasty: radial forearm free flap (RFFF) and anterolateral thigh flap (ALT) to determine an overall PFL rate following these procedures. Next, we sought to synthesize the currently available information regarding the anatomic location, extent, etiology, and management of PFL. We then utilized this information to inform a discussion on the current literature and outline future directions regarding risk mitigation and the management of PFL following phalloplasty for the treatment of gender dysphoria.





**Figure 1.** Results after radial forearm free flap phalloplasty



**Figure 2.** Result after antero-lateral thigh flap phalloplasty<sup>[33,34]</sup>

## METHODS

A systematic literature review of all articles published on the topic of phalloplasty up to and including March 2020 was performed using the PubMed database. Our search was designed to capture all published studies that fulfill our inclusion criteria of presenting primary data on the flap-related outcomes of RFFF or ALT phalloplasty as a gender-affirming surgical procedure. RFFF and ALT were defined as the main flap used for shaft creation. Exclusion criteria included: case reports, review articles; phalloplasty performed for reasons other than gender-affirming care; phalloplasty performed using techniques that did not include either RFFF or ALT, or did not specify the technique used for reconstruction; and articles that did not include flap outcomes in the dataset (i.e., urologic outcomes following phalloplasty, prosthesis outcomes following phalloplasty). Articles published in any journal in both the English and German languages were considered. Data were collected on patient demographics, flap design, partial flap loss, total flap loss, timing of glansplasty, location of flap loss and management thereof.

The search term used was “phalloplasty”. All titles were screened by the senior author and a second author with experience in the field of transgender surgery. Articles that met the exclusion criteria were dismissed. All remaining articles were then screened for abstract content by four reviewers. Any additional article that met the exclusion criteria was subsequently removed. The remaining articles were then formally reviewed and all articles that met the inclusion criteria were selected for this review. By including the date range for each article, we tried to make sure there was no double reporting from the same institution and patients were not accidentally counted twice. If a later publication included the time period of an earlier one, then the earlier publication was excluded. See [Figure 3](#) for an overview of the systematic review and [Tables 1-3](#) for results.

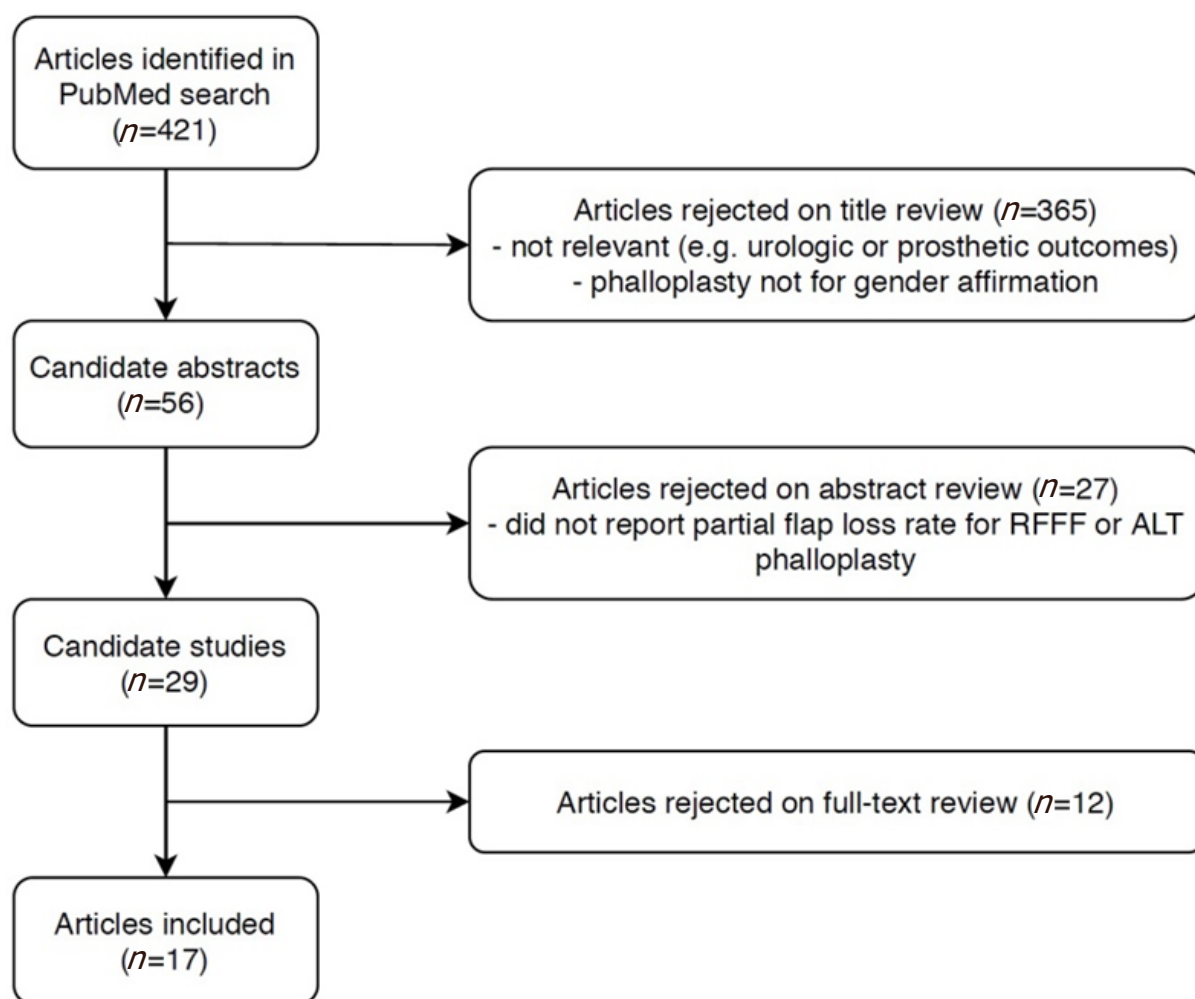
## RESULTS

A total of 421 publications were identified in the initial literature review. Of these, 56 abstracts were relevant to phalloplasty for the treatment of gender dysphoria and thus were screened systematically. 29 papers were determined to meet the inclusion criteria and were fully reviewed. Following full review, only 17 papers had RFFF and/or ALT phalloplasties in the specified cohort and clearly indicated rates of partial flap loss [[Figure 3](#)]. All articles were retrospective case series. A total of 1,199 phalloplasties, consisting of 836 RFFF and 210 ALT flaps, were identified in these 17 papers. The collective PFL rate for all flap types included in these studies was 6.1% [[Table 1](#)]<sup>[13-29]</sup>.

Subgroup analysis was performed to determine the rate of PFL in RFFF and ALT flaps respectively. On subgroup analysis, articles that did not specify flap type when reporting partial PFL rates were excluded<sup>[13,21,26]</sup>. A total of 780 RFFF were identified from 11 articles, with a PFL rate of 4.5% [[Table 2](#)]<sup>[14,15,17-19,22-25,27,29]</sup>. A total of 182 ALT flaps were identified from four articles, with a PFL rate of 7.1% [[Table 3](#)]<sup>[14,16,20,28]</sup>.

Only 4/17 papers commented on the anatomic location of PFL. All four of these papers pertained to RFFF phalloplasty (RFFFP) and reported necrosis of the distal tip. No papers commented on the exact extent of PFL when an anatomic location was mentioned, though Fang *et al.*<sup>[18]</sup> reported 10% reduction in length of the neophallus as an outcome for one patient following PFL.

Only 4/17 papers commented on the management of PFL when it occurred. Two of these papers pertained to RFFF and two pertained to ALT. Management of PFL following RFFFP was reported as healing by secondary intention or debridement and VAC therapy followed by skin grafting by Song *et al.*<sup>[27]</sup>, or repeat RFFFP by Baumeister *et al.*<sup>[15]</sup>. Management of PFL following ALT flap phalloplasty was reported as split-thickness skin grafting by van der Sluis *et al.*<sup>[28]</sup>, or debridement and skin grafting by D'Arpa *et al.*<sup>[16]</sup>. No outcome articles included any photographs.



**Figure 3.** Flowchart demonstrating the process of identifying, including and rejecting studies to be utilized in this systematic review

## DISCUSSION

Traditionally, phalloplasty as a surgical treatment for gender dysphoria has only been offered by a small group of surgeons at a few select centers around the world. These groups have been instrumental in developing the techniques that we are currently using today. The body of literature that has amassed over the past four decades focuses mainly on the various potential donor sites and grouped outcomes of a variety of technical modifications, with a persisting majority consensus that the radial forearm presents the current donor site of choice. Typically, the anterolateral thigh is the most common alternative to the radial forearm. Amassed experience with these two donor sites for phalloplasty in the transgender population has allowed phalloplasty to go from a relatively experimental procedure to a somewhat standardized set of procedures to achieve masculine-appearing and functional external genitalia. However, in the grand scheme of reconstructive surgery, phalloplasty is still a relatively rare procedure. Due to the various donor sites utilized across the world and the breadth of technical variations on flap design, harvest, shaping, and different variations regarding surgical stages, the literature lacks further insight into the various aspects of phalloplasty. A large portion of the literature focuses on reporting the successful use of various donor sites, with the take-home message that phalloplasty can be performed with success. As a surgical community treating patients with gender dysphoria, we are just beginning to progress past this more superficial perspective. It is imperative that we move beyond just broad reporting of complication rates and begin to include data on the severity of specific complications along with the impact and management

**Table 1. Overall literature review results including all studies that included results for partial flap loss in either RFFF or ALT phalloplasty**

Authors, years	Institution	Dates collected	Total # patients	No. RFFF	No. ALT	Mean age	Smoking	Type of flap (n)	Flap design	Giansplasty timing	Flap take back rate	Complete flap loss complication rate (%)	Partial flap loss complication rate (%)	Flap necrosis location	Management of partial flap loss
Matti <i>et al.</i> <sup>[25]</sup> , 1988	West Middlesex University Hospital	1983-1985	5	5	0	NR	NR	RFFF (5)	TWT	NR	NR	2/5 (40%)	1/5 (20%)	NR	NR
Hage <i>et al.</i> <sup>[21]</sup> , 1993	Free University Amsterdam	1984-1991	24	6	0	33	NR	SIEA (15), Rectus abdominus (4), RFFF (6)	Hinged, TWT, free flap	NR	NR	4/24 (16.6%)	8/24 (33.3%)	NR	NR
Fang <i>et al.</i> <sup>[19]</sup> , 1994	Veterans General Hospital, Taipei	1988 - April 1993	56	56	0	NR	NR	RFFF (56)	TWT (28); prefabrication of neurethra with tubed vaginal mucosa graft (28)	NR	NR	1/56 overall (1.8%); 1/28 TWT (3.6%); 0/28 prefab (0%)	6/56 overall (10.7%); 6/28 TWT (21.4%); 0/28 prefab (0%)	Distal ulnar portion	NR
Fang <i>et al.</i> <sup>[18]</sup> , 1999	Veterans General Hospital and National Yang-Ming University, Taipei	Jul 1994 - Dec 1996	22	22	0	NR	NR	RFFF (22)	Prefabrication of neurethra with tubed vaginal mucosa graft	2-3 weeks later	NR	0/22 (0%)	1/22 (4.5%)	Tip	NR
Felici <i>et al.</i> <sup>[20]</sup> , 2006	Azienda Ospedaliera 'S. Camillo-Forlanini', Rome	2003 - 2004	7	0	6	NR	5/7 (71%)	ALT (6), suprapubic (1)	ALT shaft, periclitoreal cutaneous neurethra	2 months later	NR	0/7 (0%)	0/7 (0%)	N/A	N/A
Leriche <i>et al.</i> <sup>[24]</sup> , 2008	University of Lyon	1986-2002	56	56	0	30	NR	RFFF (56)	TWT	Delayed	1 take back for arterial compromise, went to contralateral DIEA	3/56 (5.4%)	2/56 (3.6%)	Distal tip necrosis	NR
Kim <i>et al.</i> <sup>[22]</sup> , 2009	Dong-A University, Korea	1991-2005	40	40	0	34	NR	RFFF (40)	Osteocutaneous TWT	Immediate	NR	1/40 (2.5%)	3/40 (7.5%)	NR	NR
Song <i>et al.</i> <sup>[27]</sup> , 2011	Singapore General Hospital	1999-2009	19	19	0	NR	NR	RFFF (19)	Prelaminated neurethra, Osteocutaneous RFFF	Immediate	NR	2/19 (10.5%)	3/19 (15.8%)	Tip necrosis (2), Distal flap loss (1)	Healing by secondary intention (2) Debridement and VAC therapy + skin grafting (1)

Baumeister <i>et al.</i> <sup>[15]</sup> , 2011	Markus Krankenhaus	1993 - 2009	252	135	0	40	NA	RFF (135), groin (119), fibula (2), scapula (1), pedicled randomized (2)	TWT	Delayed	10	7/252 overall (2.7%); 2/135 RFFFP (1.5%)	12/252 overall (4.7%); RFFFP (8.9%)	Shaft (1)	Repeat radial forearm for shaft
Wirthmann <i>et al.</i> <sup>[29]</sup> , 2018	AGAPLESION Markus hospital	January 1993 - December 2015	229	232	0	33.8	105/229 (45.9%)	Gottlieb & Levine (93.9%); Chang & Hwang (3.2%)	TWT	Immediate with Gottlieb design	NR	7/232 (3.0%)	2/232 (0.9%)	NR	NR
van der Sluis <i>et al.</i> <sup>[28]</sup> , 2017	VU University Medical Center, Amsterdam	January 2008 - December 2015	19	0	19	36.5	6/19 (31.5%)	Composite stage pedicled ALT with RFFFP urethra	TWT (single- ALT with RFFFP urethra)	NR	NR	3/19 overall (15.8%); 1/19 ALT (5.2%); 2/19 RFFFP neourethra (10.5%)	1/19 overall (5.2%); 1/19 ALT (5.2%); 0/19 RFFFP neourethra (0%)	NR	Partial necrosis of the ALT flap occurred in one patient, which was salvaged with split- thickness skin grafting of an area of 2 x 1 cm
Ascha <i>et al.</i> <sup>[14]</sup> , 2018	Private Practice, San Francisco	April 2013 - July 2016	213	149	64	36.7 RFFFP; ALT 36.2 ALT 2/149 RFFFP (3.0%)	0/64 ALT (0%); 2/149 RFFFP (3.0%)	Pedicled ALT (64); RFFFP (149)	TWT	RFF: Immediate ALT: Delayed	NR	NR	10/213 overall (4.7%); 5/64 ALT (7.8%); 5/149 RFFFP (3.4%)	NR	NR
De Wolf <i>et al.</i> <sup>[17]</sup> , 2019	Ghent	NA	27 (15 had supercharging)	27	0	NA	NA	RFFFP (27)	TWT	1 week later	NR	0/27 (0%)	0/27 (0%)	N/A	N/A
Küntschner <i>et al.</i> <sup>[23]</sup> , 2019	Elisabeth Klinik	2011-2017	39	39	0	34.8	20/39 (51.3%)	RFFFP (39)	Prelamination with skin graft	Delayed	NR	2/39 (5.1%)	0/39 (0%)	NR	NR



D'Arpa <i>et al.</i> <sup>[16]</sup> , 2019	University of Ghent	2004- 2017	93	29 in combo with ALT	93	36	None	All ALT with some combination of RFFF (29), SCIP (38), previous phalloplasty flap (6)	Shaft only, TWT, combination with skin graft, second flap	1 week later	NR	7/93 overall (7.5%); 0/5 TWT (0%); 0/8 prelam (0%); 0/7 shaft- only (0%); 7/73 composite (14.3%); 6/73 (8.2%); [3/29 ALT + RFFF (10.3%); 4/38 ALT + SCIP (13.2%); (10.5%); 0/6 ALT + previous flap (0%)]	7/93 overall (7.5%); 0/5 TWT (0%); 0/8 prelam (0%); 1/7 shaft- only (0%); (14.3%); 6/73 (8.2%); [0/29 ALT + RFFF (0%); 5/38 ALT + SCIP (13.2%); 1/6 ALT + previous flap (16.7%)]	NR	Debridement, skin grafting
Namba <i>et al.</i> <sup>[26]</sup> , 2019	Okayama University	2001- 2018	15	9 in combo with others	6	NR	None	All some combination of RFFF (9), DIEP (9), SCIP (4), ALT (6)	Flap combination (2-3 different flaps per phallus i.e., bilateral SCIP or RF + ALT + SCIP)	NR	4/15 (26.6%) (reoperation for re- anastomosis)	0/15 (0%)	1/15 (6.7%)	RF flap	NR
Al-Tamimi <i>et al.</i> <sup>[13]</sup> , 2019	Vrije Universiteit Amsterdam, Belgrade, Miami, Ghent, Bordeaux, Montreal, Helsinki, Crane Surgical Services	NR	83	41	22	32	26/83 (31%)	RFFF (41), ALT (22), latissimus dorsi (8), gracilis (5), DIEP (4), groin (2), lateral upper arm (1)	Various	NR	NR	1/83 (1.2%)	16/83 (19.2%)	ALT flap	NR
Total	-	-	1199	836/1199 (69.7%)	210/1999 (17.5%)	-	-	-	-	-	-	40/1199 (3.3%)	73/1199 (6.1%)		

RFFF: radial forearm free flap phalloplasty; ALT: anterolateral thigh flap; NR: not reported; RFFF: radial forearm free flap; TWT: tube within a tube design; SIEA: superior inferior epigastric artery; DIEP: deep inferior epigastric perforator; SCIP: superficial circumflex iliac perforator

**Table 2. Extracted data for RFFFP partial flap loss used for shaft reconstruction**

<b>Radial forearm free flap phalloplasty</b>					
<b>Flap design</b>	<b># patients</b>	<b>Total flap loss (%)</b>	<b>Partial flap loss (%)</b>	<b>Necrosis location</b>	<b>Management</b>
TWT	672	16/672 (2.4%)	31/672 (4.6%)	-	-
Matti <i>et al.</i> <sup>[25]</sup> , 1988	5	2/5 (40%)	1/5 (20%)	NR	NR
Fang <i>et al.</i> <sup>[19]</sup> , 1994	28	1/28 (3.6%)	6/28 (21.4%)	Distal ulnar portion	NR
Leriche <i>et al.</i> <sup>[24]</sup> , 2008	56	3/56 (5.4%)	2/56 (3.6%)	Distal tip necrosis	NR
Kim <i>et al.</i> <sup>[22]</sup> , 2009	40	1/40 (2.5%)	3/40 (7.5%)	NR	NR
Baumeister <i>et al.</i> <sup>[15]</sup> , 2011	135	2/135 (1.5%)	12/135 (8.9%)	Shaft	Repeat RFFFP
Wirthmann <i>et al.</i> <sup>[29]</sup> , 2018	232	7/232 (3.0%)	2/232 (0.9%)	NR	NR
Ascha <i>et al.</i> <sup>[14]</sup> , 2018	149	NR	5/149 (3.4%)	NR	NR
De Wolf <i>et al.</i> <sup>[17]</sup> , 2019	27	0/27 (0%)	0/27 (0%)	N/A	N/A
Prefab urethra	108	4/108 (3.7%)	4/108 (3.7%)	-	-
Fang <i>et al.</i> <sup>[19]</sup> , 1994	28	0/28 (0%)	0/28 (0%)	N/A	N/A
Fang <i>et al.</i> <sup>[18]</sup> , 1999	22	0/22 (0%)	1/22 (4.5%)	Tip	NR
Song <i>et al.</i> <sup>[27]</sup> , 2011	19	2/19 (10.5%)	3/19 (15.8%)	Tip necrosis (2); Distal flap loss (1)	Healing by secondary intention (2) Debridement and VAC therapy + skin grafting (1)
Küntschner <i>et al.</i> <sup>[23]</sup> , 2019	39	2/39 (5.1%)	0/39 (0%)	N/A	NR
Total	780	20/780 (2.6%)	35/789 (4.5%)	-	-

RFFFP: radial forearm free flap phalloplasty; TWT: tube within a tube design; NR: Not reported; N/A: not applicable; VAC: vacuum-assisted closure of wound

**Table 3. Extracted data for ALT partial flap loss used for shaft reconstruction**

<b>Anterolateral thigh flap phalloplasty</b>						
<b>Flap design</b>	<b># patients</b>	<b>Pedicled vs. free</b>	<b>Total flap loss (%)</b>	<b>Partial flap loss (%)</b>	<b>Necrosis location</b>	<b>Management</b>
TWT	69		0/69 (0%)	5/69 (7.2%)	-	-
Ascha <i>et al.</i> <sup>[14]</sup> , 2018	64	Pedicled	NR	5/64 (7.8%)	NR	NR
D'Arpa <i>et al.</i> <sup>[16]</sup> , 2019	5	Pedicled*	0/5 (0%)	0/5 (0%)	N/A	NA
Prefab urethra	8		0/8 (0%)	0/8 (0%)	-	-
D'Arpa <i>et al.</i> <sup>[16]</sup> , 2019	8	Pedicled*	0/8 (0%)	0/8 (0%)	N/A	N/A
Composite	98		10/98 (10.2%)	7/98 (7.1%)	-	-
Felici <i>et al.</i> <sup>[20]</sup> , 2006	6	Free	0/6 (0%)	0/6 (0%)	N/A	N/A
van der Sluis <i>et al.</i> <sup>[28]</sup> , 2017	19	Pedicled	1/19 complete ALT loss (5.2%) 2/19 complete RFFF neourethral losses (10.5%)	1/19 (5.2%)	NR	Partial necrosis of the ALT flap occurred in one patient, which was salvaged with split-thickness skin grafting of an area of 2 cm × 1 cm
D'Arpa <i>et al.</i> <sup>[16]</sup> , 2019	73	Pedicled*	7/73 (9.6%) [3/29 ALT + RFFF (10.3%); 4/38 ALT + SCIP (10.5%); 0/6 ALT + previous flap (0%)]	6/73 (13.2%) [0/29 ALT + RFFF (0%); 5/38 ALT + SCIP (13.2%); 1/6 ALT + previous flap (16.7%)]	NR	Debridement, skin grafting
Shaft-only	7		0/7 (0%)	1/7 (14.3%)	-	-
D'Arpa <i>et al.</i> <sup>[16]</sup> , 2019	7	Pedicled*	0/7 (0%)	1/7 (14.3%)	NR	NR
TOTAL	182		10/182 (5.5%)	13/182 (7.1%)	-	-

TWT: tube within a tube design; NR: not reported; N/A: not applicable; ALT: anterolateral thigh flap; RFFF: radial free flap; SCIP: superficial circumflex iliac perforator

of said complications. This has, in part, been done more successfully regarding the urologic complications following phalloplasty, but less so for flap-related complications. Total flap loss remains the most feared complication of phalloplasty. This particular complication is self-explanatory, “total” meaning the entire flap is non-viable. Additionally, “partial flap loss” has been routinely reported, but few studies mention how “partial” is defined. “Partial” can refer to a variety of flap involvement, spanning from a simple issue such as minor marginal flap necrosis to a major complication with large volumes of tissue loss leading to multiple revision surgeries and impaired long-term aesthetic and functional outcomes. This was demonstrated in the literature review with the term “partial flap loss” being used to describe both small areas of distal necrosis and cases that mandated an additional free flap. This reinforces the vague definition of “partial flap loss” that plagues the current literature. Beyond the issue of a lack of consistent and meaningful reporting, there is very little offered in the literature regarding the mitigation of PFL in phalloplasty procedures. Based on this, we felt that a systematic review was a good first step to assess what we currently know about PFL, specifically regarding location, extent, and management of PFL.

### **Etiology and reduction of risk for development of partial flap loss**

While the current data is lacking for some of the following statements, we would like to suggest the following etiologies for PFL in RFFFP and ALT phalloplasty, respectively.

(A) For all flaps:

- (1) Patient selection: It is well established that certain medical co-morbidities or drug/substance abuse can affect PFL rate (e.g., peripheral vascular disease and diabetes, smoking and cocaine, *etc.*)<sup>[30]</sup>.
- (2) Flap design that includes dimensions beyond the capacity of perforators included in the flap and therefore relies on random pattern circulation for areas of tissue that are too far removed from the included perfasomes.
- (3) Technical error by inadvertently excluding or injuring perforating vessels during flap design and harvest.

(B) Specific to RFFFP:

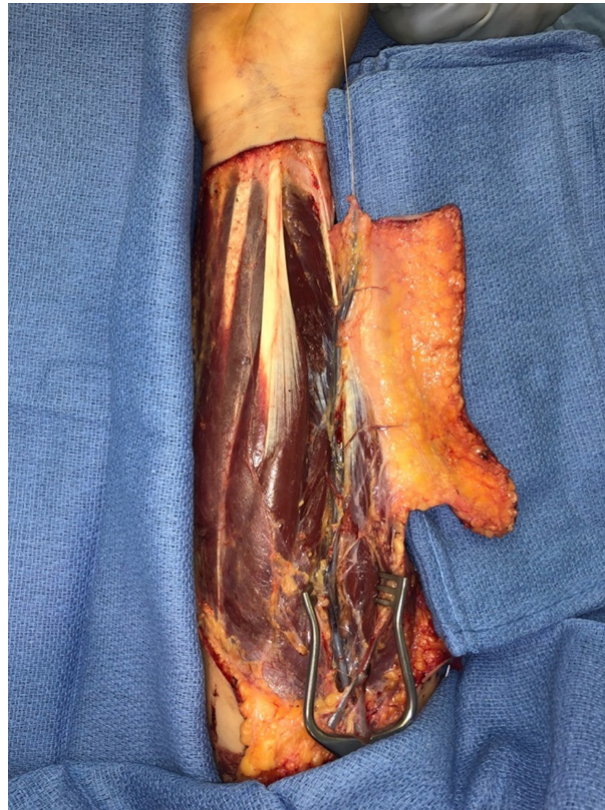
- (1) Failure to include the critical and sparse proximal radial artery perforators during flap harvest and only relying only on distal perforators [Figure 4].
- (2) Positioning of the flap design that fails to place the radial artery as close to the center of the flap as possible.

(C) Specific to ALT phalloplasty:

- (1) Failure to include or the absence of distal perforators.
- (2) Inability to position the flap distal enough on the thigh allowing adequate pedicle length for transposition while still including an adequate number of perforators to support the volume of tissue harvested.
- (3) Excessive intra-operative thinning or excessive size of flap harvested with tissue take beyond the perfusion capacity of the perfasomes.
- (4) Not delaying the flap when there is concern regarding adequacy of perfusion. Delay will assist in flap maturation and improve perfusion characteristics.

### *A1 patient selection*

The mean age of patients included in the systematic review was 34.6 years. Given the increasing accumulation of medical co-morbidities with age, this speaks to the fact that overall, this is a predominantly healthy patient population. Unfortunately, none of the articles mentioned the age of patients suffering from PFL. Smoking is a well-established risk factor for PFL and other associated wound healing issues at both the recipient and donor sites<sup>[30]</sup>. We were surprised to find that some centers offer phalloplasty to active



**Figure 4.** Elevation of radial forearm free flap with proximal radial artery perforator

smokers. We strongly advise that patients have a negative cotinine test three months prior to phalloplasty surgery. Although typical recommendations are to stop smoking 4-6 weeks prior to an elective surgery from a physiologic standpoint, smoking cessation comes with marked psychological stress and as such, it is advisable to deal with this significant life stressor prior to adding the mental and physical stress of complex surgery. Diabetes should be well controlled, with a Hb1Ac within normal limits. While obesity itself is not known to be a risk factor for PFL, it can make the microvascular aspect of the surgery difficult or impossible. We recommend that ideally, patients have a BMI < 30, although we evaluate any patient with a BMI < 35 as fat distribution is a greater determining factor than BMI alone. In the studies that did report on BMI, none assessed whether increased BMI was a risk factor for PFL. It is important for future research to include all relevant co-morbidities and patient demographics. It would be impactful if this information was included both for the total number of patients as well as for the subgroups suffering complications. This would allow future meta-analyses to be performed in order to perform subgroup analysis and identify risk factors for PFL, as well as other complications. It is important to identify modifiable pre-operative risk factors. As previously stated, complication rates are extremely high following phalloplasty surgery. Although these surgeries are essential to this patient population, there is time in the pre-operative assessment period to introduce lifestyle modifications and optimize the medical management of co-morbidities to optimize patient condition, and in doing so, hopefully minimize complication rates. The first step in this process is to identify the risk factors that appear to correlate with specific complications.

#### *A2 flap dimensions*

The dimensions of the RFFF and ALT flap used for phalloplasty are significantly larger than those of a standard RFFF or ALT. This is especially true when performing a tube within tube (TWT) phalloplasty. The ideal TWT flap dimensions are at least 16 cm in width and 16 cm or more in length. These dimensions are

far beyond most traditionally-described ALT and radial forearm flaps, pushing the limits of the perforators and their associated perfasomes to perfuse the flap via linking vessels. De Wolf *et al.*<sup>[17]</sup> recognized this issue and utilized intraoperative indocyanine green angiography to identify RFFF that that would benefit from supercharging and did so by using the posterior interosseous artery. In these cases, the anastomosis is performed to the palmar branch of the radial artery distally or in an end-to-side fashion proximally. In their study of 29 patients, 15 had supercharging of the radial forearm flap as described above, resulting in no cases of PFL.

In cases utilizing the ALT flap, dimensions are often even wider than those of the radial forearm. This is because the ALT flap is significantly thicker with a greater proportion of adipose tissue when compared to the radial forearm. This additional bulk often requires a wider skin paddle in order to be able to tube the flap around the thicker adipose tissues. Care has to be taken to include as many perforators as possible in order to support this increased flap volume (see section C1 ALT - absence of or failure to include distal perforators). Intuitively, it makes sense that if an ALT or RFFF is only used for shaft-only and composite flaps, the PFL rate would be lower due to smaller dimensions. The current literature shows a trend in that direction except for the shaft only ALT, which had a markedly higher rate of PFL [Table 3]. However, this is based on a single study that may have been an outlier. Future studies should include flap dimensions to account for lengths that may vary between cohorts and the number of perforators included.

### *A3 technical errors*

Obviously, any technical errors diminishing perfusion increase the risk for partial or total flap loss. For RFFF, a sub-fascial dissection between the flexor carpi radialis and past the superficial radial nerve can assist in protecting the radial artery and its associated perforators. During ALT harvest, care has to be taken to include all possible perforators, including those with a complex intramuscular course due to the large dimension of the flap taken and its associated volume of tissue. The time spent meticulously dissecting out all viable perforators will be well-spent by decreasing the risk of areas of PFL as much as possible. Failure to convert to a free flap in case of a short pedicle or failure to create a wide enough tunnel are other technical aspects to consider.

### *B1 RFFFP - proximal perforator inclusion*

In a traditional antegrade radial forearm flap, only the distal perforators are captured. In the RFFFP, it is important to also capture the proximal perforators<sup>[7]</sup> [Figure 4]. There is usually one distinct large perforator entering the flap that may exit the radial artery proximal to the proximal flap edge. If unaware of its location and importance, it is easily divided. In our practice, we have realized that this perforator may even take a brief intramuscular or intratendinous course and care should be taken to identify this perforator and dissect it out. Some authors advocate keeping a dermal bridge between the shaft and the proximal urethral extension to further improve blood supply to this watershed region. In the literature, the most commonly reported region of PFL was the distal radial border of the flap; this correlates with our own experience<sup>[16]</sup>. The vascular territory supplied by perforators of the posterior interosseous artery correlates anatomically to the dorsal and radial aspect of the radial forearm flap. Therefore, the previously mentioned strategy of supercharging with use of the posterior interosseous artery may be an ideal strategy to avoid PFL in this location<sup>[16]</sup>.

### *B2 RFFFP - location*

None of the review articles comment on the exact placement of the RFFFP design on the forearm in regards to the location of the radial artery. Most articles that discuss technique do, however, comment on the benefit of placing the urethra over the ulnar side of the forearm due to the sparse amount of hair follicles in this region. Although a valuable point regarding hair follicle density, this is not always the best strategy in regards to optimizing flap perfusion. In the case of a small forearm circumference, this may



lead to placement of the flap that is relatively too radial with eccentric location of the radial artery. This can increase the risk of local areas of ischemia and flap loss. In our practice, in patients with a forearm circumference of less than 15.5 cm we place the flap further ulnar beyond the least hair-bearing area to allow the radial artery to be more centralized. We have no data to support this claim, but it makes logical sense to have the perforators closer to the edge that is most affected by PFL (dorsoradial) and hopefully capture enough linking vessels to allow for perfusion of the entire flap.

#### *C1 ALT - absence of or failure to include distal perforators*

When it comes to ALT phalloplasty, the tissue of the distal aspect of the thigh has two very important benefits compared to harvesting the ALT from the mid-thigh: (1) thinner and more pliable tissue; and (2) a longer vascular pedicle allowing for a comfortable transfer to the genital region. The obvious downside however, is that more distal placement of the flap design often does not allow capture of the proximal and often more sizeable perforators. Additionally, distal perforators are more often intramuscular and necessitate a more difficult intramuscular dissection. Pre-operative CT angiography (CTA) can assist decision-making by identifying the laterality that is most likely to have numerous perforators in the desired flap location and have the highest take off of the lateral femoral circumflex artery, allowing for greater pedicle length for flap transposition<sup>[31]</sup>. In addition to CTA, we routinely perform hand-held Doppler exams in our clinic to document the location of audible perforators. Even in ideal circumstances, it is typical that the most sizeable perforators will often enter the proximal third of the flap. A strategy discussed at oral presentations by the Buncke clinic group (San Francisco) is to include a de-epithelized proximal dermal extension to capture perforators located proximally to the flap design.

#### *C2 ALT - excessive intraoperative thinning*

In most circumstances, the ALT is too bulky for TWT phalloplasty. Even in thin patients, the dimensions of the flap often get too bulky. In these cases, intraoperative thinning of the subscarpal fat layer is a useful strategy to allow for a more natural aesthetic result. We favor doing primary shaping rather than secondary liposuction or lipectomy. However, this is not without consequence. As the lateral femoral cutaneous nerve runs along this fascial plane, it jeopardizes innervation to the neophallus and, if done too excessively, can impede the subcutaneous connecting vascular plexus. In our experience, however, judicious thinning allows for decreased tension on the tissues when they are tubed and comes with improved overall flap vascularity by reducing the overall bulk and size of the flap.

#### *C3 - Failure to delay the flap*

Despite meticulous pre-operative medical and physiological optimization and surgical planning, the size of the flap may be just too big for the few available perforators on which the flap can be elevated. Therefore, it is our opinion that a low threshold should be held to delay a flap that appears congested or marginally perfused in the operating room. We have performed this successfully in two cases. Of course, we cannot know if the flaps would have been adequately perfused if transferred and shaped primarily as originally planned. However, especially with a TWT flap, it makes logical sense that a flap that is already mildly congested will not endure additional strain and insult. In these two cases, we took the patient back on postoperative days 5 and 7, respectively. They both did well and the flaps appeared less congested at the time of take back. There is little risk to flap delay and it may avoid disastrous complications for the patient. A phalloplasty is not like a standard flap in that the shaping is much more aggressive. The flap is being tubed, often twice on itself. Therefore, the vascular inflow and outflow must be robust. If relying only on random pattern perfusion through choke vessels outside of the perfasomes of the flap, the tubing of the flap will almost certainly lead to vascular issues. Therefore, delay should be considered if absolute confidence does not exist in regards to flap vascularity. The overall quality of a reconstruction should never be compromised in an effort to cut down on surgical stages; if necessary, delay.

### Location and extent of partial flap loss

The location of PFL will dictate both its management and morbidity. Distal radial edge necrosis of a RFFFP can be easily managed with excision and skin grafting. This differs from necrosis in a more critical area such as the proximal urethral extension, which may lead to fistula and stricture formation and the need for multiple reconstructive procedures. In the literature, there was a glaring deficiency in clearly outlining both the anatomic location as well as the extent of PFL. Only 4 of 17 studies reported on the anatomic location of PFL - all mentioned the tip or shaft (distal tissue) and none commented on the loss of tissue in the region of the proximal urethra. This matches our own experience and may be an indication that the proximal urethra may have better blood supply than is often feared [Figure 5]. A discussion on the anatomic location of PFL when referring to the ALT flap is complicated by the confounding factor that surgeons often place the urethra on different sides of the ALT flap design. For example, we like to place the urethra on the lateral aspect of the thigh, while other centers place it medially<sup>[32]</sup>. We believe the vascularity to be better laterally since the angle of the entry point of the perforators point laterally and inferiorly.

Most studies that reported on the timing of glansplasty have delayed it for the ALT cohort as the perforators enter the flap more proximally than they do in a RFFF. The studies with the highest amount of PFL unfortunately did not include this detail. Even if all studies included the timing of glansplasty, we would also need to know if this was done in a sub- or intra-dermal layer as this may affect the outcome.

### Management of partial flap loss

Despite our best efforts, PFL will likely always be a possibility in the field of phalloplasty due to the large flap dimensions necessary for construction of the neophallus. The management of PFL will very much depend on both the anatomic location and extent of tissue loss. When PFL occurs in the urethra, there can be difficulty identifying its occurrence in a timely fashion since this part of the reconstruction is buried either within the shaft or, in the case of single-stage phalloplasty, in the prepubic region. This may delay diagnosis and only become apparent in the setting of an infection or stricture.

#### *Urethral loss*

The management of urethral loss depends on the extent of involvement. Smaller segments can be managed by excision and re-anastomosis, or a two-stage urethroplasty using buccal mucosa grafts. Larger segments of urethral loss may necessitate additional tissue transfer, which adds to the donor site burden and potentially increases the overall complication profile. At our institution, we perform a staged approach to phalloplasty as developed by the St. Peter Andrology Institute. In this strategy, the urethra is marsupialized adjacent to the clitoral shaft at the first stage and as such, is amenable to visible inspection in the postoperative period [Figure 6]. At the second stage, the urethra is connected to the native urethra using vulvar tissue. To allow for this, the ipsilateral labia minora is resected during the first surgery. If done in this fashion, any ischemia can be identified promptly and the urethra can be shortened and marsupialized at a higher level if necessary [Figure 7].

#### *Shaft loss*

The management strategy regarding the loss of shaft tissue also depends on the extent of involvement. Larger areas may require an additional free flap. In the case of full thickness tissue loss, we favor placement of Integra (Integra, Princeton NJ) followed by the application of a full thickness skin graft. Skin grafting alone may suffice in the setting of partial thickness tissue loss [Figure 5].

### CONCLUSION

The current literature is inconsistent with reports on PFL, and details regarding the anatomic location, extent, and management of PFL are often lacking. This is further complicated by the wide variety of donor sites used for phalloplasty, such that interpretation of the literature becomes even more difficult. In this

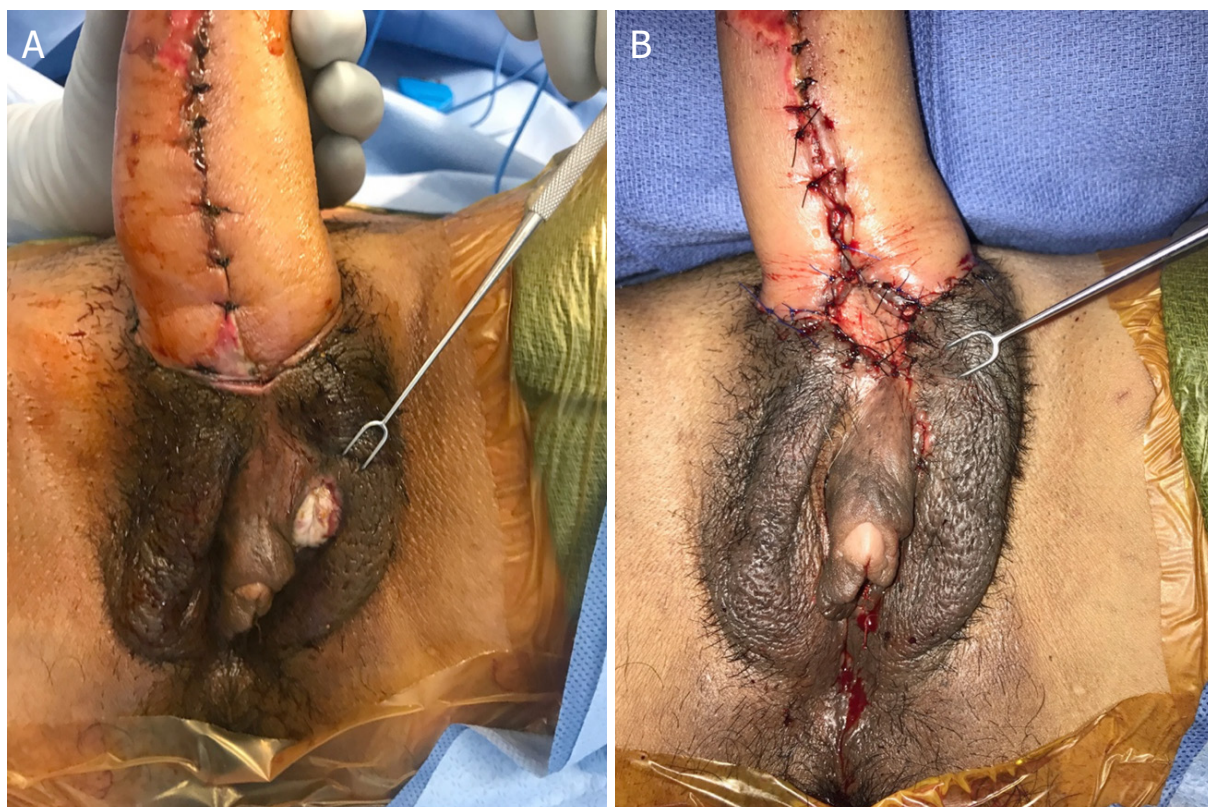


**Figure 5.** An example of partial flap loss in radial forearm free flap phalloplasty at the distal radial aspect of the flap. A: debridement of the flap and placement of Integra (Integra, Princeton, NJ); B: status post full thickness skin graft (FTSG); C: healed appearance following FTSG<sup>[34,35]</sup>





**Figure 6.** Appearance at the second stage of a Big Ben style two-stage phalloplasty. Note the marsupialized pars pendulans urethra<sup>[33,34]</sup>



**Figure 7.** Example of partial flap loss of the proximal urethral extension in an anterolateral thigh flap phalloplasty. After stage two, the patient developed a fistula that was amenable to outpatient repair; voiding now and has received a semi-malleable implant. A: visible ischemic urethral flap; B: status post marsupialization at a more superior location<sup>[35]</sup>

systematic review, we focused on the RFFF and ALT flaps used for shaft creation. There seems to be a higher rate of PFL in the ALT cohort than the RFFF cohort (7.1% *vs.* 4.5%). While the rate of total flap loss is historically higher in free flaps compared to pedicled flaps, PFL is not dictated by the type of flap, but rather the dimensions and volume of tissue harvested on a finite number of perforators. Thus, while total flap loss is attributed largely to technical failure, PFL may be considered more of a failure of design. Moreover, the nature of free flaps allows more flexibility regarding vessel lie and pedicle inset, while pedicled flaps may be more constrained three-dimensionally. When discussing PFL, future studies should report on the above variables and strive to include all pertinent patient demographics, flap characteristics, and outcomes. It is only through the identification of risk factors for PFL that appropriate strategies to mitigate and treat this complication will become apparent.

## DECLARATIONS

### Authors' contributions

Manuscript preparation, literature search, data review, creation of tables: Cylinder I

Data review, creation of tables: Heston A

Data review, creation of tables: Jedrzejewski B

Manuscript preparation, data review: Sikora Z

Manuscript preparation, data review: Peters B

Manuscript preparation, data review: Berli JU

### Availability of data and materials

The authors confirm that the data supporting the findings of this study are available within the article.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

©The Author(s) 2020.

## REFERENCES

1. Gillies H. Congenital absence of the penis: with embryological considerations. *Br J Plast Surg* 1948;1:8-28.
2. Kim S, Dennis M, Holland J, Terrell M, Loukas M, et al. The anatomy of abdominal flap phalloplasty for transgender surgery. *Clin Anat* 2018;31:181-6.
3. Puckett CL, Reinisch JF, Montie JE. Free flap phalloplasty. *J Urol* 1982;128:294-7.
4. Chang TS, Hwang WY. Forearm flap in one-stage reconstruction of the penis. *Plast Reconstr Surg* 1984;74:251-8.
5. Hage JJ, De Graaf FH. Addressing the ideal requirements by free flap phalloplasty: some reflections on refinements of technique. *Microsurgery* 1993;14:592-8.
6. Djordjevic ML, Bencic M, Kojovic V, Stojanovic B, Bizic M, et al. Musculocutaneous latissimus dorsi flap for phalloplasty in female to male gender affirmation surgery. *World J Urol* 2019;37:631-7.
7. Gottlieb LJ. Radial forearm. *Clin Plast Surg* 2018;45:391-8.
8. Xu KY, Watt AJ. The pedicled anterolateral thigh phalloplasty. *Clin Plast Surg* 2018;45:399-406.
9. Zaheer U, Granger A, Ortiz A, Terrell M, Loukas M, et al. The anatomy of free fibula osteoseptocutaneous flap in neophalloplasty in



- transgender surgery. *Clin Anat* 2018;31:169-74.
10. Nikolavsky D, Hughes M, Zhao LC. Urologic complications after phalloplasty or metoidioplasty. *Clin Plast Surg* 2018;45:425-35.
  11. Nikolavsky D, Yamaguchi Y, Levine JP, Zhao LC. Urologic sequelae following phalloplasty in transgendered patients. *Urol Clin North Am* 2017;44:113-25.
  12. Morrison SD, Shakir A, Vyas KS, Kirby J, Crane CN, et al. Phalloplasty: a review of techniques and outcomes. *Plast Reconstr Surg* 2016;138:594-615.
  13. Al-Tamimi M, Pigot GL, van der Sluis WB, van de Grift TC, van Moorselaar RJA, et al. The surgical techniques and outcomes of secondary phalloplasty after metoidioplasty in transgender men: an international, multi-center case series. *J Sex Med* 2019;16:1849-59.
  14. Ascha M, Massie JP, Morrison SD, Crane CN, Chen ML. Outcomes of single stage phalloplasty by pedicled anterolateral thigh flap versus radial forearm free flap in gender confirming surgery. *J Urol* 2018;199:206-14.
  15. Baumeister S, Sohn M, Domke C, Exner K. Phalloplasty in female-to-male transsexuals: experience from 259 cases. *Handchir Mikrochir Plast Chir* 2011;43:215-21.
  16. D'Arpa S, Claes K, Lumen N, Oieni S, Hoebeke P, et al. Urethral reconstruction in anterolateral thigh flap phalloplasty: a 93-case experience. *Plast Reconstr Surg* 2019;143:382-92e.
  17. De Wolf E, Claes K, Sommeling CE, Opsomer D, Cherubino M, et al. Free bipedicle radial forearm and posterior interosseous artery perforator flap phalloplasty. *J Sex Med* 2019;16:1111-7.
  18. Fang RH, Kao YS, Ma S, Lin JT. Phalloplasty in female-to-male transsexuals using free radial osteocutaneous flap: a series of 22 cases. *Br J Plast Surg* 1999;52:217-22.
  19. Fang RH, Lin JT, Ma S. Phalloplasty for female transsexuals with sensate free forearm flap. *Microsurgery* 1994;15:349-52.
  20. Felici N, Felici A. A new phalloplasty technique: the free anterolateral thigh flap phalloplasty. *J Plast Reconstr Aesthet Surg* 2006;59:153-7.
  21. Hage JJ, Bouman FG, de Graaf FH, Bloem JJ. Construction of the neophallus in female-to-male transsexuals: the Amsterdam experience. *J Urol* 1993;149:1463-8.
  22. Kim SK, Lee KC, Kwon YS, Cha BH. Phalloplasty using radial forearm osteocutaneous free flaps in female-to-male transsexuals. *J Plast Reconstr Aesthet Surg* 2009;62:309-17.
  23. Küntschner AM, Kilian M, Bull S, Kuntschener MV. The radial forearm flap with a prelaminated urethra: analysis of complications based on the Clavien-Dindo classification. *Handchir Mikrochir Plast Chir* 2019.
  24. Leriche A, Timsit MO, Morel-Journel N, Bouillot A, Dembele D, et al. Long-term outcome of forearm free-flap phalloplasty in the treatment of transsexualism. *BJU Int* 2008;101:1297-300.
  25. Matti BA, Matthews RN, Davies DM. Phalloplasty using the free radial forearm flap. *Br J Plast Surg* 1988;41:160-4.
  26. Namba Y, Watanabe T, Kimata Y. Flap combination phalloplasty in female-to-male transsexuals. *J Sex Med* 2019;16:934-41.
  27. Song C, Wong M, Wong CH, Ong YS. Modifications of the radial forearm flap phalloplasty for female-to-male gender reassignment. *J Reconstr Microsurg* 2011;27:115-20.
  28. van der Sluis WB, Smit JM, Pigot GLS, Buncamper ME, Winters HAH, et al. Double flap phalloplasty in transgender men: surgical technique and outcome of pedicled anterolateral thigh flap phalloplasty combined with radial forearm free flap urethral reconstruction. *Microsurgery* 2017;37:917-23.
  29. Wirthmann AE, Majenka P, Kaufmann MC, Wellenbrock SV, Kasper L, et al. Phalloplasty in female-to-male transsexuals by gottlieb and levine's free radial forearm flap technique-a long-term single-center experience over more than two decades. *J Reconstr Microsurg* 2018;34:235-41.
  30. Hwang K, Son JS, Ryu WK. Smoking and flap survival. *Plast surg (Oakv)* 2018;26:280-5.
  31. Annen AW, Heston AL, Dugi DD III, Dy GW, Bluebond-Langner R, et al. Masculinizing genital surgery: an imaging primer for the radiologist. *AJR Am J Roentgenol* 2020;214:W27-36.
  32. Chen ML, Safa B. Single-stage phalloplasty. *Urol Clin North Am* 2019;46:567-80.
  33. Danker S, Esmonde N, Berli JU. "Staging" in Phalloplasty. *Urol Clin North Am* 2019;46:581-90.
  34. Heston AL, Esmonde NO, Dugi DD, Berli JU. Phalloplasty: techniques and outcomes. *Transl Androl Urol* 2019;8:254-65.
  35. Esmonde N, Bluebond-Langner R, Berli JU. Phalloplasty flap-related complication. *Clin Plast Surg* 2018;45:415-24.

Review

Open Access



# Environmental aging of the skin: new insights

Karen E. Burke

Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, NY 10022, USA.

**Correspondence to:** Dr. Karen E. Burke, Department of Dermatology, Icahn School of Medicine at Mount Sinai, River Court Building, 429 East 52nd Street, New York, NY 10022, USA. E-mail: kebmddphd@gmail.com

**How to cite this article:** Burke KE. Environmental aging of the skin: new insights. *Plast Aesthet Res* 2020;7:59.  
<http://dx.doi.org/10.20517/2347-9264.2020.154>

**Received:** 26 Jul 2020 **First Decision:** 10 Aug 2020 **Revised:** 19 Aug 2020 **Accepted:** 22 Sep 2020 **Published:** 24 Oct 2020

**Academic Editor:** Salvador Gonzalez **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

The appearance of aging is determined primarily by extrinsic factors through exposure to environmental sunlight and airborne pollution. That solar ultraviolet B ( $\lambda = 290\text{-}320\text{ nm}$ ) directly causes photoaging (with wrinkles, dryness, and mottled pigmentation) and skin cancer has been recognized for decades; the contribution by ultraviolet A ( $\lambda = 320\text{-}400\text{ nm}$ ) was only more recently understood. New research further implicates visible light ( $\lambda = 400\text{-}700\text{ nm}$ ) as well as the heat rays of infrared radiation ( $\lambda > 800\text{ nm}$ ). Particularly in urban environments, airborne pollutants such as ozone ( $\text{O}_3$ ), polycyclic aromatic hydrocarbons, particulate matter (PM) in smog, and tobacco smoke contribute to photoaging and skin cancer. Furthermore, exposure simultaneously to both solar ultraviolet (UV) and these pollutants results in even greater synergistic damage. The volatile pollutants generate reactive oxygen species which oxidize surface lipids leading to deeper damaging inflammatory reactions. PM carries high concentrations of environmental organic compounds and trace metals. These pollutant-laden particles deliver toxins to the skin transcutaneously through hair follicles and through the blood after respiratory inhalation. The predominant natural mechanism of clearing these xenobiotic chemicals is through the ligand-activated transcription factor the arylhydrocarbon receptor (AHR) found on all skin cells. AHR activity regulates keratinocyte differentiation and proliferation, maintenance of epidermal barrier function, melanogenesis, and immunity. With chronic activation by UV exposure and pollutants, AHR signaling contributes to both extrinsic aging and carcinogenesis.

**Keywords:** Ultraviolet-induced aging, pollution, smoking, ozone, skin cancer, particulate matter, aryl hydrocarbon receptor



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



## INTRODUCTION

Today we live far longer than those in generations before us. The life expectancy of an individual born after 1990 is about 80 years, a substantial increase over the life expectancy of 65 years in those born in 1945. According to the 2018 United States Census Bureau Projections, of the approximately 36% of our present population now over 65 years old, at least a quarter will live to be over 95 years old. Thus it is imperative that our medical research goals emphasize not only lifespan, but also healthspan - maintaining our energy, mind, body, and happiness for all of these extra years.

Tantamount to healthspan is protecting our skin, the largest organ of our body and the only organ we see - our calling card to the world and shell of interface with external insults. We must be vigilant in protecting our skin from the external onslaught of environmental damage from solar ultraviolet (UV), visible light (VL), and infrared (IR) radiation and airborne pollutants. Science and technology have given us more leisure time so that we can travel to sunny climates and high altitudes where we stay outdoors for many hours swimming, sailing, or skiing, exposing ourselves not only to direct sunlight, but also virtually doubling our UV exposure from the indirect reflection of water, sand, and snow. Also we increasingly live in cities where we are surrounded by the ubiquitous pollutants of traffic and industry outdoors, and cigarette smoke, cleaning agents, and heating and cooking fuel indoors. All of these pollutants - including ozone (O<sub>3</sub>), polycyclic aromatic hydrocarbons (PAHs), volatile organic compounds (VOCs), and particulate matter (PM) - cause direct oxidative damage that leads to inflammatory responses, resulting in unattractive premature aging of the skin and deforming, dangerous skin cancer. Furthermore, recent research has proven that UV exposure not only directly harms the skin, but synergistically enhances the damage rendered by airborne pollutants<sup>[1]</sup>.

Recent research has elucidated the precise cellular and molecular mechanisms of the cutaneous damage and of the innate destructive as well as protective responses. A new understanding of how the arylhydrocarbon receptor (AHR) processes xenobiotic insults from UV exposure as well as from airborne pollutants is explained. New evidence demonstrates that topical antioxidants (especially vitamin C, vitamin E, and L-selenomethionine) can indeed not only prevent but also partially reverse extrinsic aging of the skin, though stringent criteria in formulation are required to achieve and maintain optimal delivery, activity, and stability.

## SOLAR PHOTOAGING

The first protective response of skin to solar UV radiation is production of melanin. After exposure to UV radiation, there is an immediate increase in tyrosinase activity accompanied by an increase in the size of melanosomes. Subsequent exposure results in an increase in the number of active melanocytes with increased transfer of Stage IV melanosomes to keratinocytes. The density of melanosomes in chronically sun-exposed skin is up to double that of non-sun exposed skin<sup>[2]</sup>. Within minutes after exposure to ultraviolet A (UVA) radiation, there is immediate pigmentary darkening (IPD) - probably due to oxidation of pre-existing melanin and melanin precursors. Because this fades within 20-30 min, it provides no protection. Protective delayed tanning is seen 24 h to 72 h after exposure to UVA + ultraviolet B (UVB) by the mechanisms described above<sup>[3]</sup>.

All tanning is actually photodamage - extrinsic aging of the skin. In children, the delayed tanning fades within several weeks after terminating summer exposure, though some freckles may persist. However, individuals with no visible hyperpigmentation show the subclinical photodamage of hyperpigmentation when examined with a medical UVA Wood's light ( $\lambda = 367$  nm), as seen in the 45-year-old woman shown in Figure 1<sup>[3]</sup>. With chronic UV exposure, this hyperpigmentation becomes the clearly visible, with unattractive solar lentigines seen commonly in older individuals, especially outdoor sports enthusiasts or those with outdoor professions.



**Figure 1.** Subclinical photodamage in a 45-year-old female. No photodamage of hyperpigmentation can be seen unless examined with a Wood's light ( $\lambda = 367$  nm), revealing hyperpigmentation from previous sun exposure. VISIA ultraviolet photograph. (Printed with permission by Canfield Imaging Systems, Parsippany, NJ 07054.)

Individuals with light, sun-sensitive skin (Fitzpatrick phototypes I and II) are at high risk for UV-induced photoaging and skin cancer. The pheomelanin synthesized in response to UV actually produces reactive oxygen species (ROS), leading to increased photoaging and skin cancers, especially in red-heads. Also, damaging DNA photoproducts are produced by chemo-excitation of melanin derivatives, even long after UV exposure<sup>[4]</sup>. In addition, most red-haired individuals have dysfunction of their melanocortin-1 receptor (MC1R) which hampers the possibility of developing a tan.

Studies with confocal microscopy have demonstrated that individuals of Fitzpatrick skin types I and II not only have lower melanin but also significantly fewer dermal papillae than those with higher Fitzpatrick skin types and darker skin<sup>[5]</sup>. With age, there is an even greater disparity between light and dark skin in density of dermal papillae, with increasingly fewer in light-skinned individuals as they age. This is a less-recognized manifestation of photoaging, probably contributing to the crepe-like texture of extrinsically aged skin in light-skinned phototypes.

### UV radiation

Only about 5% of solar terrestrial radiation is UV radiation. Of the UV component, 95% is UVA and 5% is UVB. The primary chromophore of UVB ( $\lambda = 290-320$  nm) radiation is DNA which suffers specific "UVB fingerprint mutations" resulting in intrastrand pyrimidine dimers<sup>[6]</sup>. Further mutations are incited by the generation of ROS. With cumulative UVB damage and natural aging, there is a decreased ability for nucleotide excision repair so that mutations persist, directly leading to pre-cancerous actinic keratoses and skin cancers<sup>[6]</sup> - basal cell carcinomas (BCCs), squamous cell carcinomas (SCCs), and malignant melanomas (MMs) - of which SSCs and MMs are potentially lethal.

Ninety-five percent of our daily UV exposure is UVA ( $\lambda = 320\text{-}450\text{ nm}$ ) which, unlike UVB, is not filtered by glass and does not vary seasonally in intensity. Like UVB, UVA exposure results in “signature mutations” with T  $\rightarrow$  G transversions and formation of specific pyrimidine dimers<sup>[6]</sup>. UVA generates ROS with resultant DNA mutations, in particular oxidation of guanosine to 8-hydroxy-deoxyguanosine (8-OHdG). UVA penetrates the skin more deeply than does UVB: UVA reaches the epidermal stem-cell rich basal layer and the dermis to inactivate the tumor suppressor p53 gene and to decrease immune functions so that early skin cancers are not recognized. Only extremely high exposure to UVA might directly initiate skin cancer, while the lower doses of UVA to which we are exposed do inhibit the normal immune response so that UVB-initiated skin carcinomas proliferate unchecked. Furthermore, it is UVA that activates dermal matrix metalloproteinases (MMP) to degrade collagen and elastic tissue, resulting in the wrinkled, crepe-like, and saggy quality of photo-damaged skin.

### High-energy visible blue light

Of the solar radiation that reaches the earth’s surface, 39% is visible light (VL, with  $\lambda = 400\text{-}700\text{ nm}$ ) with the high-energy VL in the low wavelength range (blue light). We are exposed to the high-energy VL not only from sunlight but also from computer screens and smart phones! Early observations by Kollias *et al.*<sup>[7]</sup> showed that VL does induce skin pigmentation that can last for 10 weeks. Further research demonstrated by exposure to a xenon-mercury lamp, that VL (up to 470 nm) induces IPD, as does UVA-I<sup>[8]</sup>. The peak IPD response is at wavelengths  $\lambda = 300\text{-}500\text{ nm}$ , including all UVA-II ( $\lambda = 320\text{-}340\text{ nm}$ ), UVA-I ( $\lambda = 340\text{-}400\text{ nm}$ ), and visible blue light ( $\lambda = 400\text{-}500\text{ nm}$ )<sup>[9]</sup>. This tanning (with erythema) was only seen in dark-skinned individuals of Fitzpatrick skin type IV-VI, probably because of the large amount of melanin in their skin; light-skin type I individuals showed no tanning after VL exposure<sup>[10]</sup>. The corresponding coinciding erythema may be because as melanin absorbs VL, thereby generating heat to cause vasodilation. More exposure to VL leads to darker and more sustained pigmentation as seen also in UV-induced post-inflammatory hyperpigmentation.

Exposure of human skin equivalents to VL has been shown *in vitro* and in human skin *ex vitro* to generate ROS leading to induction of pro-inflammatory cytokines and MMP-1 and MMP-9<sup>[11]</sup>. Dose-dependent generation of hydrogen peroxide was also measured after exposure to VL. Certainly this oxidative insult, the inflammatory cascades, and the MMPs destroy dermal matrix to contribute to extrinsic photoaging.

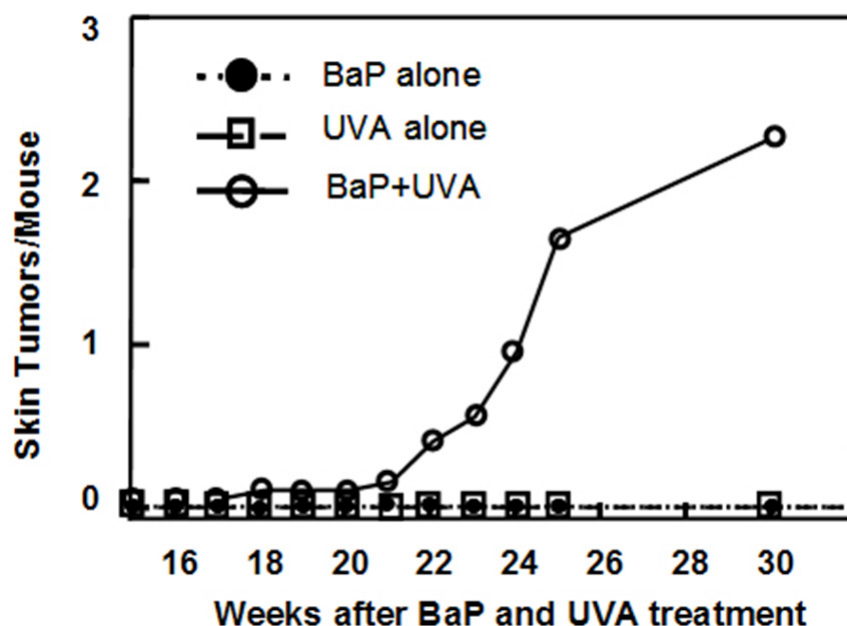
### IR solar radiation

Almost 50% of the solar energy reaching the earth’s surface is IR or heat energy: IR-A ( $\lambda = 700\text{-}1400\text{ nm}$ ), IR-B ( $\lambda = 1400\text{-}3000\text{ nm}$ ), and IR-C ( $\lambda = 3000\text{ nm} - 1\text{ mm}$ ). Solar terrestrial radiation is about 30% IR-A. IR-B and IR-C do not penetrate deeply into the skin, but they do contribute to heating the skin. Direct sunlight can raise the temperature of human skin from 37 °C to 40 °C, with darker skin types IV-VI responding to IR exposure with greater rises in temperature than experienced by light-skinned individuals (types I and II). Although more than 65% of incipient IR-A penetrates to the dermis and 10% to the subcutaneous fat, normal (non-excessive) exposure does not raise the skin’s temperature<sup>[12]</sup>.

The reaction to IR-A varies with skin type: In darker skin, melanin synthesis is stimulated with little effect in dermal MMPs, while in lightly pigmented skin, collagen in the dermal extracellular matrix is altered not only by destruction through activation of MMPs, but also by a direct reduction in synthesis<sup>[12]</sup>. Thus lightly pigmented skin manifests wrinkles and a crepe-like quality, while darkly pigmented skin responds with increased solar lentigos - both suffering from premature extrinsic aging. In addition, chronic exposure to IR-A induces angiogenesis and unattractive erythema *ab igne* as seen in “bakers’ arms” and “glassblowers’ faces”<sup>[12]</sup>.

Although the clinical manifestations of photoaging of the skin are similar after UV and IR-A exposure, the mechanisms of damage are different. IR-A affects the mitochondrial electron transport chain, increasing





**Figure 2.** Squamous cell carcinoma multiplicity (number of tumors/mouse) in Skh:1 female mice after topical application of BaP (8 nmol) followed by exposure to ultraviolet A (UVA) (40 kg/m<sup>2</sup>) three times weekly for 25 weeks. For 5 weeks after treatments were discontinued, tumors were still counted (Wang *et al.*<sup>[1]</sup>). (Printed with consent of the authors.). BaP: benzo[a]pyrene

mitochondrial superoxide anion production<sup>[13]</sup>. In human fibroblasts *in vitro*, IR-A induces genes regulating apoptosis, extracellular matrix, calcium regulation, and other signaling<sup>[14]</sup>. Solar elastosis is markedly exacerbated by IR-A in two ways: (1) stimulation of MMP-12 which specifically degrades elastin; and (2) abnormal production of the major components of elastin-fibrillin-1,2 and tropoelastin<sup>[13]</sup>. Along with degradation of collagen, this accumulation of abnormal, clumped elastin in the dermis is the paramount cause of the crepe-like, saggy, wrinkled appearance of photoaged skin.

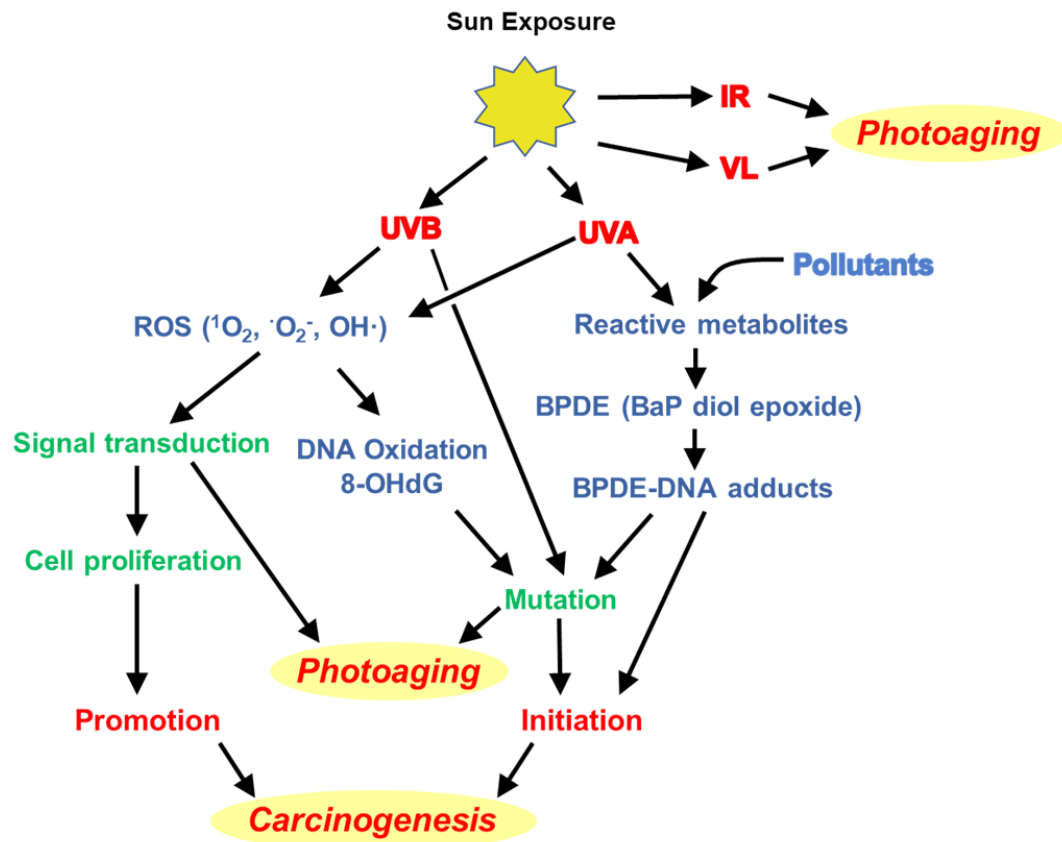
### SYNERGY: UVA AND POLLUTANTS

Atmospheric pollutants fill the air in modern cities. Outside, traffic exhausts, power plants and factories pollute, and, inside, stoves, heaters, fireplaces, candles, and cigarette smoke. Organic and inorganic compounds are emitted. PAHs are ubiquitous in our air, water, and food. The most significant PAH in urban environments is benzo[a]pyrene (BaP). These PAHs are absorbed onto and within the larger PM pollutants. When inhaled or absorbed after direct contact with mucosa or skin, these PAHs are metabolized to quinones which generate ROS.

Today, the levels of PAHs are lower than those of 40-50 years ago, well below a toxic or carcinogenic level. However, with simultaneous exposure of the skin to PAHs and UVA radiation, synergistic interactions increase oxidative damage, leading to increased extrinsic damage to the skin with resultant photoaging and skin cancer.

In mouse model experiments, exposure to low doses of BaP alone, or to UVA alone, does not initiate skin cancer. However, exposure to 1.8% of the carcinogenic dose of BaP with simultaneous exposure to 12.5% of the carcinogenic dose of UVA could initiate skin cancer<sup>[1,15]</sup>, as shown in Figure 2 (this exposure to UVA is comparable to 2 h of midday sunlight on a summer day in New York City).

Synergistic damage was further demonstrated *in vitro*. High concentrations of d-OHdG and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) were generated when calf-thymus DNA was exposed to UVA after incubation with BaP;



**Figure 3.** Mechanisms of solar-induced photoaging and carcinogenesis of the skin. Solar ultraviolet B (UVB) directly mutates cellular DNA to cause photoaging and to initiate carcinogenesis. Ultraviolet A (UVA) interacts synergistically with pollutants to form DNA-adducts, initiating carcinogenesis. Both UVA and UVB generate ROS that oxidize DNA to form 8-OHdG, leading directly to photoaging and tumor initiation. ROSs further activate signal transduction of inflammatory cytokines and matrix-destructive enzymes, which exacerbate photoaging and cellular proliferation to promote carcinogenesis. Exposure to solar (and technological) VL and IR heat also induces photoaging of the skin. (Modified from Saladi *et al.*<sup>[18]</sup> with consent of authors.). ROS: reactive oxygen species; 8-OHdG: 8-hydroxy deoxyguanosine; VL: visible light; IR: infrared light

in contrast, only minimal concentrations were measured when exposed to UVB after BaP<sup>[16]</sup>. Cells exposed to UVA after having been incubated with BaP increased 8-OHdG by a factor of 17, while exposure to UVB resulted in only an increase by a factor of 3<sup>[1]</sup>. Another experiment exposed human keratinocytes to UVA after incubation with BaP: H<sub>2</sub>O<sub>2</sub> production increased 6-fold compared to only a factor of 1.2 after exposure to UVB<sup>[1]</sup>. In the skin, all of this oxidative damage manifests clinically as photoaging and proclivity to carcinogenesis.

With exposure to UV, BaP forms diol epoxide (BPDE) which combines covalently to DNA to form BPDE-DNA adducts that directly initiate carcinogenesis<sup>[17]</sup>. Exposure to UVA generates more than double the number generated by exposure to UVB<sup>[18]</sup>. These adducts directly produce ROS. Furthermore, the BaP is recycled within cells, a reaction enhanced by UVA exposure, making even small amounts of BaP extremely damaging. This sequence of reactions leading to photoaging of the epidermis and dermis as well as to initiation and promotion of skin cancer is shown in Figure 3.

## OZONE

In the stratosphere (10-30 miles above the earth's surface), O<sub>3</sub> protects us by blocking dangerous solar UVC and dangerous high-energy UVB. On the other hand, low level tropospheric O<sub>3</sub> causes significant oxidative

damage, particularly to the skin. The average level of  $O_3$  on the earth's surface is about 0.05-0.1 ppm, quite low in comparison with the stratosphere with concentrations of 10 ppm. However, especially during the summer, in densely populated cities such as Mexico City (with the highest levels) and even Rome and Paris, industry and traffic emit VOCs including nitrogen oxides, methane, carbon monoxide, and sulfuric compounds which accumulate, causing a progressive increase in  $O_3$  concentrations up to 0.8 ppm.

Although  $O_3$  cannot penetrate the skin, it oxidizes lipids on the skin's surface, thereby triggering destructive inflammatory cascades in deeper cellular layers, turning on genes to produce inflammatory cytokines - interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF- $\alpha$ ), transforming growth factor beta (TGF- $\beta$ ), cyclooxygenase-2 (COX-2), intercellular adhesion molecule (ICAM), and vascular cell adhesion molecule (VCAM)<sup>[19,20]</sup> - all of which have been measured in response to surface  $O_3$ . This inflammatory onslaught certainly disrupts dermal cells and extracellular matrix to exacerbate photoaging<sup>[21]</sup>. Further damage is rendered by  $O_3$  generation of the protein adduct 4-hydroxy-2-nonenal (HNE) and carbonyl protein adducts<sup>[22]</sup>.

Direct proof that  $O_3$  exposure contributes substantially to accelerating extrinsic aging was demonstrated in Skh:1 hairless mice<sup>[22,23]</sup>. With exposure to  $O_3$ , dermal MMP-2 (a gelatinase that digests collagen I and IV) and MMP-12 (an elastase) are activated; MMP-9 (a gelatinase with activity similar to MMP-2) is activated only in older mice<sup>[23]</sup>.  $O_3$  further inhibits activation of inhibitors of MMPs<sup>[24]</sup>, thus increasing matrix degradation with resultant manifestations of dermal aging: Wrinkles, crepe-like skin, and sagging due to loss of support, seen histologically as loss of collagen with disorganization of fibrillar alignment and loss of elastin with clumping of dysfunctional elastic tissue fragments. This degradation of firm extracellular matrix allows and promotes enhanced tumor growth<sup>[25]</sup>.

Defending against this damage are antioxidants vitamin C and vitamin E on the skin's surface. However, exposure to  $O_3$  rapidly depletes these essential antioxidants<sup>[26]</sup>, probably because the resident levels of these vitamins are rapidly depleted by quenching the initial onslaught of  $O_3$ -induced oxidative damage. Thus, the first antioxidant defense is lost. Another protective mechanism is the induction of epidermal heat shock proteins (HSP) sequentially: HSP 27 (at 2 h), HSP 70 (at 12 h), and HSP 32 (maximally at 24 h). HSP 27 actually increases 20-fold and HSP 70 by 8-fold in response to  $O_3$ . These HSPs may mitigate the oxidative harm from surface  $O_3$ <sup>[19]</sup>. Further defending against all of the  $O_3$ -induced oxidative stress is activation of the antioxidant transcription factor nuclear factor of kappa-light-chain-enhancers of activated B-cells (NF- $\kappa$ B) in keratinocytes as well as in dermal cells, as demonstrated *in vitro* and *in vivo*<sup>[19,22]</sup>.

A recently published epidemiological study from two cohort groups in Germany (of 2013 Caucasian men and women)<sup>[27]</sup> provides direct proof that exposure to tropospheric  $O_3$  does indeed exacerbate extrinsic skin aging. Five-year cumulative residential exposure to ambient  $O_3$  was calculated for each of the two neighborhoods (within a 5-digit postal code). Course wrinkles and hyperpigmentation (solar lentigines) on the forehead, under eyes, crow's feet area, and upper lip and facial lentigines were quantitated by visual scoring according to the validated Score of Intrinsic and Extrinsic Skin Aging (SCINEXA<sup>TM</sup>)<sup>[27]</sup>. Correlation of course facial wrinkles with cumulative neighborhood  $O_3$  exposure was confirmed, but increased pigmented lentigines were not noted. This correlation was independent of other possible environmental confounders and airborne pollutants including particulate matter.

## PARTICULATE MATTER

Even the earliest humans suffered from particulate matter exposure: Dust storms from the Sahara, and pollen and particulate fecal matter from grazing herds on the savannah caused respiratory, cardiovascular, and skin disease. Today, city environments are filled with larger PM<sub>10</sub> and PM<sub>2.5</sub> particles ( $\leq 10 \mu\text{m}$  and  $\leq 2.5 \mu\text{m}$  diameter, respectively) of soot, primarily emitted by diesel engines, factories, power plants, and

incinerators as well as smaller ultrafine particles (UFPs) ( $\leq 100$  nm,  $0.1 \mu\text{m}$ , diameter) from traffic. Up to  $475 \text{ kg/km}^2$  are spewed into urban air each year<sup>[28]</sup>. Even rural dwellers suffer from nearby highways and inside furnaces and fireplaces as well as from forest fires and wind-blown dust. Polluting compounds (PAHs,  $\text{NO}_2$ ,  $\text{SO}_2$ , and trace minerals) cover the surfaces and are incorporated into the cores of the PMs<sup>[29,30]</sup>. These PMs contacting skin are absorbed to the mid-stratum corneum, as shown by tape-stripping chimney sweeps<sup>[31]</sup>. UFPs not only penetrate the skin transdermally and through hair follicles<sup>[32,33]</sup>, but also are inhaled through the lung to enter pulmonary circulation; PM contaminants can be measured in the blood within one hour and can remain for weeks<sup>[34]</sup>.

Direct damage to the skin by larger PMs was shown by exposing reconstituted human epidermis *in vitro* to concentrated  $\text{PM}_{2.5}$ <sup>[35]</sup>. Oxidation of surface lipids and apoptosis correlated with increasing dose and exposure time. The impact of larger  $\text{PM}_{10}$  pollution has been demonstrated by *in vitro* exposure of human dermal fibroblasts to  $\text{PM}_{10}$  for 24 h<sup>[36]</sup>. Surprisingly, 1,977 genes were expressed, most significantly pro-inflammatory genes for interleukins (IL) IL-1 $\beta$ , IL-6, IL-8, and IL-33, resulting in increases especially in IL-6 and -8, cytochrome (CYP) P450 (CYP1A1, CYP1B1), and MMP-1 and -3, and accompanied by substantial decreases in transforming growth factor- $\beta$  (TGF- $\beta$ ) and in collagen I and elastin mRNA. All of these factors directly result in the crepe-like, wrinkled, and sagging quality of extrinsic aging.

The mechanism of PM cutaneous insult is by oxidation of surface lipids with resultant ROS that activate inflammation through transcription factors such as NF- $\kappa\text{B}$ , turning on gene transcription for cytokines and IL-1 $\alpha$ , IL-8, COX-1 and -2<sup>[37,38]</sup>. In particular, PMs containing quinones or trace metals localize in keratinocyte mitochondria, altering the mitochondrial ultrastructure with first dilation and vacuolization and loss of cristae, then thickening and shrinking<sup>[39]</sup>.

## VOCS

VOCs such as car exhaust with benzene and industrial emissions (mainly tetrachloroethylene) pollute the outdoor environment, while organic solvents in paints, varnishes, refinishing chemicals, glues, cleaning agents, cosmetics (especially nail polish) such as aliphatic hydrocarbons (formaldehyde and acetone) fill indoor closed spaces. Indoor pollution is particularly exacerbated in all homes in winter when windows are kept closed, and throughout the year in cities where skyscrapers and other buildings have sealed windows to maintain energy conservation. This VOC-contaminated air is recirculated, leading to “sick building syndrome.” Workers experience conjunctivitis, rhinitis, atopic dermatitis, eczema, and other contact or irritant dermatitis, particularly after reconstruction, painting, installation of new rugs and draperies, and after thorough cleaning. These airborne irritants contribute to extrinsic aging of exposed skin, particularly on the hands, neck, and face.

## TOBACCO SMOKE

A major indoor pollutant is cigarette (and cigar and pipe) smoke which exposes not only the smoker but also others to the “sidestream” smoke. In the closed environments of our modern world - with urban skyscrapers and airplanes and mass transit of subways - the VOCs and tobacco contaminants linger. In a cinema which had been designated “nonsmoking” for over 5 years, 35 tobacco-related chemicals were measured, carried inside by smokers’ hair, skin, and clothes - leading to “third-hand exposure”<sup>[40]</sup>.

More than 4,700 different chemicals are released by burning tobacco<sup>[41]</sup>, some as PM (including nicotine, quinone, and benzopyrenes), some as VOCs (PAHs,  $\text{CO}_2$ , CO, formaldehyde, nitrosamine and many others). One dominant component of tobacco smoke, 4-aminobiphenyl, is particularly dangerous because it forms adducts on hemoglobin, albumin, and collagen<sup>[42]</sup>. These adducts remain for the “lifetime” of each component: On hemoglobin, adducts remain for 120 days at levels 5.5 times higher in smokers than

nonsmokers; on albumin, adducts remain for 30 days, and on collagen, for up to 10 months. Oxygen delivery is impeded and collagen structure is disrupted, leading to extrinsic aging of the skin.

The long-term exposure of mice to cigarette smoke and UV results in barrier disruption, with transepidermal water loss, erythema, telangiectasis, decreased elasticity, visible crepe-like skin, and an increased incidence of benign epitheliomas and squamous cell carcinomas<sup>[43]</sup>. Increased mRNA for the metalloproteinases MMP-1 and -3 with consequent breakdown of collagen I and III<sup>[44]</sup> as well as of elastic tissue is seen, accelerating extrinsic aging.

## MECHANISM OF DETOXIFICATION: THE AHR

AHR evolved to detoxify food and airborne toxins. The AHR is found on cells of the gastrointestinal tract, pulmonary epithelium, and is especially highly expressed on all cells of the skin - keratinocytes, fibroblasts, and melanocytes as well as on regulatory T-cells and dendritic cells. The AHR recognizes and binds xenobiotic pollutants, then signals gene transcription of enzymes and inflammatory cytokines to metabolize these foreign substances<sup>[45]</sup>. The AHR plays a key role in barrier function, pigmentation regulation responding to environmental onslaughts, and skin inflammation<sup>[46]</sup>. Specific ligands activate specific genes. In the epidermis, UVB is absorbed by the amino acid tryptophan to form the photoproduct 6-formylindolo[3,2-b]carbazole (FICZ) and indoles which bind with high affinity to the AHR to induce the protective signaling cascade. Other potent ligands are the PAHs of tobacco smoke and city pollution as well as pesticide components such as tetrachlorodibenzo-p-dioxin (TCDD), the contaminant of Agent Orange, and even normal skin flora such as bacteria (non-pathogenic as well as pathogenic *staphylococci*) and yeasts (*Malassezia furfur*).

As seen in [Figure 4](#), the inactive AHR is incorporated into a multi-protein complex in the cellular cytoplasm. Binding of the pollutant ligand generates a conformational change that breaks this complex so that ligand-AHR enters the nucleus where it dimerizes with AHR nuclear translocator (ARNT). This dimer is a transcription factor which binds to genes to induce DNA transcription. AHR can selectively induce transcription of NF- $\kappa$ B, leading to inflammatory cytokines such as NRF2 (which activates antioxidants) as well as to cell cycle (proliferation) and immune modulators.

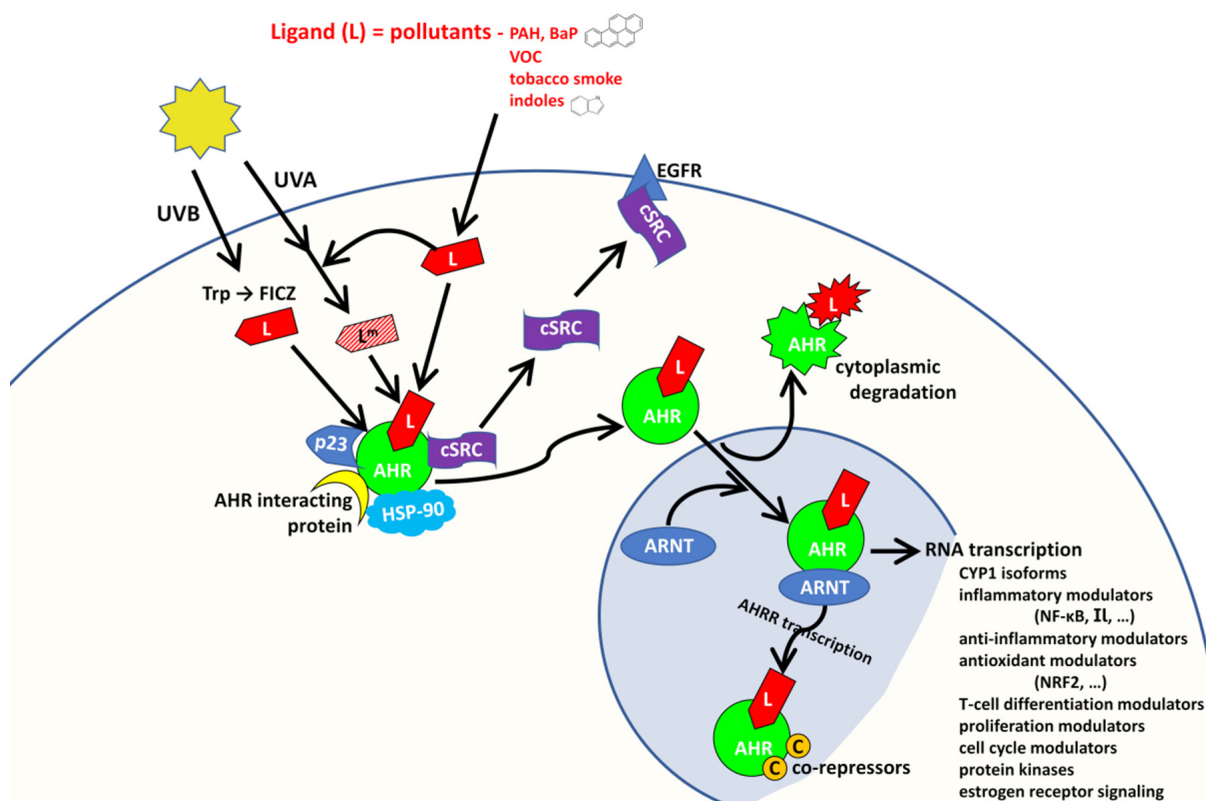
Alternatively, the ligand-AHR can induce the AHRR gene which inactivates the AHR by transcribing co-repressors<sup>[47]</sup>. Other inhibition of AHR is regulated by expulsion of the ligand-AHR from the nucleus for degradation in the cytoplasm. Thus overstimulation by the pollutant-ligand-AHR complex is curtailed. The co-chaperones that are released by the dissociation of the initial cytoplasm AHR complex after ligand binding lead to important cellular reactions: The heat shock proteins which are co-chaperoned by p23 stabilize or refold proteins that act to mitigate cellular stress; p23 also binds to the progesterone receptor that transports progesterone to the nucleus; the oncogene cSRC binds to the EGFR leading to activation of cSRC kinase and carcinogenesis.

## EPIDEMIOLOGICAL CORRELATION OF EXTRINSIC SKIN AGING AND POLLUTION

### Outdoor pollution

Vierkötter *et al.*<sup>[48]</sup> published an impressive epidemiological study showing the impact of urban pollution on aging skin on four-hundred Caucasian women (70-80 years old) who had previously enlisted in a study of the effect of industrial pollution on pulmonary aging. Half lived in urban Rohr and half in rural Bergen. All were evaluated by the SCINEXA scale which distinguishes intrinsic from extrinsic aging. Childhood sun exposure and smoking history were evaluated for each patient, and exposure to traffic and industrial emission at each individual's home were measured. The researchers concluded that traffic-generated PM nanoparticles produced 16% more lentigines on the forehead and 17% more on the cheeks for each quartile





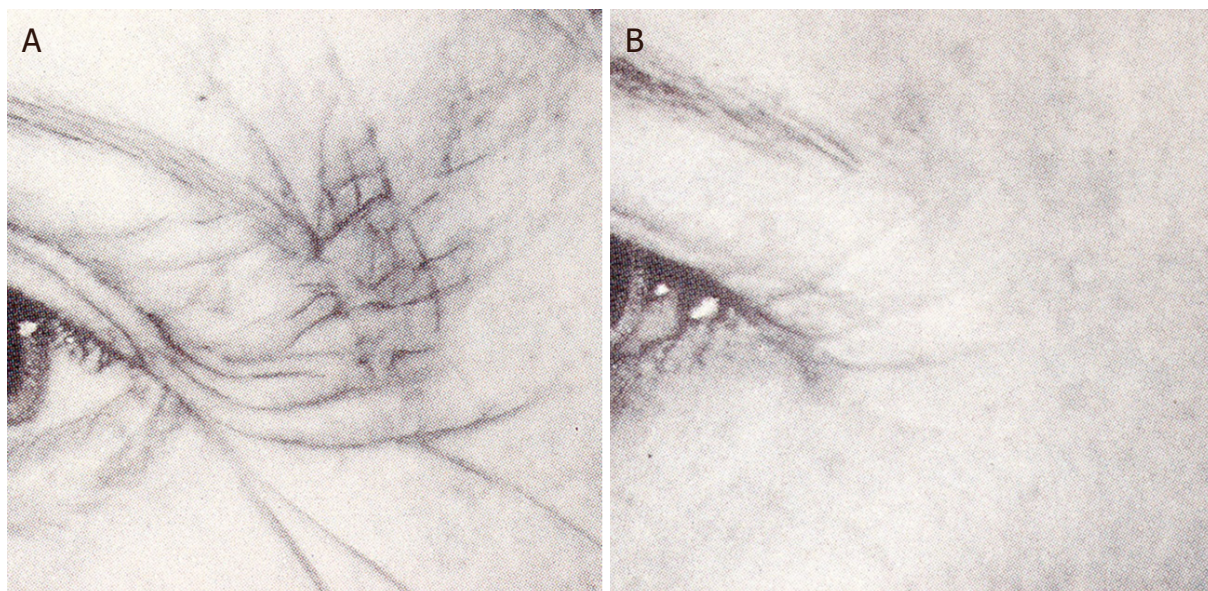
**Figure 4.** The aryl hydrocarbon receptor (AHR). The AHR is found in the cytoplasm bound to protein chaperone molecules, including AHR interacting protein, heat shock protein-90, the p53 protein, and the tyrosine kinase oncogene cSRC. When a pollutant ligand binds to this complex, conformational changes disassemble the complex so that the ligand-AHR unit translocates into the nucleus where it dimerizes with ARNT. The ligand-AHR-ARNT entity induces transcription of target genes, specific for each xenobiotic ligand. Overstimulation by the ligand-AHR complex is prevented (1) by RNA transcription of the AHR repressor (AHRR) gene to synthesize co-repressors; and (2) by cytoplasmic degradation of the ligand-AHR entity after expulsion from the nucleus. After release from the initial cytoplasmic AHR complex, the chaperone protein oncogene cSRC binds to the epidermal growth factor receptor (EGFR), activating the cSRC protein kinase to induce carcinogenesis. (Modified from Figure 1 of Vogeley *et al.*<sup>[47]</sup> with the author's consent.). cSRC: "cellular sarcoma", photo oncogene tyrosine-protein kinase; ARNT: AHR nuclear translocator

increase in pollution measured. Concentrated soot increased lentigines by 27% on the forehead and 20% on the cheeks. Women living within 100 m of a busy road showed an increase of 25% and 20% in forehead and cheeks lentigines, respectively, for each interquartile increase in pollution.

A further study looked at the VOC NO<sub>2</sub> released by traffic, known to cause lung damage and lung cancer<sup>[49]</sup>. Facial cheek lentigines were evaluated in women over 50 years old - 806 European Caucasians and 1,072 Chinese, making this the largest study to date of traffic-induced lentigines. Indeed, NO<sub>2</sub> exposure increased lentigines in both cohorts. Lentigines develop at a younger age in Asians despite their cultural avoidance of sun exposure (in contrast to many Caucasians). This proclivity for lentigines may be due to a particular genetic marker in the SLC45A2 gene similarly noted in Japanese, a marker known to be involved in melanin synthesis<sup>[50]</sup>.

### Indoor pollution

Cigarette smoke is the most significant indoor contaminant; second-hand side-stream smoke permeates indoor space and is recycled in closed environment; third-hand smoke is carried by the hair, skin, and clothes of smokers entering a closed space<sup>[40]</sup>. In 1969, Henry Daniell recognized that smokers look older than non-smokers<sup>[51]</sup>. Several epidemiological papers confirm this observation<sup>[52,53,54]</sup>. Analysis shows that men who smoke have 2.3 times as many wrinkles as nonsmoking males, and women have 3.1 times as



**Figure 5.** The 52-year old smoker (A) has more severe, deeper periorbital wrinkles than her 57-year-old non-smoking cousin (B). These cousins are neighbors who experienced the same environmental conditions throughout their lives (reprinted from *Great Skin for Life*, with consent of the author)

many as non-smoking women<sup>[53]</sup>. Smokers' skin displays extensive aging with a leathery texture, dryness, deep wrinkles, a crepe-like quality, and sagging. This appearance of prematurely aged skin can be clearly seen in Figure 5. The 52-year-old smoker has far more severe periorbital wrinkles than her non-smoking 57-year-old cousin and neighbor<sup>[55]</sup>. A further dramatic demonstration of the ravages of photoaging in smokers was shown in photographs of identical twins with a greater than 5-year difference in smoking<sup>[56]</sup>. Especially upper eyelid skin redundancy, lower lid bags, molar bags, nasolabial folds, upper and lower lip wrinkles, and jowls are markedly worse in the twin who smoked.

The other primary sources of indoor pollution, especially in underdeveloped countries, is cooking and heating with coal or wood. In China, more than 20% and up to 70% of households cook with solid fuel<sup>[57]</sup>, causing chronic obstructive pulmonary disease, compromised lung function, and lung cancer<sup>[58]</sup>. To study the effect on the appearance of the skin, Li *et al.*<sup>[59]</sup> compared 405 women (of age 30 to 70 years) from northern China with 857 women from southern China, using the standardized SCINEXA evaluation. Correcting for age, cooking and heating with solid fuel significantly increased severe facial wrinkling by 5%-8% and by 75% the fine wrinkles of the hands.

Not only do underdeveloped countries suffer with indoor pollutants, but also throughout the world, gas stoves spew pollutants (primarily nitrogen dioxide and carbon monoxide) which make indoor air factors dirtier than outdoors, as reported by The Guardian (United Kingdom) recently. Candles and fireplaces further contribute. No specific studies on the effect of these frequent enjoyments on aging skin have been completed as yet, but certainly we know that these airborne contaminants are detrimental to skin.

## SKIN CANCER

The most severe and devastating manifestation of photoaging is skin cancer. Here there is no doubt that environmental pollution directly initiates and propagates skin cancer. The first realization of environmental pollutants causing skin damage was in London in 1775 when Dr. Percivall Pott correlated SCC on the underside of the scrotum after exposure to soot in young boy chimney sweeps<sup>[60]</sup>.

That UVB directly initiates all types of skin cancer - SCC, BCC, and MM - has been proven beyond doubt. UVA promotes skin cancer by generating specific DNA mutations, by production of labile ROS and subsequent oxidation of DNA, and by suppressing the immune response. As described above and shown in [Figure 2](#), UVA also interacts synergistically with environmental airborne pollutants to form mutagenic DNA adducts leading to skin carcinogenesis.

The highly complex regulation of cellular physiology by the AHR and its influence on the initiation and propagation of skin cancer has been excellently reviewed by Vogeley *et al.*<sup>[47]</sup>. Some downstream reactions initiated by AHR suppress or inhibit initiation and promotion of carcinogenesis; others stimulate carcinogenesis. The regulation is highly specific for each xenobiotic pollutant, and concentration and interaction with UVA certainly contribute.

UVB invokes the AHR by generating the photoproducts, most prominently FICZ. The AHR-FICA complex initiates rapid metabolism of FICZ to limit carcinogenesis. On the other hand, high levels of UVB trigger other cytokines which stimulate SCC growth. Nitrosamines and aromatic amines from tobacco smoke and high levels of PAHs encountered with high industrial exposure activate the AHR, often leading to downstream metabolism of these xenobiotics to genotoxins within keratinocytes. AHR activation by these pollutants in Langerhans cells changes these antigen-presenting dendritic cells from stimulatory to regulatory, enhancing immunosuppression to allow tumor growth. Vietnam veterans had an increased incidence of melanoma after exposure to the Agent Orange carcinogen TCDD<sup>[61]</sup>, which through the AHR-TCDD complex initiated reactions that alter the microenvironment by activating destructive MMPs<sup>[62]</sup>, inducing angiogenesis, and increasing cancer cell motility<sup>[63]</sup>.

Perhaps the most studied clinical correlation of skin (and lung) cancer to environmental pollutants is the correlation to cigarette smoking. Smokers statistically have twice as many melanomas as non-smokers, 1.5 times as many cutaneous SCCs, 15 times as many SCCs of the lip, and 78 times as many oral epithelial cancers. Another direct chemical correlation of the incidence of mortality of skin cancer induced by environmental exposure was studied in São Paulo, Brazil<sup>[64]</sup>. Indeed, higher PM<sub>10</sub> pollution is directly associated with an increased incidence of skin cancer.

## CONCLUSION

The appearance of aging on our skin is primarily caused by exposure to solar radiation and environmental airborne pollutants. Recent research has elucidated new insights into the molecular mechanisms of skin damage and natural defenses. We now realize that not only is UVB the main instigator of photoaging, but also UVA as well as visible light and long wavelength IR heat contribute to extrinsic aging. Environmental airborne pollutants - particularly the PAHs released by industry and traffic, O<sub>3</sub>, VOCs, PM coated with and containing xenochemicals - all directly cause the hallmark appearance of premature aging of the skin. Furthermore, exposure to UVA and airborne pollutants simultaneously causes synergistic damage and accelerated extrinsic aging with increased carcinogenesis.

The new epidemiologically-proven realizations about the specific causes of damage can teach how to prevent or lessen the adverse results of exposure. Avoidance of sun exposure and use of broad-spectrum sunscreen; improvements in technology to minimize industrial pollution and decreasing traffic emissions; stopping smoking; avoiding cooking and heating with fossil fuels in closed, indoor spaces; choosing rural over urban dwellings if possible - these all reduce the risk. In Korea, indoor air quality was improved in nine kindergarten classes, decreasing PM<sub>10</sub> significantly from 182.7 µg/m<sup>3</sup> to 73.4 µg/m<sup>3</sup><sup>[28]</sup>. The European Health Event project improved indoor air quality by optimizing ventilation, filtering outdoor air, and controlling indoor sources of pollution (especially by prohibiting smoking)<sup>[65]</sup>. Industry is developing technology to be ecofriendly - for example, the use of compressed natural gas as fuel instead of coal and petroleum decreases PM pollution.



All environmental exposure - whether from solar energy or airborne toxins - leads to skin damage by generation of ROS, either directly or via the AHR receptor. Our bodies' primary protective mechanism is our complex endogenous antioxidant network which is dependent upon external supplementation - either orally, topically, or both. These antioxidant-induced reactions have been shown to combat extrinsic aging of the skin and skin cancer. Indeed, the resident epidermal antioxidants vitamin C and vitamin E are depleted 55% and 25%, respectively, as they protect surface skin after exposure to high concentrations of O<sub>3</sub><sup>[26]</sup>.

Antioxidants can best be replenished or delivered to the skin by topical application giving far higher concentrations than attained by oral ingestion. For example, L-ascorbic acid (vitamin C) 15% gives 27-40 times the skin concentration resulting from high oral intake<sup>[66]</sup>, d- $\alpha$ -tocopherol 5% (the only one of 32 forms of vitamin E that is effective on skin), by a factor of 12<sup>[67]</sup>, and selenium (L-selenomethionine 0.05%), by a factor of 8<sup>[68]</sup>. These three antioxidants have been extensively studied and proven to prevent and even reverse photoaging of the skin in mice<sup>[67,68,69]</sup>, pigs<sup>[70]</sup>, and humans<sup>[71,72]</sup>, and to inhibit UV-induced skin cancer in mice<sup>[67,68,69,73]</sup>. However, stringent criteria are required for topical formulations to be stable, to be successfully absorbed, as to be active as antioxidants. Esterified forms of vitamins C<sup>[66]</sup> and E<sup>[67]</sup> are not absorbed transcutaneously, and the ester is not reduced to the -OH form required for antioxidant activity. Only the isomer d- $\alpha$ -tocopherol is effective; the other 31 isomers (eight "dl" isomer configurations and four  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  forms) are not<sup>[67]</sup>. High concentrations are required, optimally 15%-20% for l-ascorbic acid<sup>[72]</sup> and 2%-5% for d- $\alpha$ -tocopherol<sup>[67]</sup>. Biologic L-selenomethionine is the optimal form of the multiple valence forms of selenium (at concentrations of 0.02%-0.05%)<sup>[68]</sup>. Combining L-ascorbic acid (15%) with  $\alpha$ -tocopherol (1%) gives four-fold protection against clinical minimal erythema dose (MED) and against thiamine dimer formation alone<sup>[74]</sup>; addition of another plant antioxidant ferulic acid (0.5%) yields 8-fold protection<sup>[72]</sup>. This formulation was shown to prevent the up-regulation of oxidative and inflammatory markers in human skin explants exposed to UV plus O<sub>3</sub> and diesel engine exhaust<sup>[75]</sup>. Many other topical antioxidants are being actively studied, including lycopene<sup>[76]</sup>, genistein<sup>[77]</sup>, plant-derived phenolic compounds (such as green tea, pomegranate, grape, and cocoa), and certain marine algae<sup>[78]</sup>.

Current research is also focused on understanding at a cellular level how the AHR binds and metabolizes xenochemicals, leading to complex cascades of protective or damaging inflammatory reactions, depending upon the specific pollutant, simultaneous exposure to UVA and to other pollutants, and concentration of these foreign substances. This understanding may lead to the discovery of methods to modulate the AHR response in order to prevent and protect the skin from extrinsic damage.

## DECLARATIONS

### Acknowledgements

The author would like to thank Xueyan Zhou, MD, MS, for assistance with literature research and Heather Nolan, MA, for excellent artistic rendition of the figures, literature research, and editing of text.

### Authors' contributions

The sole author wrote this review article.

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

The author declared that there are no conflicts of interest.

## Ethical approval and consent to participate

Not applicable.

## Consent for publication

Subjects shown in [Figures 1 and 5](#) did give consent.

## Copyright

© The Author(s) 2020.

## REFERENCES

1. Wang Y, Saladi R, Wei H. Synergistic carcinogenesis of chemical carcinogens and long wave-length UVA radiation. *Trends Photochem Photobio* 2001;10:31-45.
2. Gilchrist BA, Blog FB, Szabo G. Effects of aging and chronic sun exposure on melanocytes in human skin. *J Invest Dermatol* 1979;73:141-3.
3. Bologna JL, Orlow SJ. Chapter 65 - Melanocyte Biology. In: Bologna JL, Schaffer JV, Cerroni L, editors. *Dermatology Fourth Edition*. New York: Elsevier; 2018. pp. 1075-86.
4. Premi S, Wallisch S, Mano CM, Weiner AB, Bacchiocchi A, et al. Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. *Science* 2015;347:842-7.
5. Lagarrigue SG, George J, Questel E, Lauze C, Meyer N, et al. In vivo quantification of epidermis pigmentation and dermis papilla density with reflectance confocal microscopy: variations with age and skin phototype. *Exp Dermatol* 2012;21:281-6.
6. Cadet J, Douki T. Formation of UV-induced DNA damage contributing to skin cancer development. *Photochem Photobiol Sci* 2018;17:1816-41.
7. Kollias N, Baqer A. An experimental study of the changes in pigmentation in human skin *in vivo* with visible and near infrared light. *Photochem Photobiol* 1984;39:651-9.
8. Rosen CF, Jacques SL, Stuart ME, Gange RW. Immediate pigment darkening: visual and reflectance spectrophotometric analysis of action spectrum. *Photochem Photobiol* 1990;51:583-8.
9. Pathak MA, Riley FC, Fitzpatrick TB. Melanogenesis in human skin following exposure to long-wave ultraviolet and visible light. *J Invest Dermatol* 1962;39:435-43.
10. Mahmoud BH, Ruvalo E, Hexsel CL, Liu Y, Owen MR, et al. Impact of long-wavelength UVA and visible light on melanocompetent skin. *J Invest Dermatol* 2010;130:2092-7.
11. Liebel F, Kaur S, Ruvalo E, Kollias N, Southall MD. Irradiation of skin with visible light induces reactive oxygen species and matrix-degrading enzymes. *J Invest Dermatol* 2012;132:1901-7.
12. Cho S, Shin MH, Kim YK, Seo JE, Lee YM, et al. Effects of infrared radiation and heat on human skin aging *in vivo*. *J Inv Dermatol Symp Proc* 2009;14:15-9.
13. Schroeder P, Calles C, Benesova T, Macaluso F, Krutmann J. Photoprotection beyond ultraviolet radiation--effective sun protection has to include protection against infrared a radiation-induced skin damage. *Skin Pharmacol Physiol* 2010;23:15-7.
14. Calles C, Schneider M, Macaluso F, Benesova T, Krutmann J, et al. Infrared a radiation influences the skin fibroblast transcriptome: mechanisms and consequences. *J Invest Dermatol* 2010;130:1524-36.
15. Kelfkens G, de Gruijl FR, van der Leun JC. Tumorigenesis by short-wave ultraviolet A: papillomas versus squamous cell carcinomas. *Carcinogenesis* 1991;12:1377-82.
16. Shyong EQ, Lu Y, Goldstein A, Lebwahl M, Wei H. Synergistic enhancement of H<sub>2</sub>O<sub>2</sub> production in human epidermoid carcinoma cells by benzo[a]pyrene and ultraviolet A radiation. *Toxicol Appl Pharmacol* 2003;188:104-9.
17. Casale GP, Singhai M, Bhattacharya S, RamaNathan R, Roberts KP, et al. Detection and quantification of depurinated Benzo[a]pyrene-adducted DNA bases in the urine of cigarette smokers and women exposed to household coal smoke. *Chem Res Toxicol* 2001;14:192-201.
18. Saladi R, Austin L, Gao D, Lu Y, Phelps R, et al. The combination of benzo[a]pyrene and ultraviolet A causes an *in vivo* time-related accumulation of DNA damage in mouse skin. *Photochem Photobio* 2003;77:413-9.
19. Fuks KB, Woodby B, Valacchi G. Skin damage by tropospheric ozone. *Der Hautarzt* 2019;70:163-8.
20. Valacchi G, Sticozzi C, Belmonte G, Cervellati F, Demaude F, et al. Vitamin C compound mixtures prevent ozone-induced oxidative damage in human keratinocytes as initial assessment of pollution protection. *PLoS One* 2015;10:e0131097.
21. Pittayapruek P, Meephansan J, Prapapan O, Komine M, Ohtsuki M. Role of matrix metalloproteinases in photoaging and photocarcinogenesis. *Int J Mol Sci* 2016;17:868.
22. Valacchi G, Pagnin E, Okamoto T, Corbacho AM, Olano E, et al. Induction of stress proteins and MMP-9 by 0.8 ppm of ozone in murine skin. *Biochem Biophys Res Commun* 2003;305:741-6.
23. Fortino V, Maioli E, Torricelli C, Davis P, Valacchi G. Cutaneous MMPs are differently modulated by environmental stressors in old and young mice. *Toxicol Lett* 2007;173:73-9.
24. Caley MP, Martins VLC, O'Toole EA. Metalloproteinases and wound healing. *Adv Wound Care* 2015;4:225-34.
25. Xu X, Wang Y, Chen Z, Sternlicht MD, Hidalgo M, et al. Matrix metalloproteinase-2 contributes to cancer cell migration on collagen.



- Cancer Res 2005;65:130-36.
26. Thiele JJ, Traber MG, Tsang K, Cross CE, Packer L. In vivo exposure to ozone depletes vitamins C and E and induces lipid peroxidation in epidermal layers of murine skin. *Free Radic Biol Med* 1997;23:385-1.
  27. Fuks KB, Hüls A, Sugiri D, Altug H, Vierkötter A, et al. Tropospheric ozone and skin aging: Results from two German cohort studies. *Environ Int* 2019;124:139-44.
  28. Puri P, Nandar SK, Kathuria S, Ramesh V. Effects of air pollution on the skin: a review. *Indian J Dermatol Venereol Leprol* 2017;83:415-23.
  29. Soeur J, Bolandi JP, Chollet C, Denat L, Dimitrov A, et al. Photo-pollution stress in skin. Traces of Pollutants (PAH and particulate matter) impair redox homeostasis in keratinocytes exposed to UVA1. *J Dermatol Sci* 2017;8:162-9.
  30. Donaldson K, Tran L, Jimenez LA, Duffin R, Newby DE, et al. Combustion-derived nanoparticles: a review of their toxicology following inhalation exposure. *Part Fibre Toxicol* 2005;2:10-4.
  31. Kammer R, Tinnerberg H, Briksson K. Evaluation of a tape-stripping technique for measuring dermal exposure to pyrene and benzo[a]pyrene. *J Environ Monit* 2011;13:2165-71.
  32. Lademann J, Otberg N, Jacobi U, Hoffman RM, Blume-Peytavi U. Follicular penetration and targeting. *J Invest Dermatol Symp Proc* 2005;10:301-3.
  33. Jin SP, Li Z, Choi EK, Lee S, Kim YK. Urban particulate matter in air pollution penetrates into the barrier-disrupted skin and produces ROS-dependent cutaneous inflammatory response *in vivo*. *J Dermatol Sci* 2018;91:175-83.
  34. Moreau M, Ouellet N, Ayotte P, Bouchard M. Effects of intravenous benzo[a]pyrene dose administration on levels of exposure biomarkers, DNA adducts, and gene expression in rats. *J Toxicol Environ Health A* 2018;78:166-84.
  35. Magnani ND, Muresan XM, Belmonte G, Cervellati F, Sticozzi C, et al. Skin damage mechanisms related to airborne particulate matter exposure. *Toxicol Sci* 2016;149:227-36.
  36. Park SY, Byun EJ, Lee JD, Kim S, Kim HS. Air pollution, autophagy, and skin aging: Impact of particulate matter (PM<sub>10</sub>) on human dermal fibroblasts. *Int J Molec Sci* 2018;19:2727.
  37. Marchini T, Magnani ND, Paz ML, Vanasco V, Tasat D, et al. Time course of systemic oxidative stress and inflammatory response induced by an acute exposure to Residual Oil Fly Ash. *Toxicol and Applied Pharmacol* 2014;274:274-82.
  38. Cervellati F, Benedusi M, Manarini F, Woodby B, Russo M, et al. Proinflammatory properties and oxidative effects of atmospheric particle components in human keratinocytes. *Chemosphere* 2020;240:124746-53.
  39. Zhang Y, Zheng L, Tuo J, Liu Q, Zhang X, et al. Analysis of PM<sub>2.5</sub>-induced cytotoxicity in human HaCaT cells based on a microfluidic system. *Toxicol In Vitro* 2017;43:1-8.
  40. Sheu R, Stöner C, Ditto JC, Klüpfel T, Williams J, et al. Human transport of thirdhand tobacco smoke: A prominent source of hazardous air pollutants into indoor nonsmoking environments. *Environ Stud* 2020;6:eay4109.
  41. Valacchi G, Sticozzi C, Pecorelli A, Cervellati F, Cervellati C, et al. Cutaneous responses to environmental stressors. *Ann NY Acad Sci* 2012;1271:75-81.
  42. Bryant MS, Vineis P, Skipper PL, Tannenbaum SR. Haemoglobin adducts of aromatic amines in people exposed to cigarette smoke. *IARC Sci Publ* 1998;89:133-136.
  43. Pavlou P, Rallis M, Deliconstantinos G, Papaioannou G, Grando S. In-vivo data on the influence of tobacco smoke and UV light on murine skin. *Toxicol and Indus Health* 2009;25:231-9.
  44. Yin L, Morita A, Tsuji T. Alternations of extracellular matrix induced by tobacco smoke extract. *Arch Dermatol Res* 2000;292:183-94.
  45. Larigot L, Juricek L, Dairou J, Coumoul X. AhR signaling pathways and regulatory functions. *Biochim Open* 2018;7:1-9.
  46. Haarmann-Stemmann T, Esser C, Krutman J. The Janus-faced role of aryl hydrocarbon receptor signaling in the skin: Consequences for prevention and treatment of skin disorders. *J Invest Dermatol* 2015;135:2572-6.
  47. Vogeley C, Esser C, Tüting T, Krutmann J, Haarmann-Stemmann T. Role of the aryl hydrocarbon receptor in environmentally induced skin aging and skin carcinogenesis. *Intl J Molec Sci* 2019;20:6005-26.
  48. Vierkötter A, Schikowski T, Ranft U, Sugiri D, Matsui M, et al. Airborne particle exposure and extrinsic aging. *J Invest Dermatol* 2010;130:2719-26.
  49. Nakamura M, Morita A, Seité S, Haarmann-Stemmann T, Grether-Beck S, et al. Environment-induced lentigines: formation of solar lentigines beyond ultraviolet radiation. *Exper Dermatol* 2015;24:407-11.
  50. Vierkötter A, Krämer U, Sugiri D, Morita A, Yamamoto A, et al. Development of lentigines in German and Japanese women correlate with variants in the SLC45A2 gene. *J Invest Dermatol* 2012;132:733-6.
  51. Daniell JW. Smooth tobacco and wrinkled skin. *NPJM* 1969;280:53.
  52. Kadunce DP, Burr R, Gress R, Kanner R, Lyon JL, et al. Cigarette smoking: risk factor for premature facial wrinkling. *Ann Intern Med* 1991;114:840-4.
  53. Ernster VL, Grady D, Miike R, Black D, Skelby J, et al. Facial wrinkling in men and women, by smoking status. *Am J Public Health* 1995;85:78-82.
  54. Aizen E, Gilhar A. Smoking effect on skin wrinkling in the aged population. *Int J Dermatol* 2001;40:431-3.
  55. Burke KE. Great Skin for Life. London: Hamlyn Books Ltd, An imprint of Reed International; 1996. p. 59.
  56. Okada HC, Alleyne B, Varghai K, Kinder K, Guyuron B. Facial changes caused by smoking: A comparison between smoking and nonsmoking identical twins. *Plast Reconstr Surg* 2013;132:1085-92.
  57. Jin Y, Zhou Z, He G, Wei H, Liu J, et al. Geographical, spatial, and temporal distributions of multiple indoor air pollutants in four Chinese provinces. *Environ Sci Technol* 2005;39:8431-9.
  58. Zhang JJ, Smith KR. Household air pollution from coal and biomass fuels in China: measurements, health impacts, and interventions.

- Environ Health Perspect 2007;115:848-35.
59. Li M, Vierkötter A, Schikowski T, Hüls A, Ding A, et al. Epidemiological evidence that indoor air pollution from cooking with solid fuels accelerates skin aging in Chinese women. *J Dermatol Sci* 2015;79:148-54.
  60. Mukherjee, S. The emperor of all maladies - a biography of cancer. New York: Scribner; 2010. p. 247.
  61. Akhtar FZ, Garabrant DH, Ketchum NS, Michalek JE. Cancer in US air force veterans of the Vietnam War. *J Occup Environ Med* 2004;46:123-36.
  62. Villano CM, Murphy KA, Akintobi A, White LA. 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induces matrix metalloproteinase (MMP) expression and invasion in A2058 melanoma cells. *Toxicol Appl Pharmacol* 2006;210:212-24.
  63. Contador-Troca M, Alvarez-Barrientos A, Barrasa E, Rico-Leo EM, Catalina-Fernandez L, et al. The dioxin receptor has tumor suppressor activity in melanoma growth and metastasis. *Carcinogenesis* 2013;34:2683-93.
  64. Yanagi Y, Assunção JV, Barrozo LV. The impact of atmospheric particulate matter on cancer incidence and mortality in the city of São Paulo, Brazil. *Cad Saude Publ* 2012;28:1737-48.
  65. Asikainen A, Carrer P, Kephelopoulous S, Fernandes E de O, Wargocki P, et al. Reducing burden of disease from residential indoor air exposures in Europe (HEALTHVENT project). *Environ Health* 2016;15 Suppl 1:35.
  66. Pinnell SR, Yang HS, Omar M, Monteiro-Riviere N, DeBuys HV, et al. Topical L-ascorbic acid: percutaneous absorption studies. *Dermatol Surg* 2001;27:137-42.
  67. Burke KE, Clive J, Combs GF Jr, Commisso J, Keen CL, et al. The effects of topical and oral vitamin E on pigmentation and skin cancer induced by ultraviolet irradiation in Skh:2 hairless mice. *Nutr Cancer* 2000;38:87-97.
  68. Burke KE, Combs GF Jr, Gross EG, Bhuyan KC, Abu-Libdeh H. The effects of topical and oral L-selenomethionine on pigmentation and skin cancer induced by ultraviolet irradiation. *Nutr Cancer* 1992;17:123-37.
  69. Burke KE. Method for the prevention and reversal of the extrinsic aging of the skin by transdermal application of selenoamino acids and compositions therefore. US Patent Number: 5,330,757, July 19, 1994.
  70. Darr D, Combs S, Dunston S, Manning T, Pinnell S. Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. *Br J Dermatol* 1992;127:247-53.
  71. Burke KE, Combs GF Jr, French IW, Skeffington D. The effect of topical L-selenomethionine on minimal erythema dose of ultraviolet irradiation in humans. *Photoderm Photoimmun Photomed* 1992;9:52-7.
  72. Zielinski JE, Pinnell SR. Stabilized ascorbic acid compositions and methods thereof. US Patent 344052, 2004.
  73. Burke KE, Zhou X, Wang Y, Commisso J, Keen CL, et al. The effects of topical L-selenomethionine on protection against UVB-induced skin cancer when given before, during, and after UVB exposure. *J Drugs Dermatol* 2014;13:1214-23.
  74. Lin JY, Selim A, Shea CR, Grichnik JM, Omar MM, et al. UV photoprotection by combination topical antioxidants vitamin C and E. *J Am Acad Dermatol* 2003;48:866-74.
  75. Ferrara F, Woodby B, Pecorelli A, Schiavone ML, Pambianchi E, et al. Additive effect of combined pollutants to UV induced skin OxInflammation damage. Evaluating the protective topical application of a cosmeceutical mixture formulation. *Redox Biol* 2020;34:101481.
  76. Zhou X, Burke KE, Wang Y, Wei H. Dietary lycopene protects SkH-1 mice against ultraviolet B-induced photocarcinogenesis. *J Drugs Derm* 2019;18:1214-23.
  77. Burke KE. "Chapter 24: Photodamage: protection and reversal with topical antioxidants in Textbook of Cosmetic Dermatology, Fifth Edition. Boca Raton, FL: Taylor and Francis, 2017. pp. 199-213.
  78. Boo YC. Can plant phenolic compounds protect the skin from airborne particulate matter? *Antioxidants* 2019;8:379.

Review

Open Access



# Ptosis repair: external levator advancement vs. Müller's muscle-conjunctiva resection - techniques and modifications

Jacquelyn F. Laplang<sup>1</sup>, Julia Y. Kang<sup>2</sup>, Kimberly P. Cockerham<sup>2,3</sup>

<sup>1</sup>Department of Ophthalmology, Tulane University School of Medicine, New Orleans, LA 70112, USA.

<sup>2</sup>Central Valley Eye Medical Group, Stockton, CA 95207, USA.

<sup>3</sup>Department of Ophthalmology, Byers Eye Institute, Stanford University, Palo Alto, CA 94303, USA.

**Correspondence to:** Dr. Kimberly P. Cockerham, Central Valley Eye Medical Group, 36 W Yokuts, Stockton, CA 95207, USA.  
E-mail: cockerhammd@gmail.com

**How to cite this article:** Laplang JF, Kang JY, Cockerham KP. Ptosis repair: external levator advancement vs. Müller's muscle-conjunctiva resection - techniques and modifications. *Plast Aesthet Res* 2020;7:60.  
<http://dx.doi.org/10.20517/2347-9264.2020.69>

**Received:** 6 Apr 2020 **First Decision:** 24 Aug 2020 **Revised:** 7 Sep 2020 **Accepted:** 21 Oct 2020 **Published:** 6 Nov 2020

**Academic Editor:** Raúl González-García **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Surgical techniques for ptosis repair continue to evolve as we gain a better understanding of the anatomy and physiology of the eyelid. External repair by levator advancement and internal repair by Müller's muscle-conjunctiva resection are the most established surgical techniques used for acquired ptosis today. Controversy over their relative indications, advantages, and disadvantages exist. The advent of new surgical techniques and modifications has further complicated traditional algorithms that guide a surgeon towards choosing an external vs. internal approach. Specifically, the use and interpretation of pre-operative phenylephrine testing has recently been challenged. The purpose of this study is to review the evolution of external and internal ptosis repair techniques, and current trends in pre-operative evaluation and surgical management of acquired ptosis.

**Keywords:** Blepharoptosis, ptosis, levator advancement, Müller's muscle-conjunctiva resection

## INTRODUCTION

Blepharoptosis is one of the most commonly encountered eyelid disorders in ophthalmology. Management is primarily surgical; however, certain etiologies may improve with non-surgical management and



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





**Figure 1.** Before and after ptosis repair of left eye. Note the improvement in the MRD1 of the left eye post-operatively

treatment of underlying diseases. Surgical correction of ptosis has been performed for centuries with reports dating back to ancient Arabia and possibly ancient Rome<sup>[1,2]</sup>. Since then, hundreds of surgical techniques and modifications have been described [Figure 1].

Ptosis surgery generally falls into three categories: frontalis suspension techniques, external/transcutaneous repair of the levator complex, or internal/transconjunctival repair of the Müller's muscle, tarsus, conjunctiva, or levator complex. Accurate assessment of the etiology of ptosis is essential and can direct the surgeon to the most appropriate surgical options; however, there is significant variation in preoperative evaluation<sup>[3-5]</sup>. In particular, the use of phenylephrine testing to identify suitable candidates for internal repair and to estimate postoperative outcomes has recently been revisited<sup>[6,7]</sup>.

The literature is replete with reports on advantages of different surgical techniques to treat similar etiologies<sup>[8-10]</sup>. This has led to further investigations on the anatomy of the levator muscle complex and Müller's muscle in an attempt to better define their roles in eyelid elevation and guide surgical interventions<sup>[11,12]</sup>. The most significant changes in ptosis repair techniques have resulted from a better understanding of the anatomy and physiology of the eyelid. In this review, we focus on the two most favored techniques that have emerged for surgical repair of involutional ptosis: the external levator advancement and the internal, Müller's muscle-conjunctiva resection (MMCR) and their modifications.

## EXTERNAL REPAIR TECHNIQUES

The initial concept of aponeurotic repair was presented at a meeting held at the New York Eye and Ear Hospital in 1970<sup>[13]</sup>. Jones *et al.*<sup>[13]</sup> were one of the first to describe the repair of involutional ptosis by addressing the levator aponeurosis. A detailed description of the anatomy of the levator anatomy was then provided by Anderson and Beard, emphasizing the importance of understanding the anatomy of the levator aponeurosis and its attachments to achieve desirable surgical outcomes<sup>[13,14]</sup>.

Histopathological studies performed during this time further demonstrated aponeurogenic defects with preservation of Müller's muscle, emphasizing that repair of this defect will address the underlying pathology to correct the ptosis<sup>[15]</sup>. Jones *et al.*<sup>[13]</sup> were one of the first to describe repair of the aponeurosis and Anderson and Dixon identified aponeurotic repair as the procedure of choice for acquired ptosis<sup>[16]</sup>. This led to the "Age of Aponeurotic Awareness" whereby external levator repair was recommended for all cases of ptosis with preserved levator function<sup>[17]</sup>.

Several techniques and modifications for levator aponeurosis repair have since been described<sup>[18-20]</sup>. Levator advancement and levator resection are among the most commonly performed external repairs used today. In levator aponeurosis surgery a transcutaneous incision is made at the lid crease and dissection through the orbicularis muscle is performed. While elevating the skin-orbicularis flaps the septum is identified through blunt dissection and opened to reveal the preaponeurotic fat and levator aponeurosis. After evaluating and mobilizing the levator aponeurosis the tarsal plate is exposed. If disinsertion has occurred,

reattachment of the levator aponeurosis to the tarsal plate is performed or if the levator is stretched thin but still attached, a resection or advancement may be completed. This is often performed by placing a double armed 6-0 nylon suture on a spatulated needle partial thickness through the anterior surface of the tarsus corresponding to where the peak of the eyelid should be which is usually just nasal to the pupil. The length of the lamellar tarsal bite helps determine the eyelid contour. A shorter bite has an increased risk of peaking. The suture chosen varies and includes 6-0 silk, 6-0 vicryl, and 6-0 nylon, with either one central suture or a series of two or more sutures. Each needle is then passed through the levator aponeurosis and a temporary tie is placed. Intraoperative adjustments are performed as needed with the patient in sitting position to ensure desired eyelid height and contour prior to closure<sup>[21]</sup>. The patient's age and concurrent ocular co-morbidities should be taken into consideration when determining the optimal eyelid height. Younger patients may be able to tolerate small amounts of lagophthalmos, but older patients may be at risk for post-operative corneal exposure and exacerbate pre-existing ocular surface issues, especially if they have a poor Bell's reflex. The use of adjustable sutures that can be adjusted postoperatively has been described to help yield more predictable results<sup>[22,23]</sup>. A small incision technique has also been introduced and involves an 8 to 10 mm skin incision compared to the longer incisions used in conventional external ptosis repair<sup>[24,25]</sup>.

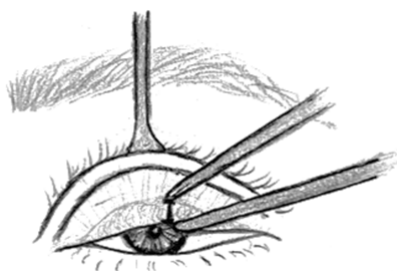
The main advantages of external repair include suitability for all degrees of ptosis, the ability to make intraoperative adjustments, preservation of the conjunctiva, and direct visualization of important anatomical structures. An external approach can also allow for removal of excessive skin and fat if needed. Disadvantages of the external approach include a steeper learning curve, less predictability, increased risk of abnormal lid contour, and longer surgical times compared to internal approaches. Although a success rate of 70%-95% is reported in the literature, up to 20% require revisions with higher revision rates for bilateral ptosis repairs<sup>[26-28]</sup>. It has been suggested that change in lid height following local anesthesia could affect a surgeon's judgement of lid position intraoperatively due to the effect of epinephrine on Müller's muscle. In addition, some patients have extensive fat infiltration of the levator muscle making securing of shortening by resection or a tucking advancement of altered muscle more challenging<sup>[29,30]</sup>.

## INTERNAL REPAIR TECHNIQUES

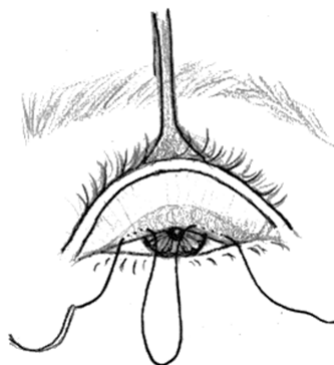
The principles of the posterior approach to ptosis repair is often attributed to Blascovic who described a posterior levator resection involving a tarsectomy in the early 1900s<sup>[31]</sup>. In 1961, Fasanella and Servat described a modification of this technique that is now well-known as the Fasanella-Servat procedure for correction mild ptosis of 3 mm or less<sup>[32]</sup>. It was initially thought that this resection involved both the levator and Müller's muscles; however, histopathological analysis demonstrated no levator, indicating that it was a tarso-conjunctival and Müller's muscle resection<sup>[32]</sup>. In the same year that Jones *et al.*<sup>[13]</sup> described the aponeuritic ptosis repair technique, Putterman *et al.*<sup>[33]</sup> introduced the MMCR technique without levator resection or tarsectomy.

MMCR was originally described for patients with mild to moderate ptosis, good levator function, and a positive response to 10% phenylephrine testing<sup>[33]</sup>. Many mechanisms have been proposed for the success of MMCR including vertical shortening of the posterior lamella, Müller's muscle or levator aponeurosis plication or advancement, or induction of cicatricial changes<sup>[34]</sup>. Marcet *et al.*<sup>[35]</sup> evaluated the histopathological changes of the eyelids in cadavers following MMCR and demonstrated that MMCR shortens the posterior lamella resulting in advancement of the levator palpebrae superioris muscle and plication of the levator aponeurosis. Traditionally, an 8 mm resection was recommended to achieve the eyelid elevation observed on positive phenylephrine testing with appropriate modifications if the eyelid elevates higher or lower than desired<sup>[33]</sup>. Several algorithms have been developed in an attempt to better predict postoperative results<sup>[36]</sup>. Since its introduction, many modifications and new techniques have also been described, including the conjunctival sparing Müller's resection, isolated mullerectomy,





**Figure 2.** The upper eyelid is everted using a Desmares retractor and the desired resection is measured using calipers



**Figure 3.** A 6-0 black silk suture is placed to mark the site



**Figure 4.** The 6-0 silk suture is tented up and the Putterman Muller's muscle-conjunctival resection clamp is placed

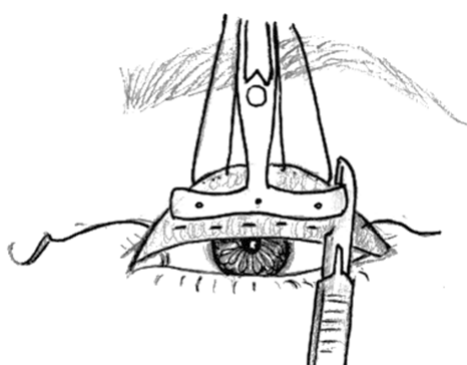
transconjunctival aponeurotic surgery without Müller's muscle resection, and transconjunctival levator plication<sup>[37-41]</sup>.

### MMCR

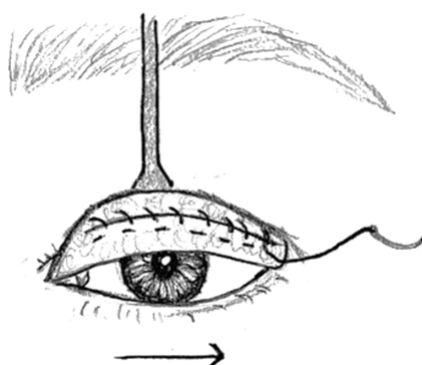
In traditional MMCR surgery a Desmares retractor is used to evert the lid and expose the posterior surface [Figure 2]. A predetermined amount of Müller's muscle and conjunctiva is measured and a marking suture is placed at this distance from the superior border of the upper tarsus [Figure 3]. A Putterman clamp (Bausch and Lomb, Storz, Manchester, MO) is placed containing the Müller's muscle and conjunctiva, and a running mattress suture is placed below the clamp in a temporal to nasal direction [Figures 4 and 5]. The tissue



**Figure 5.** A 5-0 plain gut suture is placed approximately 1.5 mm below the Putterman clamp along its entire width in a temporal to nasal direction

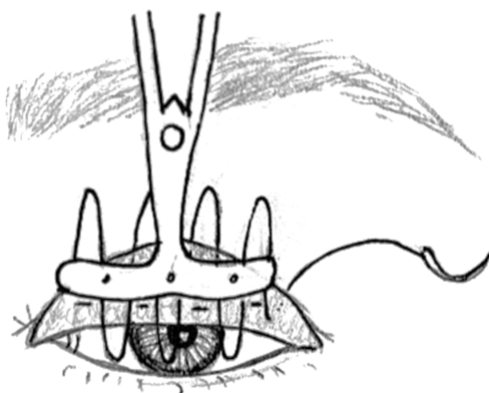


**Figure 6.** A #15 surgical blade is used to excise the tissue by cutting between the suture and the clamp

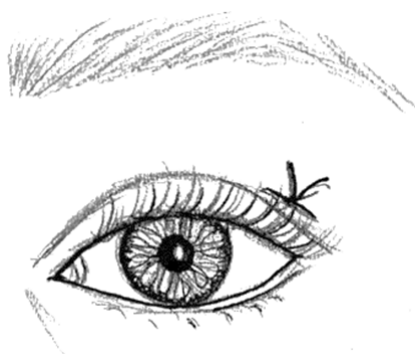


**Figure 7.** The suture is then passed in a running fashion from the nasal to temporal direction, burying the knot under the conjunctiva at the end

within the clamp is removed using a No. 15 blade avoiding the sutures [Figure 6]. The suture is then passed in a running fashion from nasal to temporal direction, burying the knot under the conjunctiva at the end [Figure 7]. Alternatively, the suture may be passed from the nasal to temporal direction with the clamp still attached and the excess conjunctiva and Muller's muscle removed with Wescott scissors [Figure 8]. Either single or double armed 6-0 or 5-0 plain gut sutures can be utilized. If using a single needle, the suture is passed through full thickness eyelid skin, run medially and then back laterally and externalized adjacent



**Figure 8.** Alternatively, the suture can be passed from the nasal to temporal direction with the clamp still attached, and the tissue is excised with Westcott scissors



**Figure 9.** The suture can also begin externally with the knot externalized through the skin, or in a blepharoplasty incision if performing this procedure concurrently

to the entry track and tied off [Figure 9]. If double armed, both needles are passed full thickness through the eyelid and taped to the skin surface, and later removed. If combined with an upper lid blepharoplasty, the skin and fat removal is performed first and then a running suture is passed from medially then back laterally and the knot is tied on the surface of the orbicularis instead and then the skin is closed in standard fashion. Rarely, a Frost suture may be needed if there is not adequate eyelid closure to temporarily protect the ocular surface<sup>[21]</sup>.

Strengths of the MMCR technique are better predictability of postoperative outcomes, lower revision rates, preservation of lid contour, no intraoperative adjustment, faster recovery, and success in anophthalmic and high-risk patients<sup>[41,42]</sup>. Success rates of 80%-100% are reported in the literature for MMCR<sup>[33,43,44]</sup>. Weaknesses of MMCR include limited amount of elevation, variable resection algorithms, and irritation from posterior placed sutures. Early concerns for a reduction in tear production due to resection of conjunctival goblet cells and accessory lacrimal has not been shown to be clinically significant<sup>[45]</sup>. Classic teaching also advises not to perform an MMCR on a patient who has a negative phenylephrine test; however, this theory is being revisited<sup>[7,46,47]</sup>. Additional elevation can be achieved by performing a tarsectomy with the MMCR that results in the distance of the eyelash margin to clamp position of 5 mm. Less than 5 mm of residual tarsus may increase the risk of upper eyelid entropion. A MMCR with tarsectomy has been performed in patients with moderate to severe ptosis and patients with a negative phenylephrine test with good levator function<sup>[48,49]</sup>.

## Open sky technique

Internal approaches can be further categorized into a “closed” technique utilizing a Putterman clamp and an “open sky” technique employing deeper surgical dissection<sup>[33,50]</sup>. In 2003, Lake *et al.*<sup>[51]</sup> introduced a modification of Putterman’s MMCR that allows for direct visualization of the Müller’s muscle prior to resection with passage of sutures through the skin crease to advance the levator muscle to the tarsus. This is achieved by identifying Müller’s muscle and removing its attachment to the levator aponeurosis, then passing a suture through the stump of Müller’s muscle, underlying conjunctiva, and upper border of the tarsus exiting through the skin crease<sup>[51]</sup>. A conjunctiva-sparing modification of this technique has also since been described<sup>[37]</sup>.

It is believed that this technique exerts its effect by effectively advancing the levator muscle through the stump of Müller’s muscle and tarsus and/or by enhancing a stretch reflex transmitted to the levator muscle<sup>[51,52]</sup>. Success with the open sky technique has also been reported in patients with suboptimal levator function<sup>[47,53]</sup>.

The main advantage of the open sky technique is the ability to adjust intra and post-operatively if overcorrected. This technique also does not require a clamp and avoids closure on the conjunctival surface, preventing irritation. Other strengths include direct visualization of Müller’s muscle to allow for maximal resection, predictable outcomes, preservation of lid contour, and high success rate<sup>[47,51]</sup>. It may also be beneficial in patients with high aponeurosis defects and patients with negative phenylephrine testing<sup>[47]</sup>.

## UTILITY OF PHENYLEPHRINE TESTING

The phenylephrine test is commonly used in preoperative assessment of ptosis; however, there is significant variation in how it is performed and interpreted. Putterman and Urist initially described phenylephrine testing as a means to identify candidates for the Fasanella-Servat and MMCR procedures<sup>[33]</sup>. They demonstrated a predictive role of phenylephrine testing for successful eyelid elevation after internal repair<sup>[33,54]</sup>. Phenylephrine testing is also used to identify subclinical contralateral ptosis whereby eyelid lowering is observed in the contralateral eyelid<sup>[55]</sup>. This is explained by Hering’s law of equal innervation, whereby surgical repair of the ptotic eyelid results in decreased innervation to the bilateral levator palpebrae muscles, unmasking ptosis of a previously compensated contralateral eyelid. If contralateral ptosis is observed, phenylephrine drops are then administered to that eye and measurements are taken. A positive Hering’s test may cause a surgeon to pursue bilateral ptosis repair to achieve better symmetry and avoid sequential surgery while other surgeons may defer surgery for the second eye<sup>[4,6]</sup>.

Traditional testing involves applying several drops of 10% phenylephrine to the superior conjunctival cul-de-sac to elicit eyelid elevation through the sympathetically innervated Müller’s muscle. However, a concern for the adverse systemic effects of 10% phenylephrine, although short-lived, has caused many surgeons to adopt the use of 2.5% phenylephrine instead<sup>[56]</sup>. A study comparing 2.5% to 10% phenylephrine demonstrated a 0.2 mm higher elevation with 10% phenylephrine that was considered clinically insignificant<sup>[57]</sup>. Further, there is no consensus on the ideal time to wait before assessing for a response although eyelid height is commonly assessed after 5 to 10 min following instillation of phenylephrine<sup>[6,7,57-59]</sup>. It was recently reported that maximal response to phenylephrine occurs within 2 min of instillation and persists for 30 min in healthy subjects<sup>[60]</sup>. In contrast, another study identified a subset of patients who responded later than 10 min to 2.5% phenylephrine, drawing attention to the possibility of incorrect identification of such patients as “non-responders”<sup>[6]</sup>.

A positive phenylephrine test has traditionally been defined as elevation of the ptotic eyelid to a cosmetically acceptable level and has served as an indication for internal ptosis repair<sup>[33]</sup>. However, the classic teaching that an internal repair should not be performed in the setting of a negative phenylephrine

test has recently been challenged<sup>[47,51,53]</sup>. Baldwin *et al.*<sup>[47]</sup> demonstrated efficacy of MMCR performed in patients with ptosis and a negative phenylephrine test using an open-sky technique. Another study reported a direct correlation of a patient's response to phenylephrine with postoperative results but identified improvement of eyelid position in patients despite a suboptimal or negative phenylephrine test<sup>[46]</sup>.

The phenylephrine test is also utilized to reveal subclinical ptosis of the contralateral eye. The degree of eyelid elevation in phenylephrine testing has also been used to determine the amount of tissue to be resected in MMCR<sup>[33]</sup>. An 8 mm MMCR is traditionally recommended to achieve the eyelid elevation observed on positive phenylephrine testing with a nomogram of 1 mm of lift achieved for every 4 mm of resection<sup>[33,61]</sup>. Several studies have since modified this formula; however significant controversy exists regarding its ability to predict postoperative outcomes with reports both confirming and negating the correlation between resection amount and post-operative results<sup>[36,62]</sup>. Further, Ben Simon *et al.*<sup>[7]</sup> found that phenylephrine testing underestimated the degree of eyelid elevation achieved surgically by 40%. Given the variability in phenylephrine testing, more studies are warranted to further characterize the responses in ptotic patients and its implications for surgical correction. The phenylephrine test is also useful to demonstrate the effect on the relative position of the eyelid height to the eyelid skin so that patients understand they may need an upper lid blepharoplasty either at the same time as the MMCR or subsequently.

## MÜLLER'S MUSCLE REVISITED

Reports of good surgical results with internal ptosis repair in phenylephrine negative patients and achievement of eyelid elevation greater than 2 mm has led to the reconsideration of the role of Müller's muscle in eyelid elevation. It has long been taught that the levator aponeurosis functions as the main transmitter of the levator muscle while Müller's muscle primarily maintains the tone of the upper eyelid and contributes an additional 2 mm to lid elevation when sympathetically activated<sup>[63]</sup>. However, many argue that this alone does not account for the degree of ptosis observed in Horner's syndrome. Further, eyelid elevations ranging from 2.5 to 4.0 mm have been reported with MMCRs of 7 to 12 mm suggesting that the Müller's muscle may serve a larger role in eyelid elevation by transmitting the action for the levator muscle to the tarsal plate<sup>[11,12,36,64]</sup>. The authors routinely utilize this approach for patients with congenital ptosis, chronic progressive external ophthalmoplegia, third nerve palsies, and myasthenia gravis, independent of the phenylephrine response.

Another proposed role of Müller's muscle in the eyelid is by functioning as a muscle spindle, creating a continuous stretch reflex of the levator muscles. Matsuo *et al.*<sup>[52]</sup> demonstrated that stretching of Müller's muscle resulted in involuntary tonic contraction of the ipsilateral or bilateral levator muscles, supporting that the Müller's muscle functions as a muscle spindle. Stretching and fatty infiltration of Müller's muscle with increasing age has also been observed on histopathological studies and could attenuate this reflex<sup>[29,65]</sup>. This could also account for the negative phenylephrine testing in some patients due to sympathetic denervation. Such fibro-fatty degeneration of Müller's muscle was recently observed on direct visualization on phenylephrine negative patients undergoing open sky MMCR<sup>[47]</sup>. Further, histopathological studies demonstrating a high concentration of alpha-2 receptors in Müller's muscle suggest that its response to phenylephrine may not represent its full role in eyelid retraction<sup>[66]</sup>.

Many attribute the success of MMCRs to incorporation of the levator muscle into the MMCR resulting in an "internal advancement" of the levator aponeurosis-müllerectomy conjunctival complex. Morris *et al.*<sup>[12]</sup> confirmed the presence of levator muscle fibers on all histopathological specimens obtained from cadavers who underwent ptosis repair using a modified internal müllerectomy approach. It is likely that a combination of the above mechanisms contributes to the success of MMCR and that results vary by surgeon and the type of internal repair performed.



## TRENDS IN CLINICAL PRACTICE

The initial introduction of external and internal repair techniques created two diverging schools of thought that have persisted for decades. Many abandoned the internal repair techniques during the “age of aponeurotic awareness”; however, the internal approach has experienced a resurgence. In a recent survey sent to ASOPRS members, 100% and 74% of respondents perform external levator repair and internal repair techniques in their practice, respectively<sup>[5]</sup>. Respondents who were greater than 15 years out of fellowship were less likely to perform internal ptosis repair, suggesting that this choice could be associated with the repair techniques preferred at the time of their training. Interestingly, 84% of respondents indicated that they will perform internal repair on patients with moderate ptosis and 32.4% of respondents will perform internal repair on patients with severe ptosis. This differs from the classic practice of performing internal repair only in patients with mild to moderate ptosis.

A similar study was sent to British Oculoplastic Surgery Society (BOPSS) members to identify practice patterns. In contrast to ASOPRS members, 76% prefer external repair techniques as first line treatment of ptosis compared to 13% using a MMCR and 11% preferred an interior white-line levator advancement technique; however, the majority reported switching to a posterior approach in the setting of a positive phenylephrine test<sup>[4]</sup>. Interestingly, only 40% of BOPSS respondents regularly use phenylephrine for ptosis assessment compared to 59.9% of ASOPRS respondents. Over half of BOPSS respondents use phenylephrine testing to assess for subclinical contralateral ptosis, to determine surgical approach, and modify surgery based on observed eyelid elevation. The majority of both BOPSS and ASOPRS respondents utilize 2.5% phenylephrine rather than 10% phenylephrine to minimize the risk of cardiac effects.

Although most surgeons perform both external and internal repair, the debate over the favored technique for surgical repair of ptosis still exists. Both internal repair using MMCR and external repair through levator advancement demonstrate high success rates for ptosis repair and each is associated with its own strengths and weaknesses<sup>[8,67]</sup>. The only randomized controlled trial to date found that external levator advancement and MMCR were both effective in repair of mild to moderate ptosis; however, MMCR provided a better cosmetic outcome and less eyelid asymmetry<sup>[68]</sup>. In a retrospective comparison of 272 surgical procedures, Ben Simon *et al.*<sup>[8]</sup> reported successful correction of involutional ptosis with both approaches although reported a higher reoperation rate in external repair (17%) compared to internal repair (< 3%). A larger retrospective review of 1,519 patients also demonstrated higher revision rate in external repair (9.5%) than internal repair (6.8%)<sup>[69]</sup>. Another retrospective study instead compared small incision external repair and the Fasanella-Servat technique, demonstrating similar efficacy and patient satisfaction with both techniques, with lower operative times using the Fasanella-Servat technique<sup>[70]</sup>. To date, no studies have compared small incision external repair with MMCR.

## CONCLUSION

The advancements in external and internal repair have added a myriad of techniques to the surgical armamentarium for correction of ptosis. Insights into the anatomy and physiology of the levator complex and Müller’s muscle have helped us better understand the mechanisms employed by different surgical approaches although much remains to be elucidated regarding their contributions to eyelid elevation. Similarly, although phenylephrine testing maintains an important role in preoperative evaluation of ptosis, success with internal approaches in patients with phenylephrine negative testing suggests that a negative test does not necessarily predict a poor surgical outcome.

Despite changing attitudes towards external vs. internal repair and their indications over time, success has been achieved with both approaches. While internal approaches have consistently demonstrated lower revision rates and better cosmetic outcomes, external approaches are still preferred for more severe ptosis.

Larger, randomized controlled studies with longer follow-up comparing the two approaches in mild to severe ptosis are needed to better identify which approach is preferred in certain situations.

## DECLARATIONS

### Authors' contributions

Conceptualization, writing, and editing of this manuscript: Laplant JF, Cockerham KP  
Medical illustrations and editing of this manuscript: Kang JY

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Servat J, Mantilla M. The history of ptosis surgery. *Adv Ophthalmic Plast Reconstr Surg* 1986;5:133-7.
2. Beard C. History of ptosis surgery. *Adv Ophthalmic Plast Reconstr Surg* 1986;5:125-31.
3. Pereira IC, Matayoshi S. Quantitative comparison of the effect of 10% phenylephrine instillation and manual elevation in patients with involutional blepharoptosis. *Semin Ophthalmol* 2017;32:172-6.
4. Mota PM, Norris JH. Review on surgical management of ptosis and the use of phenylephrine: a national survey of British Oculoplastic Surgery Society (BOPSS) UK Consultants. *Orbit* 2016;35:339-42.
5. Aakalu VK, Setabutr P. Current ptosis management: a national survey of ASOPRS members. *Ophthalmic Plast Reconstr Surg* 2011;27:270-6.
6. Barsegian A, Botwinick A, Reddy HS. The phenylephrine test revisited. *Ophthalmic Plast Reconstr Surg* 2018;34:151-4.
7. Ben Simon GJ, Lee S, Schwarcz RM, McCann JD, Goldberg RA. Muller's muscle-conjunctival resection for correction of upper eyelid ptosis: relationship between phenylephrine testing and the amount of tissue resected with final eyelid position. *Arch Facial Plast Surg* 2007;9:413-7.
8. Ben Simon GJ, Lee S, Schwarcz RM, McCann JD, Goldberg RA. External levator advancement vs Müller's muscle-conjunctival resection for correction of upper eyelid involutional ptosis. *Am J Ophthalmol* 2005;140:426-32.
9. Pang NK, Newsom RW, Oestreicher JH, Chung HT, Harvey JT, Fasanella-Servat procedure: indications, efficacy, and complications. *Can J Ophthalmol* 2008;43:84-8.
10. Park DH, Choi WS, Yoon SH, Shim JS. Comparison of levator resection and frontalis muscle transfer in the treatment of severe blepharoptosis. *Ann Plast Surg* 2007;59:388-92.
11. Bang YH, Park SH, Kim JH, Cho JH, Lee CJ, Roh TS. The role of Müller's muscle reconsidered. *Plast Reconstr Surg* 1998;101:1200-4.
12. Morris CL, Morris WR, Fleming JC. A histological analysis of the Müllerectomy: redefining its mechanism in ptosis repair. *Plast Reconstr Surg* 2011;127:2333-41.
13. Jones LT, Quickert MH, Wobig JL. The cure of ptosis by aponeurotic repair. *Arch Ophthalmol* 1975;93:629-34.
14. Anderson RL, Beard C. The levator aponeurosis. Attachments and their clinical significance. *Arch Ophthalmol* 1977;95:1437-41.
15. Dortzbach RK, Sutula FC. Involutional blepharoptosis. A histopathological study. *Arch Ophthalmol* 1980;98:2045-9.
16. Anderson RL, Dixon RS. Aponeurotic ptosis surgery. *Arch Ophthalmol* 1979;97:1123-8.
17. Anderson RL. Age of aponeurotic awareness. *Ophthalmic Plast Reconstr Surg* 1985;1:77-9.

18. McCord CD, Seify H, Codner MA. Transblepharoplasty ptosis repair: three-step technique. *Plast Reconstr Surg* 2007;120:1037-44.
19. Scuderi N, Chiummariello S, De Gado F, Alfano C, Scuderi G, Recupero SM. Surgical correction of blepharoptosis using the levator aponeurosis-Müller's muscle complex readaptation technique: a 15-year experience. *Plast Reconstr Surg* 2008;121:71-8.
20. Singh D. Orbicularis plication for ptosis: a third alternative. *Ann Ophthalmol (Skokie)* 2006;38:185-93.
21. Black EH, Nesi FA, Gladstone G, Levine MR, Calvano CJ. Smith and Nesi's ophthalmic plastic and reconstructive surgery. 3rd ed. New York: Springer-Verlag New York; 2012.
22. Collin JR, O'Donnell BA. Adjustable sutures in eyelid surgery for ptosis and lid retraction. *Br J Ophthalmol* 1994;78:167-74.
23. Meltzer MA, Elahi E, Taupeka P, Flores E. A simplified technique of ptosis repair using a single adjustable suture. *Ophthalmology* 2001;108:1889-92.
24. Frueh BR, Musch DC, McDonald HM. Efficacy and efficiency of a small-incision, minimal dissection procedure versus a traditional approach for correcting aponeurotic ptosis. *Ophthalmology* 2004;111:2158-63.
25. Lucarelli MJ, Lemke BN. Small incision external levator repair: technique and early results. *Am J Ophthalmol* 1999;127:637-44.
26. Brown BZ. Ptosis revision. *Int Ophthalmol Clin* 1989;29:217-8.
27. McCulley TJ, Kersten RC, Kulwin DR, Feuer WJ. Outcome and influencing factors of external levator palpebrae superioris aponeurosis advancement for blepharoptosis. *Ophthalmic Plast Reconstr Surg* 2003;19:388-93.
28. Linberg JV, Vasquez RJ, Chao G. Aponeurotic ptosis repair under local anesthesia. *Ophthalmology* 1988;95:1046-52.
29. Cahill KV, Buerger GF Jr, Johnson BL. Ptosis associated with fatty infiltration of Müller's muscle and levator muscle. *Ophthalmic Plast Reconstr Surg* 1986;2:213-7.
30. Zhang L, Li B, Li L, Li Y, Zhang Y, Li DM. Pathological features of levator aponeurosis in patients with involutional blepharoptosis. *Zhonghua Yan Ke Za Zhi* 2018;54:671-7.
31. von Blaskovics L, Kreiker A. Eingriffe am Auge. 1st ed. Stuttgart: Enke; 1938.
32. Fasanella RM, Servat J. Levator resection for minimal ptosis: another simplified operation. *Arch Ophthalmol* 1961;65:493-6.
33. Putterman AM, Urist MJ. Müller muscle-conjunctiva resection. Technique for treatment of blepharoptosis. *Arch Ophthalmol* 1975;93:619-23.
34. Buckman G, Jakobiec FA, Hyde K, Lismann RD, Hornblass A, Harrison W. Success of the Fasanella-Servat operation independent of Müller's smooth muscle excision. *Ophthalmology* 1989;96:413-8.
35. Marcet MM, Setabutr P, Lemke BN, et al. Surgical microanatomy of the müller muscle-conjunctival resection ptosis procedure. *Ophthalmic Plast Reconstr Surg* 2010;26:360-4.
36. Dresner SC. Further modifications of the Müller's muscle-conjunctival resection procedure for blepharoptosis. *Ophthalmic Plast Reconstr Surg* 1991;7:114-22.
37. Khooshabeh R, Baldwin HC. Isolated Müller's muscle resection for the correction of blepharoptosis. *Eye (Lond)* 2008;22:267-72.
38. Ichinose A, Tahara S. Transconjunctival levator aponeurotic repair without resection of Müller's muscle. *Aesthetic Plast Surg* 2007;31:279-84.
39. Singh D, Singh K, Singh SK, Singh RS. Sutureless levator plication by conjunctival route: a new technique. *Compr Ther* 2006;32:240-7.
40. Patel V, Salam A, Malhotra R. Posterior approach white line advancement ptosis repair: the evolving posterior approach to ptosis surgery. *Br J Ophthalmol* 2010;94:1513-8.
41. Saha K, Leatherbarrow B. Conjunctival sparing Müller's muscle resection for the management of blepharoptosis in the anophthalmic patient. *Clin Exp Ophthalmol* 2011;39:478-9.
42. Michels KS, Vagefi MR, Steele E, et al. Müller muscle-conjunctiva resection to correct ptosis in high-risk patients. *Ophthalmic Plast Reconstr Surg* 2007;23:363-6.
43. Carruth BP, Meyer DR. Simplified Müller's muscle-conjunctival resection internal ptosis repair. *Ophthalmic Plast Reconstr Surg* 2013;29:11-4.
44. Patel RM, Aakalu VK, Setabutr P, Putterman AM. Efficacy of Müller's muscle and conjunctiva resection with or without tarsectomy for the treatment of severe involutional blepharoptosis. *Ophthalmic Plast Reconstr Surg* 2017;33:273-8.
45. Dailey RA, Saulny SM, Sullivan SA. Müller muscle-conjunctival resection: effect on tear production. *Ophthalmic Plast Reconstr Surg* 2002;18:421-5.
46. Wee SW, Lee JK. Clinical outcomes of conjunctiva-Müller muscle resection: association with phenylephrine test-negative blepharoptosis and dry eye syndrome. *J Craniofac Surg* 2014;25:898-901.
47. Baldwin HC, Bhagey J, Khooshabeh R. Open sky Müller muscle-conjunctival resection in phenylephrine test-negative blepharoptosis patients. *Ophthalmic Plast Reconstr Surg* 2005;21:276-80.
48. Samimi DB, Erb MH, Lane CJ, Dresner SC. The modified fasanella-servat procedure: description and quantified analysis. *Ophthalmic Plast Reconstr Surg* 2013;29:30-4.
49. Perry JD, Kadakia A, Foster JA. A new algorithm for ptosis repair using conjunctival Müllerectomy with or without tarsectomy. *Ophthalmic Plast Reconstr Surg* 2002;18:426-9.
50. Werb A. Ptosis. *Aust J Ophthalmol* 1976; 4:40-3.
51. Lake S, Mohammad-Ali FH, Khooshabeh R. Open sky Müller's muscle-conjunctiva resection for ptosis surgery. *Eye (Lond)* 2003;17:1008-12.
52. Matsuo K. Stretching of the Mueller muscle results in involuntary contraction of the levator muscle. *Ophthalmic Plast Reconstr Surg* 2002;18:5-10.
53. Cohen AJ, Weinberg DA. Müller's muscle-conjunctival resection for blepharoptosis with poor levator function. *Ophthalmic Surg Lasers*

- Imaging Retina* 2002;33:491-2.
54. Shields M, Putterman A. Blepharoptosis correction. *Curr Opin Otolaryngol Head Neck Surg* 2003;11:261-6.
  55. Gay AJ, Salmon ML, Windsor CE. Hering's law, the levators, and their relationship in disease states. *Arch Ophthalmol* 1967;77:157-60.
  56. Stavert B, McGuinness MB, Harper CA, Guymer RH, Finger RP. Cardiovascular adverse effects of phenylephrine eyedrops: a systematic review and meta-analysis. *JAMA Ophthalmol* 2015;133:647-52.
  57. Glatt HJ, Fett DR, Putterman AM. Comparison of 2.5% and 10% phenylephrine in the elevation of upper eyelids with ptosis. *Ophthalmic Surg. Lasers Imaging Retina* 1990;21:173-6.
  58. Ayala E, Gálvez C, González-Candial M, Medel R. Predictability of conjunctival-Müllerectomy for blepharoptosis repair. *Orbit* 2007;26:217-21.
  59. Putterman AM. Re: Phenylephrine test protocol. *Ophthalmic Plast Reconstr Surg* 2018;34:396.
  60. Ramesh S, Mancini R. Dynamic analysis of Müller's muscle response to phenylephrine. *Ophthalmic Plast Reconstr Surg* 2016;32:46-8.
  61. Putterman AM, Fett DR. Müller's muscle in the treatment of upper eyelid ptosis: a ten-year study. *Ophthalmic Surg Lasers Imaging Retina* 1986;17:354-60.
  62. Weinstein GS, Buerger GF. Modifications of the Müller's muscle-conjunctival resection operation for blepharoptosis. *Am J Ophthalmol* 1982;93:647-51.
  63. Carraway JH. Surgical anatomy of the eyelids. *Clin Plast Surg* 1987;14:693-701.
  64. Kakizaki H, Malhotra R, Selva D. Upper eyelid anatomy: an update. *Ann Plast Surg* 2009;63:336-43.
  65. Collin JRO, Beard C, Wood I. Experimental and clinical data on the insertion of the levator palpebrae superioris muscle. *Am J Ophthalmol* 1978;85:792-801.
  66. Esmali-Gutstein B, Hewlett BR, Pashby RC, Oestreicher J, Harvey JT. Distribution of adrenergic receptor subtypes in the retractor muscles of the upper eyelid. *Ophthalmic Plast Reconstr Surg* 1999;15:92-9.
  67. Thomas GN, Chan J, Sundar G, Amrith S. Outcomes of levator advancement and Müller muscle-conjunctiva resection for the repair of upper eyelid ptosis. *Orbit* 2017;36:39-42.
  68. Saonanon P, Sithanon S. External levator advancement versus Müller muscle-conjunctival resection for aponeurotic blepharoptosis: a randomized clinical trial. *Plast Reconstr Surg* 2018;141:213e-9.
  69. Chou E, Liu J, Seaworth C, et al. Comparison of revision rates of anterior- and posterior-approach ptosis surgery: a retrospective review of 1519 Cases. *Ophthalmic Plast Reconstr Surg* 2018;34:246-53.
  70. Sohrab MA, Lissner GS. Comparison of Fasanella-Servat and small-incision techniques for involutional ptosis repair. *Ophthalmic Plast Reconstr Surg* 2016;32:98-101.

Original Article

Open Access



# Metoidioplasty using labial advancement flaps for urethroplasty

Toby R. Meltzer, Nick O. Esmonde

The Meltzer Clinic, Scottsdale, AZ 85253, USA.

**Correspondence to:** Dr. Toby R. Meltzer, The Meltzer Clinic, 7025 N Scottsdale Rd. Ste 302, Scottsdale, AZ 85253, USA.  
E-mail: tmeltzer@tmeltzer.com

**How to cite this article:** Meltzer TR, Esmonde NO. Metoidioplasty using labial advancement flaps for urethroplasty. *Plast Aesthet Res* 2020;7:61. <http://dx.doi.org/10.20517/2347-9264.2020.122>

**Received:** 22 May 2020 **First Decision:** 12 Aug 2020 **Revised:** 2 Sep 2020 **Accepted:** 10 Oct 2020 **Published:** 6 Nov 2020

**Academic Editor:** Marlon E. Buncamper **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

**Aim:** A variation of the ring metoidioplasty has been performed for masculinizing transgender surgery by the senior surgeon since 2010. It does not require buccal grafts or vaginal wall flaps. An excisional vaginectomy was completed in all patients. We sought to evaluate the urologic outcomes and complications for this technique. Further, we provide a detailed technical description of the technique, including ancillary masculinizing procedures.

**Methods:** This is a retrospective, single surgeon chart review of all patients undergoing metoidioplasty from 2010 to 2020. Demographics, outcomes, and complications are reported. A self-reported patient questionnaire provided data on patient-perceived urologic outcomes.

**Results:** Ninety-one patients were included in the study, with 80 (87.9%) patients reporting ability to stand and void with a strong stream. We observed five strictures (5.5%) and one fistula (1%). Scrotoplasty with tissue expanders and testicular implants were performed in 75 (82.4%) patients, while monsplasty was performed in 54 (59%) patients.

**Conclusion:** Our technique has a low complication rate and patients report a strong urinary stream and the ability to stand in the large majority of cases. Ancillary masculinizing procedures are common. The limitations of metoidioplasty, in general, still persist, which are the small phallus size and variable ability to clear the zipper without lowering the pants to void.

**Keywords:** Metoidioplasty, urethroplasty, transgender surgery, transmasculine surgery



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.





## INTRODUCTION

The surgical treatment of gender dysphoria related to genital anatomy for transmasculine patients is largely achieved via phalloplasty or metoidioplasty. Both techniques offer masculine anatomy and the potential to urinate while standing. However, there are some inarguable downsides to phalloplasty, namely a large scar burden, more potential immediate and long-term urologic and flap complications, greater number of procedures, less predictable sensory and sexual function outcomes, and relatively few surgeons and centers providing the procedure, that motivate some patients to pursue less extensive genital reconstruction. Thus, metoidioplasty is an appealing alternative for some patients.

Metoidioplasty aims at masculinizing the genitalia by making the hypertrophied clitoris appear as a penis and may also include urethral lengthening and scrotoplasty.

While there are now several variations, most published reports of this technique describe the release of the urethral plate and dorsal suspensory ligaments of the clitoris to facilitate lengthening<sup>[1-4]</sup>. The defect created by dividing the urethral plate is typically grafted with either vaginal or buccal mucosa, and possibly buttressed with local flaps. Further, anterior vaginal wall flaps harvested during vaginectomy may be utilized for construction of the proximal portion of the pars fixa<sup>[5]</sup>.

A novel variation of the metoidioplasty technique was presented by Takamatsu *et al.*<sup>[6]</sup>, who described raising a large mucosal flap and tubularizing it in continuity with the anterior vaginal wall flap to create the pars fixa, dubbed the “labial ring flap” (also referred to here as “ring metoidioplasty”). The flap is anteriorly based and includes the urethral plate and entire mucosa of the inner labia including the tissue adjacent and posterior to the vagina and urethral meatus. Their flap design excluded the vaginal and urethral tissues and thus creates a “ring” of absent tissue within the flap that is subsequently closed. This flap allows for the entirety of the neourethra to be comprised of vascularized tissue and spares the need for additional grafts. In their series, most patients did not have a vaginectomy prior to metoidioplasty, which may have increased their occurrence of urethral complications (3 strictures and 12 fistulas in 43 patients). Here, the senior author has further modified the Takamatsu technique to eliminate the need for the vaginal flap and reconfigured the labial flap to be two large V-Y adipo-mucosal advancement flaps which form the entirety of the lengthened urethra. This paper reviews the senior author’s experience with the technique, including a technical description, perioperative care, patient demographics, surgical outcomes, and secondary procedures.

## METHODS

The study is a retrospective chart review of a single surgeon’s experience performing metoidioplasty using V-Y labial flaps between 2010 and 2020. All patients gave informed consent. Inclusion criteria were age greater than 18, diagnosis of gender dysphoria, and compliance with World Professional Association for Transgender Health guidelines for genital surgery. Most patients self-referred for a metoidioplasty. Their rationale for choosing metoidioplasty over phalloplasty was not recorded. There was no strict body mass index (BMI) “cut off” to qualify for metoidioplasty; however, if the patient was an active tobacco user that was an absolute contraindication. It is the senior author’s practice that all patients undergo vaginectomy and colpocleisis immediately prior to the metoidioplasty. Additionally, the senior author preferred all patients were established on exogenous testosterone therapy prior to surgery to realize the most benefit from clitoral hypertrophy. Patient demographics and surgical data, ancillary procedures, urologic outcomes, and complications were collected. Follow up data included self-reported urologic outcomes, using an ad hoc questionnaire that was administered in person or via phone. Patients were first asked whether they were able to stand and urinate (“yes/no”), and then whether their urinary stream was “strong” or “weak”. These questions were asked at all visits, but we chose the patient response from their most recent or final

postoperative visit. For the analysis, we generated descriptive statistics using the mean and standard deviation for continuous variables, and the frequency and percentage for binary variables using Excel (Microsoft Corp. Richmond, WA).

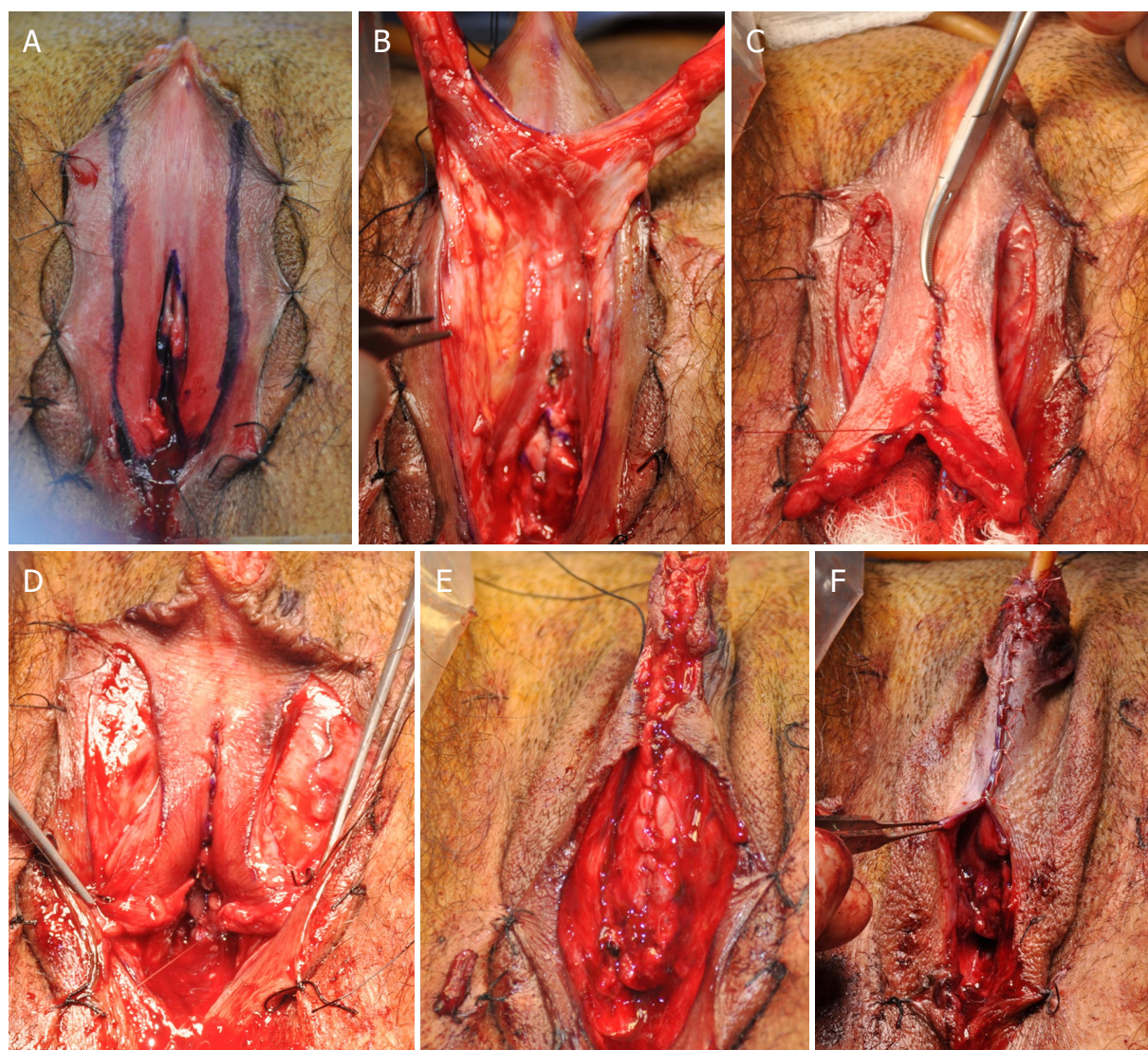
### **Perioperative care and operative technique**

The metoidioplasty begins after an excisional vaginectomy is completed with the patient in low lithotomy. If not previously done, a laparoscopic assisted hysterectomy and oophorectomy are completed before the vaginectomy. An episiotomy facilitates the vaginectomy so the posterior fourchette is already divided at this point. The vaginal lining has been removed and closed down with sutures leaving a 2-cm cuff of mucosa on the ventral urethra.

A suture is placed through the glans clitoris to maintain tension. Tacking sutures are placed on the edge of the labia minora to spread out the inner surface of the labia minora and widely expose the urethral plate. Below the level of the urethra, the flaps are each designed to be a minimum of 12 mm in width. This is measured from the innermost edge at the vaginectomy site and extends as far laterally as necessary. The medial border of the flap will extend just lateral to the urethra, making sure to preserve the location of ducts of Skene's glands (lesser vestibular glands) [Figure 1A]. The flaps will join at the midline immediately above the urethral meatus. The midline is marked from this point up to the glans clitoris, and the flap is marked on either side with a width of 12 mm (24 mm total width). If the labia minora are very small or attenuated distally, the flap can be designed to cross the labial edge over to the preputial skin. The labial edge can be unfolded later when elevating the flap and the preputial skin will be the lateral edge of the urethral flap. The lateral border of the flaps should gently curve outward above and below where the labial flaps unite in the midline. This helps to prevent an area of narrowing and reduces tension where the paired flaps are sewn together. This modification was made after early experience of this area being a repeat locus for stricture. Subsequently, stricture frequency decreased. Local anesthesia (1% lidocaine with 1:100,000 epinephrine) is infiltrated throughout the planned flaps. A cystoscope is used to confirm that there is no bladder injury from the vaginectomy and allows a percutaneous suprapubic tube to be placed under direct visualization.

Beginning posteriorly, the flaps are incised and elevated deeply (including fat) with scissors. When the flaps are elevated just superior to the urethra, the dissection then extends deeply to the corporal bodies and extends superiorly along the corporal bodies. The urethral plate is undermined releasing the ventral chordee until no restriction to straightening is felt [Figure 1B]. While upward traction is held on the clitoris, applying digital pressure to ventral clitoris one can appreciate the loss of bowstringing of the urethral plate and it should feel flat against the straightened corporal bodies. Usually, the urethral plate will be released between 3 and 5 cm in length (measured anterior to posterior). The labial flaps are incised distally beyond this, but not undermined to maintain the blood supply through the urethral plate via anterior branches of the external pudendal artery. The clitoral skin lateral to the mucosa is elevated as needed to reduce the redundancy and allow tension free wrapping of the skin over the urethra in the mobile (pendulous) portion of the clitoris. There is usually an excess of skin and this can be de-epithelialized later to help cover the neourethra. The skin around the corona of the glans is not incised, giving the penis an uncircumcised appearance. A running, locking 4-0 Monocryl (Ethicon Inc., Sommerville, NJ) suture is used to over sew the raw surface area where the urethral plate was released on the ventral corporal bodies. There are frequently several larger venous plexuses below where the flaps have been elevated proximally, which are also over sewn with 4-0 Monocryl.

With mild tension on the glans, the labial flaps should easily reach below the urethral meatus. Most often, the mucosa is then removed from the ventral portion of the urethra leaving 1-cm cuff to sew. If it appears the flaps will not reach, then a posterior flap can be elevated instead - but this has not been necessary. The



**Figure 1.** A: Standard markings for metoidioplasty technique; B: ventral chordee released and flaps elevated. Bulbospongiosus muscles and corporal body visible on deep side; C: ventral urethral repair (V-Y configuration); D: native urethra to neourethra repair; E: completed urethral repair over 14F catheter; F: paramedian skin closure

epithelium of the flaps is imbricated in all suturing of the neourethra. The medial edges of the flaps are sewn together with running 4-0 Monocryl stopping when it reaches the dorsal urethra without tension [Figure 1C]. The flaps are now sewn around the native urethra, with 3-0 interrupted Monocryl [Figure 1D]. Once completed, a 14F catheter is placed and the lateral border of the flaps are rolled over the catheter and approximated with interrupted 3-0 Monocryl (with the knots facing away from the urethral lumen), creating the ventral wall of the neourethra. As the suturing proceeds distal to the pars fixa, a 4-0 Monocryl suture is used. The urethra is repaired as far distal as possible provided there is no tension on the repair over the catheter. Depending on the size of the glans clitoris, the urethroplasty will usually terminate at or just distal to the base of the glans.

The levator ani muscles in the vaginectomy site are now approximated in the midline snugly with 2-0 Monocryl. Approximating the muscles provides hemostasis at the vaginectomy site and buttresses the ventral and proximal urethra to help prevent diverticulum and fistulas. A single TLS drain (Stryker Corp., Kalamazoo, MI) is placed along the urethra and brought out superiorly. The remainder of the vaginectomy





**Figure 2.** Scrotoplasty markings

site is closed in layers over the pars fixa. The subcutaneous loose areolar tissue can be approximated at the base of the mobile portion of the urethra. The lateral skin of the clitoris from one side is brought across the midline creating a paramedial skin closure so that it does not lay directly over the urethral repair [Figure 1E and F]. There is usually an excess of skin on either side which can be de-epithelialized before the closure and folded over the urethra for more coverage. The final skin repair is completed with interrupted 4-0 Vicryl (Ethicon Inc., Sommerville, NJ) along the edges of the clitoral skin, to wrap the neo-phallus.

If a scrotoplasty is going to be performed at the same time, a variation of the scrotoplasty described by Selvaggi *et al.*<sup>[7]</sup> is used [Figure 2]. The labia majora flaps are designed similarly, but not advanced as far anteriorly as with a phalloplasty to prevent the scrotum from engulfing and obscuring the metoidioplasty. A transverse incision around 30 mm in length is made at the base of the penis below the midline suture line to inset the tip of the labial flaps. The lateral incision stops at about the level of the base of the penis or lower if there is excessive tension for a midline repair. The upper portions of the anterior labia are reduced separately with either an inverted V to Y excision or an asymmetric triangle excision to flatten the penopubic junction and make the penis more visible and accessible.

At the completion of the procedure, the catheter in the penis is plugged and taped against the abdomen to reduce pressure on the urethral suture line. The suprapubic tube is maintained to gravity drainage. The patient is maintained on bedrest until Postoperative Day (POD) 2 to decrease the risk of bleeding. On POD 12, the Foley catheter is removed, and the patient is allowed to void through the neourethra. Total output, void times, and post-void residuals are recorded. When the post-void residuals are consistently low (< 30 mL), the suprapubic tube is removed. Occasionally, higher post-void residuals are seen and are more common after the first void in the morning. If the patients have persistently higher post-void residuals and/or slower voiding times, then tamsulosin (Sanofi-Aventis SA, Cambridge, MA) is given. It has been the senior author's experience that, for the majority of patients, tamsulosin will increase their flow rate and reduce their post-void residuals, although this is an off-label use. If effective, a two-week course of tamsulosin is prescribed and the suprapubic tube is removed. There are occasional patients who have persistently higher post-void residuals regardless with a stable trend. As a trial, the suprapubic tube is plugged for 24 h and then the post-void residual is checked prior to removal. Early strictures have not occurred; however, if there is any concern, it is prudent to leave the suprapubic catheter in place longer. The patient will continue to void through the neourethra, and the suprapubic tube is kept plugged.

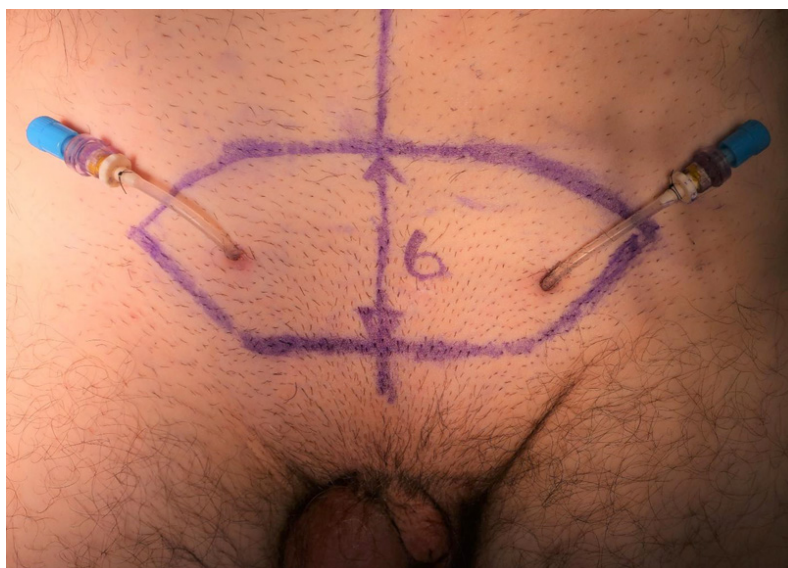


**Figure 3.** Postoperative photo showing externalized testicular tissue expander ports

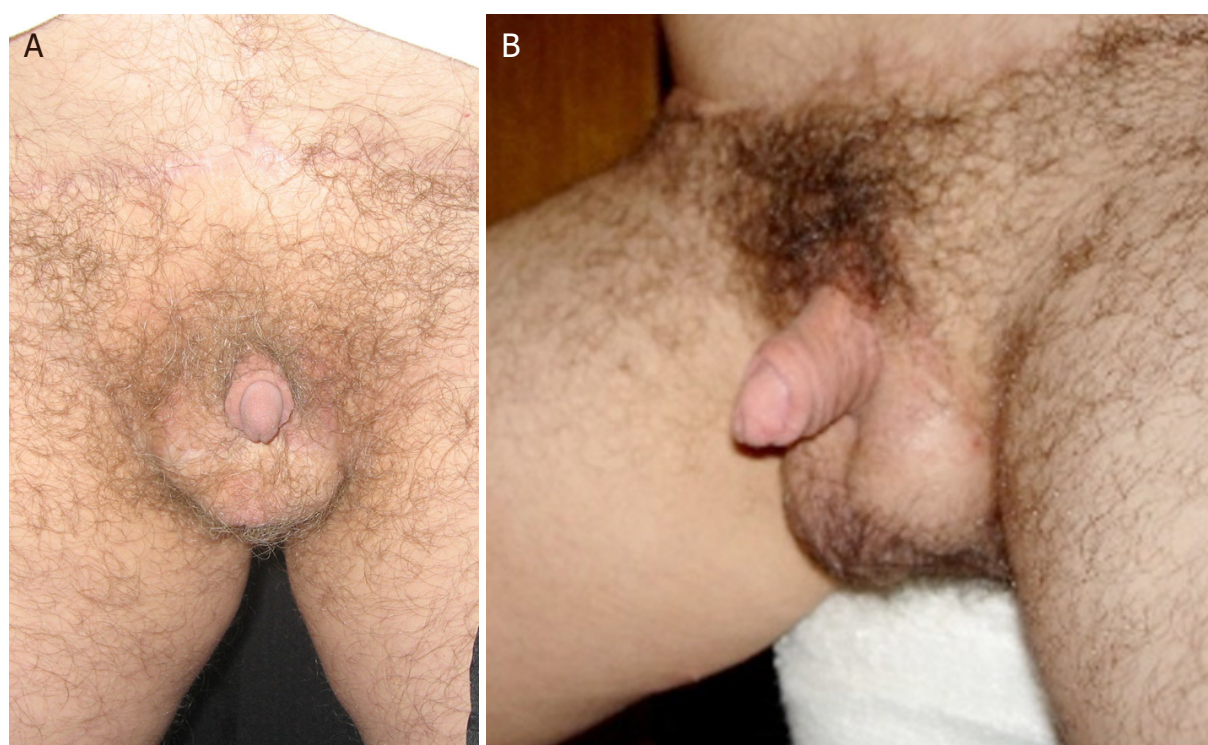
Tissue expanders have been used to enlarge the scrotum prior to implantation of testicular prostheses (Implantech, Sydney, Australia). Regardless of the timing of scrotal construction, placement of implants or tissue expanders is delayed for three months after the metoidioplasty to allow the neourethra to heal well without external pressure. If the scrotoplasty is performed as a secondary procedure, then the testicular implants or scrotal expanders can be placed at this time. Two weeks after implantation, the patient starts inflating them at home using 1-2 mL every other day. Expansion is stopped at 50 mL total in most cases. At least one month after completion of expansion, the permanent testicular implants are placed. The tubing from the expanders has been exteriorized on the mons with a two-way valve in response to some issues with compliance and irritation [Figure 3].

The reduction of the mons is another important adjunct. It masculinizes the mons by making it flatter, gives more definition to the penopubic junction, and positions the penis further forward. The mons resection is not done at the index metoidioplasty because of the anterior positioning of the mons skin and advancing the penis forward would increase the tension on the neourethra at the junction of the native urethra. Instead, it is done as an isolated procedure at least six weeks prior to metoidioplasty or at their final stage, during permanent testicular implant placement. We offer a monsplasty prior to metoidioplasty (at least six weeks) for patients with higher BMI or patients who have had significant weight loss with subsequent mons ptosis. The mons resection in this group will need to be a more extended excision. By performing this procedure, we try to avoid some of the issues that arise after monsplasty where patients are dissatisfied with appearance and/or urinary function. An earlier monsplasty thus facilitates better exposure of the phallus and improves the ability to stand and urinate after subsequent metoidioplasty. An elliptical excision of skin is designed 7 cm above the top of the penopubic junction. The limited amount of skin to be removed is estimated by grasping upwards from the lower marking [Figure 4]. The preference is to keep the final scar within the pubic hair pattern, if possible. After excising the skin, the sub-Scarpa's fat is directly excised inferior to the incision and down to the pubic symphysis using cautery. Liposuction with aggressive cannulas has also been used to reduce the fat. The dorsal suspensory ligament is released at this time. For the patients with a greater BMI, they benefit from tacking sutures with 0 Prolene (Ethicon Inc., Sommerville, NJ) around the penopubic junction, similar to procedures performed for a buried penis indication. These sutures are placed through Scarpa's fascia, close to the dermis and anchored to the pubis and anterior rectus sheath following the fat removal. This will cause visible puckering at each suture site





**Figure 4.** Monsplasty markings



**Figure 5.** A: Completed metoidioplasty, including scrotoplasty, testicular implants, and monsplasty (frontal view); B: completed metoidioplasty, including scrotoplasty, testicular implants, and monsplasty (oblique view). Patient in Figure 5B is not same patient as Figure 5A

which will resolve. These secondary procedures work to accentuate the aesthetics and function of the final metoidioplasty reconstruction [Figure 5A and B].

## RESULTS

In total, 91 patients met inclusion criteria for the study between 2010 and 2020. The mean age was 39.8 years (SD = 12.6), with all patients well established on testosterone prior to surgery [Table 1]. Of those 91

**Table 1. Demographic data (n = 91)**

Characteristic	Mean (SD) or No (%)
Age	39.8 (12.6)
BMI	25.9 (4.3)
Duration testosterone (months)	87.8 (79.8)
Previous hysterectomy and oophorectomy	46 (50.5)
Hysterectomy/oophorectomy at time of metoidioplasty	45 (49.5)

**Table 2. Secondary procedures following metoidioplasty (n = 91)**

Procedure	Mean (SD) or No (%)
Scrotoplasty*	75 (82.4)
TE + TI	75 (82.4)
TI	68 (74.7)
Monsplasty	54 (59.3)
BMI 18-25	24
BMI 25-30	19
BMI 30-35	9
BMI 35 <	2

\*Have had or are scheduled to have scrotoplasty. TE: tissue expanders; TI: testicular implants

**Table 3. Urologic complications (n = 91)**

Description	No (%)
Fistula	1 (1)
Surgical revision	0
Spontaneously closed	1 (1)
Stricture	5 (5.5)
Stricture repair	2 (2.2)
Dilation and urethrotomy	3 (3.3)

patients, 75 (82.4%) patients underwent at least one secondary procedure [Table 2]. The most common secondary procedure was placement of scrotal tissue expanders followed by scrotal implants (82.4%). A monsplasty was performed in 54 (59.3%) patients and was indicated in a wide range of BMIs. Mean follow-up for all patients was 15.4 months.

The urologic complications are listed in Table 3. The most common complication was a stricture in five patients (5.5%). Two of these strictures were treated with buccal mucosa graft at the stricture site after one internal urethrotomy and dilation failed. One of those patients had a stricture recurrence, and ultimately underwent a perineal urethrostomy and secondary closure without buccal mucosa. The three other patients were treated with intermittent self-dilation following a single urethrotomy.

There were 80 (87.9%) patients who reported being able stand and urinate with a strong stream [Table 4]. Two patients reported a strong stream, even if they were unable to urinate while standing.

While sexual function outcomes were not studied in depth, no patients reported a change in ability to orgasm by clitoral stimulation following the procedure.

## DISCUSSION

Metoidioplasty is a reliable procedure for creating a small phallus that allows patients to stand and urinate<sup>[8]</sup>. There exist several variations of this procedure around the world and relatively few practitioners. Our approach is a novel modification of the Takamatsu technique<sup>[6]</sup>. We propose that the primary

**Table 4. Self-reported urologic outcomes (n = 91)**

Description		Mean (SD) or No (%)
Able to stand and void		80 (87.9)
Quality urinary stream		
	Strong	82 (90.1)
	Okay	3 (3.3)
	Weak/erratic	3 (3.3)
	Unknown	3 (3.3)

advantages of this technique are that it achieves urethral lengthening without additional grafts or the anterior vaginal wall flap, which is prone to diverticulum and distension. Furthermore, we found that the use of tubularized vascularized flaps is less prone to stricture and fistulae, likely owing to their robust blood supply and the geometry of the suture line. Variations of the ring technique have been utilized by other centers<sup>[9,10]</sup>, but to our knowledge this is the first report to describe the technique and report on the outcomes. Indeed, we found that a large majority (87.9%) of patients were able to stand and urinate (primary outcome). Thankfully, complications were relatively rare. We found that five patients experienced either a fistula (1%) or stricture (5.5%). Two of the strictures required urethral reconstruction and three were treated with a single internal urethrotomy and intermittent self-dilation, based upon patient preference. To our knowledge, they have not had any stricture recurrence. After two early strictures in this series, the senior author modified the lateral border of the labial flaps to gently curve outward just above and below the urethra. This prevents tightness and subsequent stricture formation at this location, which is distal to the junction of native and neourethra. Secondary procedures are common with metoidioplasty, with scrotoplasty, followed by testicular tissue expanders and testicular implants being most frequently performed (82.4%). While the available data made it impossible to comment on whether patients went on to subsequent phalloplasty after metoidioplasty, the follow up we do have showed this was a very uncommon event. For the patients who did have a phalloplasty at our centers, our metoidioplasty technique is easily coapted to the neourethra of the phalloplasty.

The published outcomes for urologic complications following metoidioplasty indicate stricture rates up to 50% and fistula rates up to 75%<sup>[10-12]</sup>. This is substantially higher than what we found in our series, where strictures occurred in 5.5% of patients, and fistulas in 1%. Admittedly, there is considerable heterogeneity in the surgical techniques used and how to classify complications. This makes it hard to compare techniques and even make comparisons with phalloplasty outcomes, for those patients who are weighing the risks of each procedure<sup>[13,14]</sup>. When patients request a metoidioplasty, we believe that they are seeking the most reliable technique for standing to void, with the least recovery and scar burden. In the systematic review by Frey *et al.*<sup>[15]</sup>, 89% of patients reported the ability to stand and urinate following metoidioplasty, which is similar to our findings. We found 87.9% of patients were able to stand and urinate, and 90% of patients overall reported a strong stream. The urologic history that is completed in advance of the procedure helps identify patients who may have more trouble with urethral lengthening. Further urologic work up and urodynamic studies are indicated in patients who have frequent urinary tract infections, low flow rates, atypical voiding patterns (stopping/starting or incomplete emptying), or stress/urgency incontinence. This is helpful in identifying patients who may have problems exacerbated by the urethral lengthening and appropriately counselling them. It may also help explain an unusual voiding pattern following the procedure.

We believe there are several features of our technique which may explain the relatively low rate of urologic complications. The urethral lengthening is constructed entirely of vascularized tissue with a robust, retrograde blood supply<sup>[6,16]</sup>. The flaps are thick, with some underlying subcutaneous tissue, and are easily mobilized to create a tension free urinary conduit. Posteriorly (at the anastomosis with the native urethra), the flaps are brought together in a V to Y configuration, which adds some length to the construct.

This avoids a narrow circumferential suture line at the junction of the native and neourethra. The senior author has found this junction to be a common site for stricture, in revision surgery for patients who underwent metoidioplasty elsewhere. In our technique, we avoid different types of tissue grafts/flaps, and orient the suture line in an anterior-posterior direction, which we believe contributes to our relatively low rate of urethral complications. While the track record for buccal grafts in urethral reconstruction is well established<sup>[17]</sup>, it is, nevertheless, a graft, which takes longer to heal and is more prone to contracture and possible graft loss. Moreover, the use of buccal graft can make it difficult to get a watertight closure along the urethral lengthening. We have seen even small areas of poor graft take or healing to contribute to fistulas in this area. The donor site for buccal grafts is painful initially and carries some morbidity long term<sup>[18]</sup>. Multiple authors describe the use of the anterior vaginal wall flap<sup>[2-4,19,20]</sup>, including the original Takamatsu paper<sup>[6]</sup>. In theory, it is appealing to utilize this vascularized, local tissue. However, we have found that these flaps may be problematic. The flap - with its redundant folds and broad-based geometry<sup>[5,19]</sup> - can create a large diverticulum proximal to the urethra and cause issues with siphoning of urine and incomplete emptying<sup>[21]</sup>. This results in more post-void dribbling, increased risk for urinary tract infections, and weakened urinary stream. For patients who had the vaginal wall flaps and subsequently underwent a phalloplasty, these thin walled and distensible flaps increased the pressure needed to achieve a normal urinary stream. Due to the myriad issues, the vaginal wall flaps were abandoned in 2010 by our center.

At least one secondary procedure was performed in 82.4% of our patients, with tissue expanders followed by testicular implants being the most common [Table 2]. Tissue expansion for masculinizing surgery has been described for some time<sup>[22]</sup>, but it does not appear to be in widespread use and is even argued against by some groups<sup>[5,7]</sup>. Further, reports on the use of testicular implants in transgender men is rare. Hage *et al.*<sup>[4]</sup> reported using testicular implants alone in their series of 70 metoidioplasties. Their most common issues were malposition (50%) and implant loss (30%-35%, depending on whether the scrotoplasty was performed primarily or secondarily). While we did not record testicular complications in this study, we have found that expanding the scrotal flaps decreased our need for malposition revision. Tissue expansion improves blood supply to skin flaps, so we suspect this may also improve implant loss rates through better healing and tissue durability. At our center, we began to perform scrotoplasty at the same stage as the metoidioplasty beginning in 2017. While some worry about compromised blood supply of concurrent labial flaps for both the metoidioplasty and scrotoplasty, we did not see any evidence of this. In fact, with an anteriorly based scrotoplasty, there is a robust blood supply that can support these flaps during the first operation. In response to some injection site infections and patient compliance issues, when tissue expanders were indicated, we began externalizing the ports to allow greater ease of fill. Prior to converting to a variation of the Selvaggi technique<sup>[7]</sup>, the scrotoplasty was performed as a separate procedure a minimum of three months following the metoidioplasty. This was a posteriorly-based design and gives excellent definition to the penoscrotal junction. However, there is a tendency to make the scrotum too posterior. The Selvaggi technique has the advantage of lengthening the perineal body and advancing the scrotum forward. It is important not to advance the flaps too far forward with the perineal closure because it will engulf the penis. The fullness of the labia majora anteriorly will need to be separately reduced to flatten the penopubic junction. Reduction of the anterior labia majora is performed at the same time as the metoidioplasty and may be reduced further when the testicular implants are placed. Aggressive fat excision around the final closure is also done at this time.

Patients with higher BMIs with thicker mons or those who have had a significant weight loss can benefit from a mons resection at least six weeks prior to metoidioplasty instead of during their final stage (which is typically scrotal implant placement). This makes the phallus more accessible and can facilitate standing to void for patients who would otherwise be dissatisfied with their immediate postoperative results. Fifty-four of our patients (59%) underwent monsplasty, the majority (79.6%) of whom had a BMI less than 30.



This underscores our observation that BMI alone is not a useful indicator for who would benefit from a monsplasty postoperatively. Rather, the clinical exam and goals of the patient should guide this choice.

As phalloplasty has gained increasing attention in the gender surgery literature, metoidioplasty has not enjoyed the same ascendant profile. Achieving reliable urologic and sensory outcomes without the morbidity and time commitments of a phalloplasty is a clear benefit of metoidioplasty. Moreover, minimizing scar burden, recovery time, and distant donor sites also favor metoidioplasty. While our patient population present to consultation with a uniquely thorough level of understanding of the procedures available, this difference between phalloplasty and metoidioplasty is an essential part of the consultation. We describe a novel variation of the ring flap metoidioplasty and found that we could achieve low rates of complications while observing high rates of ability to stand and urinate with a strong stream. Concurrent scrotoplasty can be safely performed and secondary procedures are common for patients desiring complete genital masculinization. The high rate of secondary procedures also allowed us to objectively evaluate the status of the urethra at that time, if there were any voiding issues reported. There are, however, several limitations to this study. Many patients travel for these procedures, so it is possible that complications or other secondary procedures were managed elsewhere. Our patient questionnaire was not performed at a standardized time interval, so it is possible we captured these data before they developed any urologic issues. Finally, there were some modifications to the technique made over the study period, including the widening of the labial flaps, the use of external tissue expander ports, and the conversion to an anterior based scrotoplasty. This heterogeneity may make strict comparisons between techniques more difficult.

## **DECLARATIONS**

### **Acknowledgments**

The authors would like to thank Jeremy Ballard for his assistance in data acquisition.

### **Authors' contributions**

Development of surgical technique, data acquisition, study design: Meltzer TR

Data analysis and interpretation, manuscript preparation: Meltzer TR, Esmonde NO

### **Availability of data and materials**

The data used for the study will not be made publicly available. The database contains proprietary information, including intellectual property, that the authors do not wish to share.

### **Financial support and sponsorship**

None.

### **Conflicts of interest**

Both authors declared that there are no conflicts of interest.

### **Ethical approval and consent to participate**

This was an anonymous, retrospective study of a deidentified dataset. Patients provided informed consent for inclusion in research at the time of establishing care.

### **Consent for publication**

Written consent for publication was obtained.

### **Copyright**

© The Author(s) 2020.



## REFERENCES

1. Cohanad S. Extensive metoidioplasty as a technique capable of creating a compatible analogue to a natural penis in female transsexuals. *Aesthetic Plast Surg* 2016;40:130-8.
2. Djordjevic ML, Bizic M, Stanojevic D, et al. Urethral Lengthening in metoidioplasty (female-to-male sex reassignment surgery) by combined buccal mucosa graft and labia minora flap. *Urology* 2009;74:349-53.
3. Djordjevic ML, Bizic MR. Comparison of two different methods for urethral lengthening in female to male (metoidioplasty) surgery. *J Sex Med* 2013;10:1431-8.
4. Hage JJ, van Turnhout AA. Long-term outcome of metaidoioplasty in 70 female-to-male transsexuals. *Ann Plast Surg* 2006;57:312-6.
5. Hage JJ, Torenbeek R, Bouman FG, Bloem JJ. The anatomic basis of the anterior vaginal flap used for neourethra construction in female-to-male transsexuals. *Plast Reconstr Surg* 1993;92:102-8; discussion 109.
6. Takamatsu A, Harashina T. Labial ring flap: a new flap for metaidoioplasty in female-to-male transsexuals. *J Plast Reconstr Aesthet Surg* 2009;62:318-25.
7. Selvaggi G, Hoebeke P, Ceulemans P, et al. Scrotal reconstruction in female-to-male transsexuals: a novel scrotoplasty. *Plast Reconstr Surg* 2009;123:1710-8.
8. Djinovic RP. Metoidioplasty. *Clin Plast Surg* 2018;45:381-6.
9. Chen ML, Reyblat P, Poh MM, Chi AC. Overview of surgical techniques in gender-affirming genital surgery. *Transl Androl Urol* 2019;8:191-208.
10. Veerman H, de Rooij FPW, Al-Tamimi M, et al. Functional outcomes and urological complications after genital gender affirming surgery with urethral lengthening in transgender men. *J Urol* 2020;204:104-9.
11. Santucci RA. Urethral complications after transgender phalloplasty: strategies to treat them and minimize their occurrence. *Clin Anat* 2018;31:187-90.
12. Morrison SD, Shakir A, Vyas KS, Kirby J, Crane CN, Lee GK. Phalloplasty: a review of techniques and outcomes. *Plast Reconstr Surg* 2016;138:594-615.
13. Al-Tamimi M, Pigot GL, Elfering L, et al. Genital gender-affirming surgery in transgender men in the netherlands from 1989 to 2018: the evolution of surgical care. *Plast Reconstr Surg* 2020;145:153e-61.
14. Berli JU. Discussion: genital gender-affirming surgery in transgender men in the netherlands from 1989 to 2018: the evolution of surgical care. *Plast Reconstr Surg* 2020;145:162e-3.
15. Frey JD, Poudrier G, Chiodo MV, Hazen A. A systematic review of metoidioplasty and radial forearm flap phalloplasty in female-to-male transgender genital reconstruction: is the "ideal" neophallus an achievable goal? *Plast Reconstr Surg Glob Open* 2016;4:e1131.
16. Giraldo F, Mora MJ, Solano A, Abehsera M, Ferrón M, Smith JM. Anatomic study of the superficial perineal neurovascular pedicle: implications in vulvoperineal flap design. *Plast Reconstr Surg* 1997;99:100-8.
17. Elliott SP, Metro MJ, McAninch JW. Long-term followup of the ventrally placed buccal mucosa onlay graft in bulbar urethral reconstruction. *J Urol* 2003;169:1754-7.
18. Markiewicz MR, DeSantis JL, Margarone JE 3rd, Pogrel MA, Chuang SK. Morbidity associated with oral mucosa harvest for urological reconstruction: an overview. *J Oral Maxillofac Surg* 2008;66:739-44.
19. Hage JJ. Metaidoioplasty: an alternative phalloplasty technique in transsexuals. *Plast Reconstr Surg* 1996;97:161-7.
20. Stojanovic B, Bizic M, Bencic M, et al. One-stage gender-confirmation surgery as a viable surgical procedure for female-to-male transsexuals. *J Sex Med* 2017;14:741-6.
21. Hoebeke P, Selvaggi G, Ceulemans P, et al. Impact of sex reassignment surgery on lower urinary tract function. *Eur Urol* 2005;47:398-402.
22. Small MP, Becker H. Use of tissue expanders in genitourinary reconstructive surgery for transsexuals. Proceedings of the eleventh Harry Benjamin International Gender Dysphoria Association Symposium. Cleveland, OH; 1989. p. 67.

Review

Open Access



# Hyaluronic acid for lower eyelid and tear trough rejuvenation: review of the literature

Alberto Diaspro<sup>1</sup>, Giuseppe Sito<sup>2</sup>

<sup>1</sup>Department of Maxillofacial and Facial Plastic Surgery, Rigenerallab Centre for Regenerative Medicine, Turin 10134, Italy.

<sup>2</sup>Department of Aesthetic Surgery and Medicine, Pegaso University, Naples 80121, Italy.

**Correspondence to:** Dr. Alberto Diaspro, Department of Maxillofacial and Facial Plastic Surgery, Rigenerallab Centre for Regenerative Medicine, Corso Unione Sovietica 159/a, Turin 10134, Italy. E-mail: info@albertodiaspro.com

**How to cite this article:** Diaspro A, Sito G. Hyaluronic acid for lower eyelid and tear trough rejuvenation: review of the literature. *Plast Aesthet Res* 2020;7:62. <http://dx.doi.org/10.20517/2347-9264.2020.143>

**Received:** 30 Jun 2020 **First Decision:** 10 Aug 2020 **Revised:** 23 Aug 2020 **Accepted:** 23 Sep 2020 **Published:** 6 Nov 2020

**Academic Editor:** Wen-Guo Cui **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Periorbital aging has been identified as one of the most important aesthetic concerns of the face, so that lower eyelid rejuvenation has become a topic of major interest. Not every patient requires surgical blepharoplasty and selected lower eyelid problems and defects due to the aging process have been treated with hyaluronic acid (HA) gel injections since 2004. With this as the premise, the current work serves to review the published medical literature on the use of HA for lower eyelid and tear trough rejuvenation. A PubMed search was carried out in May 2020 using the search terms: "Tear trough [and] HA [and] filler"; "Tear trough [and] HA"; "HA [and] lower eyelid [and] filler"; "HA [and] lower eyelid". A large number of relevant studies were identified. Surgical management remains the gold standard for lower eyelid rejuvenation but increasingly, non-surgical correction of selected deformities with HA injection may provide a reliable option based on the available evidence. Further, prospective randomized controlled studies and systematic reviews of the literature are nevertheless desirable and a standardized, widely accepted grading system of the deformity and its treatment outcomes will allow us to codify this procedure better.

**Keywords:** Eyelid, tear trough, hyaluronic acid, rejuvenation

## INTRODUCTION

The eyes are located in the centre of the face and are crucial to its aesthetic appeal. It is therefore of major concern to patients. Because minor changes to the eyes can yield dramatic results, many aesthetic



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



procedures have been described, such as brow lifting, autologous fat transfer, surgical excision, chemical peels and lasers.

The nasojugal fold was first described by Loeb<sup>[1]</sup> in 1981. It is the groove resulting from attachment of the lower dermis to the periosteum of the infraorbital margin, which accentuates the depression of the eyelids against the heavier bulk of tissue towards the nasal wall.

However, Flowers<sup>[2]</sup> in 1993 reported and named the “tear trough deformity” with reference to its correction by techniques utilizing alloplastic implants.

The treatment options to correct a hollow, lower lid area have since been further developed: Hamra *et al.*<sup>[3]</sup> for instance, filled the trough by means of vascularised fat transposed through release of the arcus marginalis. Other surgical techniques to address orbital fat reposition have also been published<sup>[4,5]</sup> and fat injections have been proposed as a non-vascularised graft to fill the tear trough<sup>[6,7]</sup>.

The aforementioned techniques represent different degrees of invasiveness while non-surgical options allow treatment of dark pigmentation but do not successfully treat sunken lower eyelids<sup>[8,9]</sup>.

Proper understanding of the role of the orbitomalar ligament in tear trough deformities, as well as of progressively worsening midface ptosis<sup>[10]</sup>, has subsequently enabled the opportunity to inject filling agents other than fat to fill the hollow and in so doing, rejuvenate the lower eyelid area, in order to obtain the same outcome without the downtime from surgery.

Hyaluronic acid (HA) gel injections were first used for this purpose in 2005<sup>[11,12]</sup> and since then, this non-surgical, outpatient procedure has been one of the mainstays of HA filler treatment.

As long as the non-surgical procedure does not violate the key anatomical structures and relationships of the lower eyelid, it can be adopted in properly selected patients as a simple method to accomplish its rejuvenation. Not every patient requires a surgical blepharoplasty to meet their needs and selected lower eyelid deformities and defects due to aging can still be treated easily, safely, and quickly with HA injections.

This study has thus been carried out to review the published medical literature on the use of HA for lower eyelid and tear trough rejuvenation.

## DATA COLLECTION

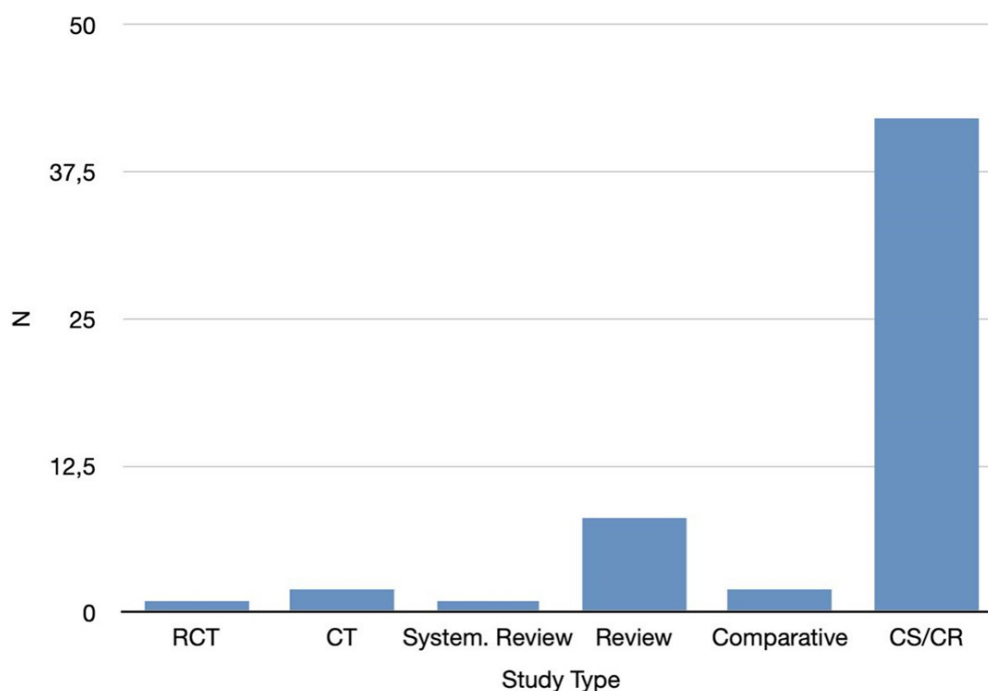
A PubMed search was performed in May 2020 using the strings: “Tear trough [and] HA [and] filler”; “Tear trough [and] HA”; “HA [and] lower eyelid [and] filler”; “HA [and] lower eyelid”, which yielded a total of 242 indexed articles.

Results were limited to human subjects, in clinical trials, randomized controlled trials, case reports, comparative studies, controlled clinical trials and multi-center studies.

No limits based on the year or language of publication was applied.

Manual review of abstracts was also performed to omit unrelated articles.

The final result included 66 articles from 2005 to 2020.



**Figure 1.** Retrieved articles: study type. RCT: Randomized Controlled Trial; CT: Controlled Trial; System. Review: Systematic Review; Comparative: Comparative Study; CS: Case Serie; CR: Case Report

## DATA DISTRIBUTION

### Study type

There were eighteen prospective studies<sup>[12-21]</sup>, twenty retrospective studies<sup>[22-31]</sup>, and one that was both retrospective and prospective<sup>[32]</sup>; the study type was not mentioned in twenty-seven<sup>[33-42]</sup>.

Among the published papers, there has only been one randomized controlled study<sup>[43]</sup> on HA injections performed to rejuvenate the tear trough area, along with two controlled trials<sup>[16,40]</sup>.

A systematic review presented with a meta-analysis<sup>[41]</sup> has been carried out in order to evaluate treatment for cicatricial ectropion including HA injection among non-surgical treatments.

Eight review articles have been written, the first in 2007<sup>[33]</sup>, then in 2010<sup>[36]</sup>, 2012<sup>[38-40]</sup> and from 2016 to 2020<sup>[44-47]</sup>, and two comparative studies were published in 2006<sup>[13,22]</sup>.

Twenty-nine papers are case-series<sup>[11,18,27-29,48-52]</sup>, thirteen are case-reports<sup>[20,30,31,37,39,53-57]</sup> whilst the remainder<sup>[26,34,35,40,58-66]</sup> are related to general content [Figure 1].

### Follow-up duration

The mean recorded follow-up was 18.2 months<sup>[11-34]</sup>, ranging from 0.75 (3 weeks)<sup>[17,27,66]</sup> to 96 months (8 years)<sup>[24]</sup>.

### Average study population

The study of Airan and Born<sup>[11]</sup> remains the largest, with 400 treated patients, followed by the paper edited by Artzi *et al.*<sup>[51]</sup>, which reviewed 351 patients.

Among the investigated articles which directly mentioned the study population<sup>[14-16,21,26,32,34,63,66-68]</sup>, the average (n) was 71.37, and ranged from 3<sup>[18]</sup> to 400<sup>[11]</sup> once case reports were excluded.

## Anatomy

Since 2012, three papers have been published with regard to anatomical topics: interestingly, those published in 2012<sup>[58]</sup> and 2013<sup>[60]</sup> were both focused on the tear trough region, whilst that in 2015<sup>[61]</sup> was targeted at anatomical knowledge applied to the midface and not only the lower eyelid.

## Classification

Two articles provided a classification system for tear trough deformities, along with a review of the available treatments<sup>[45]</sup>, and presented a series of treated patients in order to establish correlation with achieved outcomes<sup>[62]</sup>.

## Complications

Complication-related papers have been published since 2012<sup>[25,39]</sup>, and have been reported consistently in 2014<sup>[68]</sup>, 2016<sup>[51,69]</sup>, 2017<sup>[29,53,54]</sup>, 2018<sup>[69]</sup>, 2019<sup>[20,55-57,63]</sup> and 2020<sup>[70]</sup>. These are either case reports or case series, and one did not mention the size of the study population. The reported follow-up duration, when mentioned, ranged from 12 months<sup>[29]</sup> to 5 years<sup>[39,69]</sup>, with prolonged edema, the Tyndall effect and contour irregularities as the most commonly reported complications, along with diplopia<sup>[25]</sup>, and xanthelasma-like reactions<sup>[53]</sup> as a rare occurrence. Complications were also reported in several articles, even if not the focus of the study<sup>[13,17,22,28,32,71-75]</sup>, and included two cases of cellulitis and one of migraine<sup>[13]</sup>.

## Technique

Research focused on personal techniques purposed by the authors have been published since 2005. Airan and Born<sup>[11]</sup> and Kane<sup>[12]</sup> wrote the first articles ever about this topic, both in 2005; technique refinement and new trends have been published in 2006<sup>[22,23]</sup>, 2007<sup>[24,33]</sup>, 2011<sup>[16,32,76]</sup>, 2012<sup>[26,41,42,66]</sup>, 2013<sup>[73]</sup>, 2014<sup>[43]</sup>, 2015<sup>[48-50]</sup>, 2016<sup>[27,28]</sup>, 2017<sup>[17,52]</sup>, 2018<sup>[19,71,74]</sup> and 2019<sup>[21]</sup>.

Anaesthesia has been used both by local infiltration<sup>[11,19,24,31]</sup> or by topical application<sup>[13-17,48,52]</sup>.

Injections performed by needle have been described since 2005 up to 2019<sup>[11-16,21,28,33,74]</sup>, while cannula use has been reported since 2017<sup>[19,30,31,52,71]</sup> and only two studies reported both techniques<sup>[17,62]</sup>.

The injection depth is reported to be carried out directly at the orbital bony border<sup>[11,13,15,16,19,27,48,71,73,74]</sup>, either under the orbicularis oculi muscle<sup>[14,24,31,32,50,66,71,74]</sup> or subcutaneously<sup>[12,33]</sup>. The average amount of HA injected into each side is 0.56 mL and ranges from 0.2 mL<sup>[12]</sup> to 2.0 mL<sup>[44]</sup>.

## Endpoint

Of the 66 studies included for analysis, the majority<sup>[19,24,30,31,33,36,62,69,71,74]</sup> were focused on tear trough rejuvenation whilst 10 reported HA injections to treat functional impairments such as lower eyelid retraction<sup>[23,35,37,67]</sup>, cicatricial<sup>[34,41,75]</sup> and involutive ectropion<sup>[18]</sup>, and suprachoroidal buckling<sup>[40,58]</sup>.

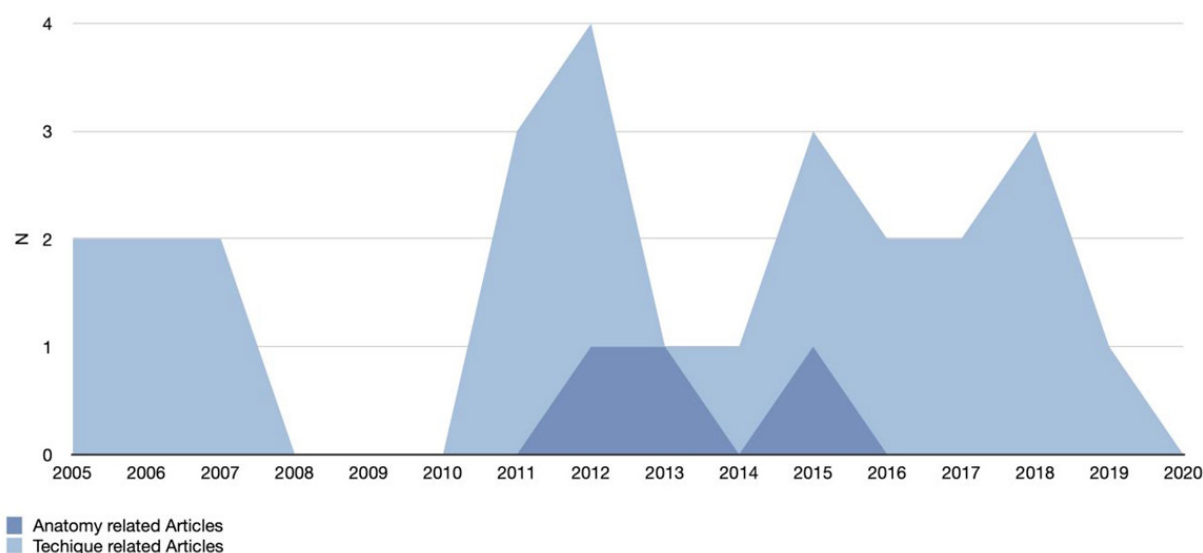
## Outcome evaluation

In 2010, two papers on objective and validated measurements of the achievable results of non-surgical tear trough rejuvenation as assessed by digital 3D photographs and cutometer were published<sup>[14-16,76]</sup>. The papers had not been presented up to 2018<sup>[74]</sup> and this topic has not been further investigated.

## DATA REVISION

This review of the medical literature on HA use for lower eyelid and tear trough rejuvenation identified a large number of related studies.





**Figure 2.** Anatomy and technical note focused articles: publication trend

The average study population was 71.37, ranging from 3<sup>[44]</sup> to 400<sup>[11]</sup> treated patients, along with a recorded mean follow-up of 18.2 months, ranging from 0.75 (3 weeks)<sup>[17,27,66]</sup> to 96 months (8 years)<sup>[24]</sup>.

It is remarkable that the first two papers ever published on the topic already had 6.5 months of retrospective follow-up of 400 treated patients<sup>[11]</sup>, and 9 months of prospective follow-up of treated patients<sup>[12]</sup>.

Even though the lower eyelid and tear trough are considered difficult sites to be injected, only two papers focused on related anatomy were published in 2012<sup>[58,60]</sup>, seven years after the technique was introduced<sup>[11,12]</sup>. Subsequently, the anatomy and the aging process of the tear trough region have started to be considered as strongly correlated to the midface<sup>[61]</sup> and thus, were no longer published as stand-alone topics [Figure 2].

The first papers focused on complications arising from lower eyelid and tear trough HA treatment were published in 2012 by Dayan *et al.*<sup>[39]</sup> and Kashkouli *et al.*<sup>[25]</sup>.

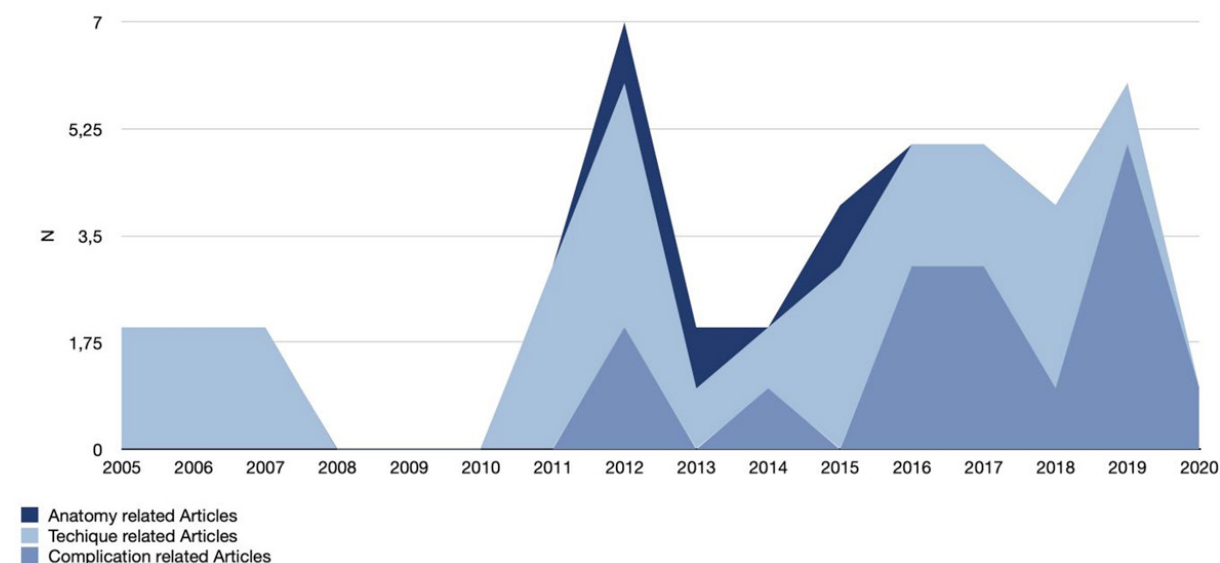
Articles focused on complications have since been constantly published<sup>[20,51,53-57,63,68,69]</sup>, up to 2020<sup>[70]</sup>, which highlights the technical complexities of this treatment that requires adequate skills for it to be properly performed [Figure 3].

However, adverse events are occasionally reported in articles that are not directly related<sup>[13,17,22,28,32,39,71]</sup>.

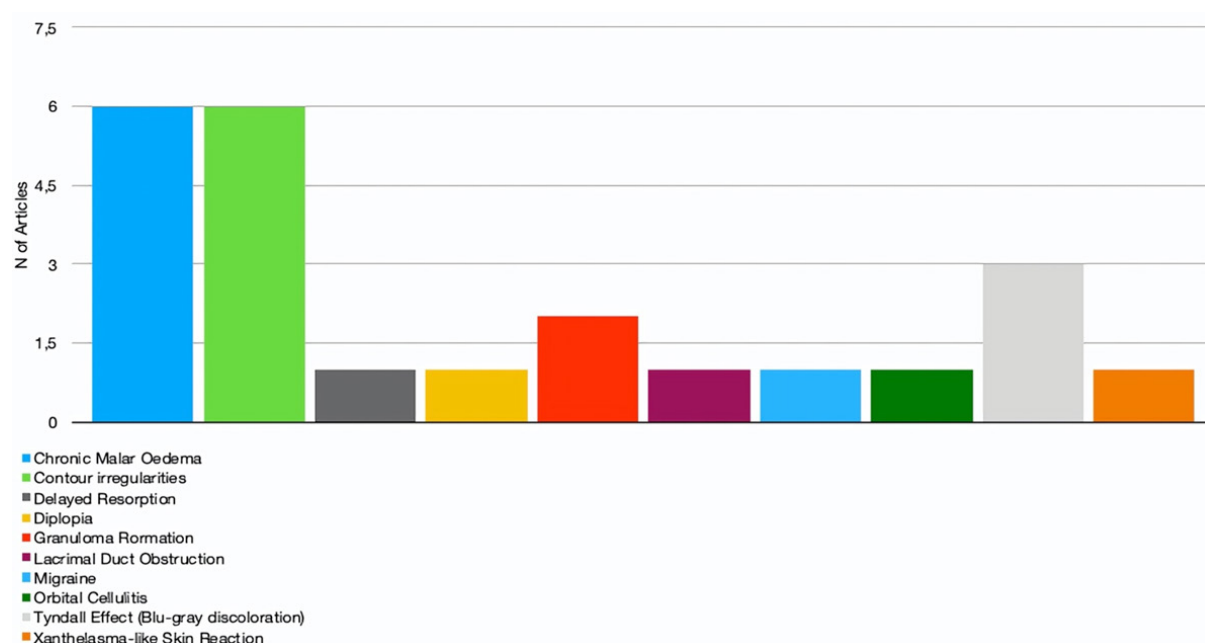
The most commonly reported complications include the Tyndall effect<sup>[51,63,69]</sup>, a blue-grey dyschromia, in up to 30.5% of cases<sup>[69]</sup>; contour irregularities<sup>[25,51,53,63,69]</sup> ranging from 4.25%<sup>[51]</sup> to 30.5%<sup>[69]</sup> of cases; and chronic malar oedema<sup>[20,54,63,68-70]</sup> that has been reported in up to 11% of cases<sup>[69]</sup>.

Minor or rarely reported complications secondary to lower eyelid and tear trough HA injections are orbital cellulitis and migraine<sup>[13]</sup>, delayed resorption of HA<sup>[39]</sup>, diplopia<sup>[25]</sup>, xanthelasma-like eyelid skin reactions<sup>[29]</sup>, granuloma formation<sup>[55,56]</sup> and lacrimal duct obstruction<sup>[57]</sup> [Figure 4].

The injection of an adequate dose of hyaluronidase has always been reported to be the best treatment<sup>[54,57,63,68-70]</sup>,



**Figure 3.** Complication focused articles: publication trend



**Figure 4.** Reported complications

even if delayed with respect to primary onset, for these complications. Therefore, injecting with a reliable and safe technique plays a pivotal role in achieving optimal outcomes.

Indeed, publications on injection techniques constitute the majority<sup>[11-22,31,33,71]</sup> of the recorded articles.

Local anaesthesia has been widely used, either topically<sup>[13-17,48,52]</sup> or by local infiltration<sup>[11,16,60,61,66]</sup>. It has to be noted that care must be taken when injecting anaesthetic solution in order to avoid untoward alteration of the contours of the lower eyelid.

Nevertheless, some authors did not report the use of any form of anaesthesia for this treatment<sup>[32,73]</sup>.

In order to avoid the complications above, it is mandatory to inject only a small quantity of HA, as the reported average amount has been 0.56 mL, whilst ranging from 0.2 mL<sup>[12]</sup> to 2.0 mL<sup>[48]</sup> per side, in a deep plane on the inferior orbital rim, either pre-periosteal<sup>[11,13,15,16,19,27,48,71,73,74]</sup> or under the orbicularis oculi muscle<sup>[14,24,31,32,50,52,66,71,74]</sup>.

Based on personal experience, the authors suggest injecting a lesser quantity of 0.2 mL to 0.45 mL per session<sup>[77]</sup>, as the aforementioned 0.56 mL could include correction of both the tear trough deformity and the palpebro-malar groove<sup>[11,13,16,20,22,30-32,60,61]</sup>.

The authors' preferred techniques are direct injection on the periosteum (GS) and retrograde injection technique deeper than the orbicularis muscle (AD).

The first requires the identification and protection of the infraorbital foramen with gentle finger pressure, followed by insertion of a 30-gauge needle into the deepest portion of the tear trough, which is always treated first by injection of the HA gel as a single bolus on the bony surface. The injected area is then gently massaged to create a natural shape and then assessed for further injections, if needed.

When a cannula is preferred, the entry point is located at the intersection between the vertical line passing through the external canthus and the line marked by the tear trough. Once inserted, it should pierce the orbicularis oculi muscle, and advanced until it reaches the bony orbital ridge. The infraorbital foramen will therefore be located deeper than the injection plane.

The cannula should then be moved medially toward the inner canthus and small amounts of HA are injected retrograde. Touch-up has been necessary within 1 month in around 20% of cases treated with cannula, normally requiring further injection of 0.1 mL to 0.3 mL of HA in order to improve the outcome of the previous session, whilst injections with needles did not require further corrections.

The choice between needle or cannula is based on personal experience and preference. While the authors experienced post-injection swelling and redness and pain in 2% to 3% of cases, less bruising and ecchymosis occur with the latter. Bruising may also cause lymphatic vessel compression with a greater risk of oedema, although it minimizes the risks of intravascular injection and embolism.

As a general rule, it is advisable not to inject medially in the inner canthus to avoid lesions or compression of the angular vessels<sup>[77]</sup>.

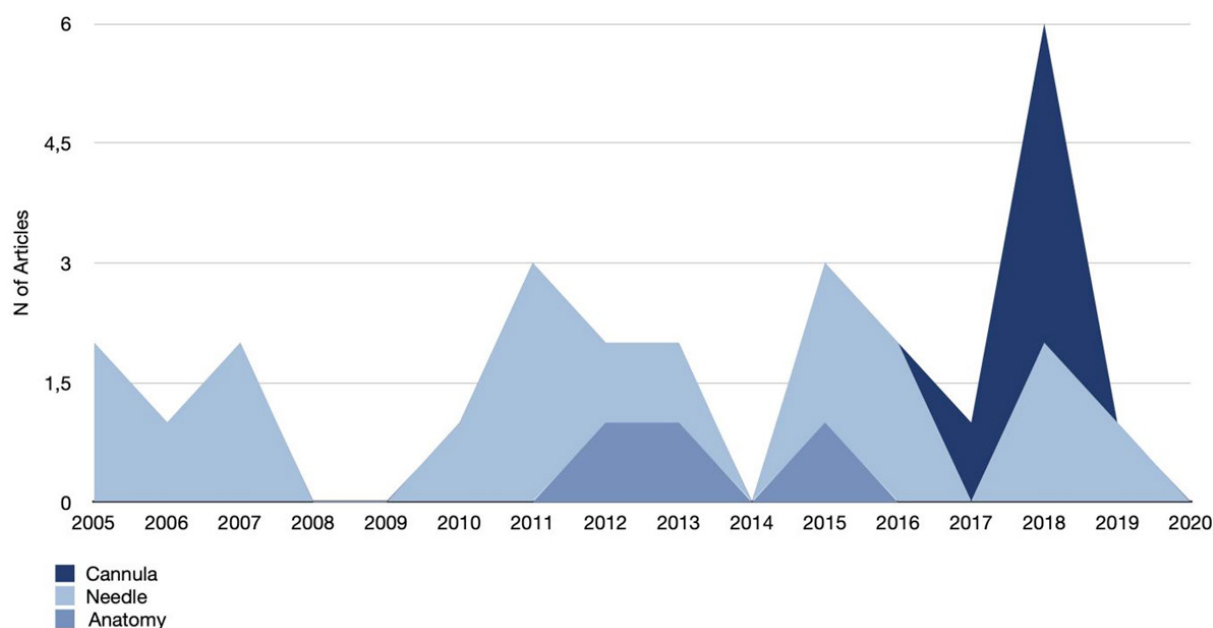
In the authors' experience, the use of cannula leads to overcorrection and the Tyndall effect in 6% to 7% of cases whilst needle injections avoid such complications; nevertheless, it has to be carried out by experienced injectors.

Subcutaneous placement of the HA is seldom reported<sup>[12,33]</sup> and has not been mentioned since 2007.

The injection can be performed by both the needle or cannula technique; it is remarkable that the technique with needle has been referred to since the very first papers in 2005, up to 2019<sup>[11-16,21,24,33,74]</sup>, while the use of the cannula was not mentioned before 2017<sup>[19,30,31,52,71]</sup>.

The purpose of this paper is not to discuss the safety of these two methods of HA injection for tear trough and lower eyelid deformities, but it is the author's opinion that growing awareness of the relevant anatomy<sup>[58,60,61]</sup> has supported the use of blunt cannulas as the safer choice for injectors, thus coinciding with the increasing rate of related papers after 2017 [Figure 5].

Finally, to highlight the reliability of HA injections for the treatment of lower eyelid deformities, 10 studies



**Figure 5.** Complications focused articles: publication trend with respect to needle and cannula use

out of the 66 reviewed in this paper focused on its use in the treatment of functional impairments such as retraction<sup>[23,35,37,67]</sup>, cicatricial<sup>[34,41,75]</sup> and involutive ectropion<sup>[18]</sup>, and suprachoroidal buckling<sup>[40,59]</sup>, while the majority<sup>[11-13,19,22,30,62,69,71,74]</sup> were, of course, focused on rejuvenation of the lower eyelid and tear trough.

With respect to the published literature, it could therefore be claimed that interest in lower eyelid and tear trough rejuvenation peaked in 2012, but has continued on an ascending trend, as summarized in Figure 6.

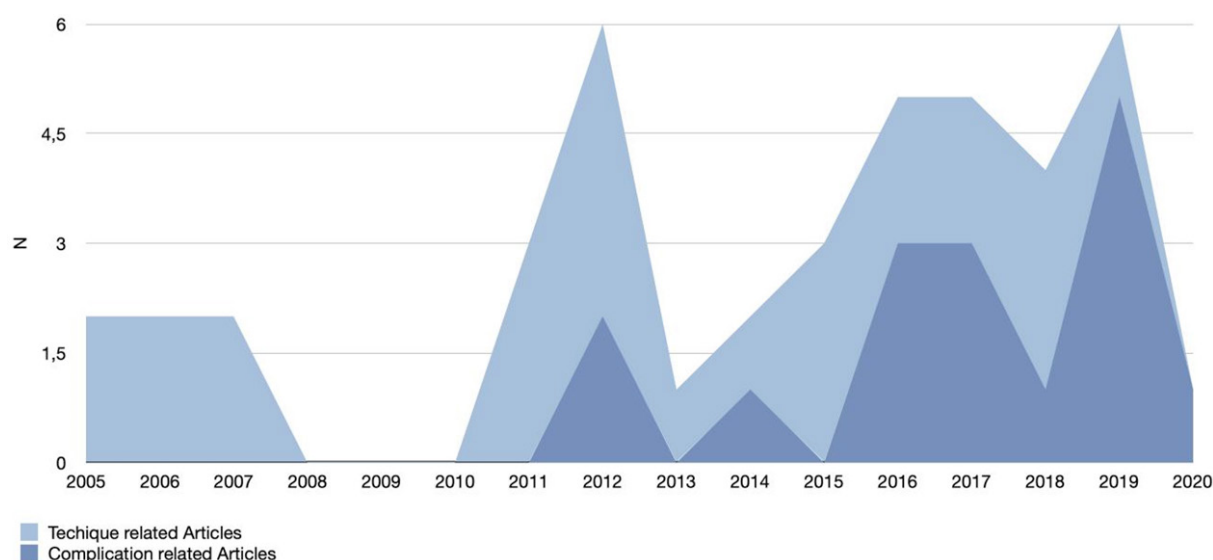
Indeed, this paper has some limitations as the presented review is non-systematic and therefore does not allow a meta-analysis of the collected data.

Furthermore, with respect to the Levels of Evidence classification of the Oxford Centre for Evidence-based Medicine (OCEBM)<sup>[78]</sup>, the average level of evidence of the evaluated studies is low, as there was only one randomized controlled study<sup>[43]</sup> on HA injections to rejuvenate the tear trough area (Level 1b), two controlled trials<sup>[16,41]</sup> (Level 4), and a systematic review along with a meta-analysis<sup>[76]</sup> (Level 3a), which has indeed been carried out in order to evaluate treatment of cicatricial ectropion and has been cited since it includes HA injections among non-surgical treatments.

The remaining literature was limited to case series, case reports and observational cohorts and lacks multicentre studies, highlighting the bias due to variances in single operator techniques.

Multiple classifications of the severity of tear trough deformities are presented<sup>[45,62]</sup>, although a standardized one is yet to be included in the current literature.

The lack of a grading system or classification directly correlates with the paucity of available articles focused on objective outcome evaluation of tear trough deformity treatment with HA injections. Digital 3D photography has been adopted as a method of choice since 2010<sup>[14,76]</sup> but except for the paper published by Cho *et al.*<sup>[74]</sup> in 2018, outcomes evaluation has only been carried out subjectively, clinically<sup>[11,12,22,33]</sup> or through bidimensional photographs<sup>[15,16,43,48,50,68]</sup>, sometimes even taken without standardized conditions<sup>[13,24,27,28,32,52,69]</sup>, the Global Aesthetic Assessment Scale as evaluated by both patients and doctors<sup>[17,21,43,48,50,66]</sup>, and



**Figure 6.** Techniques and complications focused articles: publication trend

administered questionnaires<sup>[71]</sup>, even by telephone calls<sup>[26]</sup>.

It is remarkable that only one paper has focused on instrumental follow-up and outcomes evaluation with ultrasound and this was published in 2013<sup>[73]</sup>. No other related research has been carried out since.

Whilst surgical management remains the standard for lower eyelid rejuvenation, non-surgical correction of its selected deformities, such as the tear trough by HA injections may provide a reliable and viable option based upon the presented evidence.

In the author's experience, this treatment can achieve, when properly executed, great satisfaction of both patients and doctors, due to its immediate result and longevity, as it can be assessed for up to 1 year after the injection.

To the best of the authors' knowledge, this is the first literature review related to HA use in lower eyelid for tear trough deformity correction and rejuvenation.

## CONCLUSION

This review has been performed based on the common assumption that when a method to determine the true outcomes, safety and reliability of treatments is lacking besides the mere judgement of patients and clinicians, evidence has to be incorporated into daily clinical practice by considering case reports, pilot studies and available, high-quality studies, as the authors believe that evidence based medicine is essential to provide better answers for both the patients and us healthcare professionals<sup>[79]</sup>.

Further prospective, randomized controlled studies and systematic reviews of the literature are thus desirable, along with a standardized and widely-accepted grading system of the deformity and its treatment outcomes will allow us to better codify this procedure.

## DECLARATIONS



## Acknowledgments

The authors would like to thank Giulia Tanteri, an independent medical writer, who provided English-language editing and journal styling prior to submission.

## Author's contributions

Made substantial contributions to conception and design of the study, performed data analysis and interpretation: Diaspro A

Verified the analytical methods, provided critical feedback and helped shape the research, analysis and manuscript: Sito G

Contributed to the final manuscript the final manuscript: Diaspro A, Sito G

## Availability of data and materials

Not applicable.

## Financial support and sponsorship

None.

## Conflict of interest

All authors declared that there are no conflicts of interest.

## Ethical approval and consent to participate

Not applicable.

## Consent for publication

Not applicable.

## Copyright

© The Author(s) 2020.

## REFERENCES

1. Loeb R. Fat pad sliding and fat grafting for leveling lid depressions. *Clin Plast Surg* 1981;8:757-76.
2. Flowers RS. Tear trough implants for correction of tear trough deformity. *Clin Plast Surg* 1993;20:403-15.
3. Hamra ST. Arcus marginalis release and orbital fat preservation in midface rejuvenation. *Plast Reconstr Surg* 1995;96:354-62.
4. Fante RG, Baker SR. Fat-conserving aesthetic lower blepharoplasty. *Ophthalmic Surg Lasers* 2001;32:41-7.
5. Huang T. Reduction of lower palpebral bulge by plicating attenuated orbital septa: a technical modification in cosmetic blepharoplasty. *Plast Reconstr Surg* 2000;105:2552-8; discussion 2559-60.
6. Carraway JH, Coleman S, Kane MA, Patipa M. Periorbital rejuvenation. *Aesthet Surg J* 2001;21:337-43.
7. Seiff SR. The fat pearl graft in ophthalmic plastic surgery: everyone wants to be a donor! *Orbit* 2002;21:105-9.
8. Roberts TL 3rd. Laser blepharoplasty and laser resurfacing of the periorbital area. *Clin Plast Surg* 1998;25:95-108.
9. Lieb WE, Klink T, Münnich S. CO<sub>2</sub> and erbium YAG laser in eyelid surgery. A comparison. *Ophthalmologie* 2000;97:835-41.
10. Lucarelli MJ, Khwarg SI, Lemke BN, Kozel JS, Dortzbach RK. The anatomy of midfacial ptosis. *Ophthalmic Plast Reconstr Surg* 2000;16:7-22.
11. Airan LE, Born TM. Nonsurgical lower eyelid lift. *Plast Reconstr Surg* 2005;116:1785-92.
12. Kane MA. Treatment of tear trough deformity and lower lid bowing with injectable hyaluronic acid. *Aesthetic Plast Surg* 2005;29:363-7.
13. Steinsapir KD, Steinsapir SM. Deep-fill hyaluronic acid for the temporary treatment of the naso-jugal groove: a report of 303 consecutive treatments. *Ophthalmic Plast Reconstr Surg* 2006;22:344-8.
14. Donath AS, Glasgold RA, Meier J, Glasgold MJ. Quantitative evaluation of volume augmentation in the tear trough with a hyaluronic acid-based filler: a three-dimensional analysis. *Plast Reconstr Surg* 2010;125:1515-22.
15. Viana GA, Osaki MH, Cariello AJ, Damasceno RW. Treatment of tear trough deformity with hyaluronic acid gel filler. *Arq Bras Oftalmol* 2011;74:44-7.
16. Viana GA, Osaki MH, Cariello AJ, Damasceno RW, Osaki TH. Treatment of the tear trough deformity with hyaluronic acid. *Aesthet Surg J* 2011;31:225-31.
17. Berguiga M, Galatoire O. Tear trough rejuvenation: a safety evaluation of the treatment by a semi-cross-linked hyaluronic acid filler.

- Orbit* 2017;36:22-6.
18. Mitchell DA, Lyons AB, Moy RL. Correction of cicatricial and involutional lower eyelid ectropion with hyaluronic acid. *JAAD Case Rep* 2018;4:628-30.
  19. Innocenti A, Melita D, Ghezzi S, Innocenti M. Refinements in tear trough deformity correction: intraoral release of tear trough ligaments: anatomical consideration and clinical approach. *Aesthetic Plast Surg* 2018;42:1576-81.
  20. Vasquez RAS, Park K, Braunlich K, Aguilera SB. Prolonged periorbicular edema after injection of hyaluronic acid for nasojugal groove correction. *J Clin Aesthet Dermatol* 2019;12:32-5.
  21. Hussain SN, Mangal S, Goodman GJ. The tick technique: a method to simplify and quantify treatment of the tear trough region. *J Cosmet Dermatol* 2019;18:1642-7.
  22. Goldberg RA, Fiaschetti D. Filling the periorbital hollows with hyaluronic acid gel: initial experience with 244 injections. *Ophthalmic Plast Reconstr Surg* 2006;22:335-41; discussion 341-3.
  23. Goldberg RA, Lee S, Jayasundera T, et al. Treatment of lower eyelid retraction by expansion of the lower eyelid with hyaluronic Acid gel. *Ophthalmic Plast Reconstr Surg* 2007;23:343-8.
  24. Lambros VS. Hyaluronic acid injections for correction of the tear trough deformity. *Plast Reconstr Surg* 2007;120:74S-80.
  25. Kashkouli MB, Heirati A, Pakdel F, Kiavash V. Diplopia after hyaluronic acid gel injection for correction of facial tear trough deformity. *Orbit* 2012;31:330-1.
  26. Hamman MS, Goldman MP, Fabi SG. Comparison of two techniques using hyaluronic acid to correct the tear trough deformity. *J Drugs Dermatol* 2012;11:e80-4.
  27. Wollina U. Improvement of tear trough by monophasic hyaluronic acid and calcium hydroxylapatite. *J Clin Aesthet Dermatol* 2014;7:38-43.
  28. Jiang J, Wang X, Chen R, et al. Tear trough deformity: different types of anatomy and treatment options. *Postepy Dermatol Alergol* 2016;33:303-8.
  29. Or L, Eviatar JA, Massry GG, Bernardini FP, Hartstein ME. Xanthelasma-like reaction to filler injection. *Ophthalmic Plast Reconstr Surg* 2017;33:244-7.
  30. Berros P, Armstrong BK, Foti P, Mancini R. Cosmetic adolescent filler: an innovative treatment of the “Selfie” complex. *Ophthalmic Plast Reconstr Surg* 2018;34:366-8.
  31. Bagci B. A new technique for the correction of tear trough deformity via filler injections. *Plast Reconstr Surg* 2018;6:e1901.
  32. Morley AM, Malhotra R. Use of hyaluronic acid filler for tear-trough rejuvenation as an alternative to lower eyelid surgery. *Ophthalmic Plast Reconstr Surg* 2011;27:69-73.
  33. Kane MA. Advanced techniques for using Restylane in the lower eyelids. *Aesthet Surg J* 2007;27:90-2.
  34. Fezza JP. Nonsurgical treatment of cicatricial ectropion with hyaluronic acid filler. *Plast Reconstr Surg* 2008;121:1009-14.
  35. Zamani M, Thyagarajan S, Olver JM. Functional use of hyaluronic acid gel in lower eyelid retraction. *Arch Ophthalmol* 2008;126:1157-9.
  36. Glaser DA, Patel U. Enhancing the eyes: use of minimally invasive techniques for periorbital rejuvenation. *J Drugs Dermatol* 2010;9:s118-28.
  37. Peckinpugh JL, Reddy HS, Tower RN. Large particle hyaluronic acid gel for the treatment of lower eyelid retraction associated with radiation-induced lipoatrophy. *Ophthalmic Plast Reconstr Surg* 2010;26:377-9.
  38. Stutman RL, Codner MA. Tear trough deformity: review of anatomy and treatment options. *Aesthet Surg J* 2012;32:426-40.
  39. Dayan SH, Arkins JP, Somenek M. Restylane persisting in lower eyelids for 5 years. *J Cosmet Dermatol* 2012;11:237-8.
  40. Sharad J. Dermal fillers for the treatment of tear trough deformity: a review of anatomy, treatment techniques, and their outcomes. *J Cutan Aesthet Surg* 2012;5:229-38.
  41. Rzany B, Cartier H, Kestermont P, et al. Correction of tear troughs and periorbital lines with a range of customized hyaluronic acid fillers. *J Drugs Dermatol* 2012;11:s27-34.
  42. Trevidic P. The use of blunt-tipped cannulas for tear trough correction. *J Drugs Dermatol* 2012;11:s38-40.
  43. Lim HK, Suh DH, Lee SJ, Shin MK. Rejuvenation effects of hyaluronic acid injection on nasojugal groove: prospective randomized split face clinical controlled study. *J Cosmet Laser Ther* 2014;16:32-6.
  44. Hartstein ME. Injectable adjunctive procedures for cosmesis and function. *Facial Plast Surg Clin North Am* 2016;24:139-44.
  45. Turkmani MG. New classification system for tear trough deformity. *Dermatol Surg* 2017;43:836-40.
  46. Lee S, Yen MT. Nonsurgical rejuvenation of the eyelids with hyaluronic acid gel injections. *Semin Plast Surg* 2017;31:17-21.
  47. Sharad J. Treatment of the tear trough and infraorbital hollow with hyaluronic acid fillers using both needle and cannula. *Dermatol Ther* 2020;33:e13353.
  48. Huber-Vorländer J, Kürten M. Correction of tear trough deformity with a cohesive polydensified matrix hyaluronic acid: a case series. *Clin Cosmet Investig Dermatol* 2015;8:307-12.
  49. El-Garem YF. Estimation of bony orbit depth for optimal selection of the injection technique to correct the tear trough and palpebromalar groove. *Dermatol Surg* 2015;41:94-101.
  50. Hill RH 3rd, Czyz CN, Kandapalli S, et al. Evolving minimally invasive techniques for tear trough enhancement. *Ophthalmic Plast Reconstr Surg* 2015;31:306-9.
  51. Artzi O, Loizides C, Verner I, Landau M. Resistant and recurrent late reaction to hyaluronic acid-based gel. *Dermatol Surg* 2016;42:31-7.
  52. Pascali M, Quarato D, Pagnoni M, Carinci F. Tear trough deformity: study of filling procedures for its correction. *J Craniofac Surg* 2017;28:2012-5.
  53. Teo AA, Mokhtarzadeh A, Cameron JD, Harrison AR. Late presentation of enlarging lower eyelid mass and muscle degeneration

- secondary to hyaluronic acid filler. *Ophthalmic Plast Reconstr Surg* 2017;33:S9-11.
54. Iverson SM, Patel RM. Dermal filler-associated malar edema: treatment of a persistent adverse effect. *Orbit* 2017;36:473-5.
  55. Parulan MAA, Sundar G, Lum JH, Ramachandran U. A case report on dermal filler-related periorbital granuloma formation. *Orbit* 2019;38:169-72.
  56. Wulu JA, Garcia-Rodriguez L, Prilutskiy A, Spiegel JH. The case of the eyelid silicone granulomas. *Am J Otolaryngol* 2019;40:776-8.
  57. Kalin-Hajdu E, Kersten RC. Nasolacrimal duct obstruction following hyaluronic acid rejuvenation of the tear trough. *Ophthalmic Plast Reconstr Surg* 2019;35:e14-5.
  58. Wong CH, Hsieh MK, Mendelson B. The tear trough ligament: anatomical basis for the tear trough deformity. *Plast Reconstr Surg* 2012;129:1392-402.
  59. El Rayes EN, Elborgy E. Suprachoroidal buckling: technique and indications. *J Ophthalmic Vis Res* 2013;8:393-9.
  60. Griepentrog GJ, Lemke BN, Burkat CN, Rose JG Jr, Lucarelli MJ. Anatomical position of hyaluronic acid gel following injection to the infraorbital hollows. *Ophthalmic Plast Reconstr Surg* 2013;29:35-9.
  61. Surek C, Beut J, Stephens R, Lamb J, Jelks G. Volumizing viaducts of the midface: defining the Beut techniques. *Aesthet Surg J* 2015;35:121-34.
  62. Peng PH, Peng JH. Treating the tear trough: a new classification system, a 6-step evaluation procedure, hyaluronic acid injection algorithm, and treatment sequences. *J Cosmet Dermatol* 2018;17:333-9.
  63. Zoumalan CI. Managing periocular filler-related syndrome prior to lower blepharoplasty. *Aesthetic Plast Surg* 2019;43:115-22.
  64. Tung R, Ruiz de Luzuriaga AM, Park K, et al. Brighter eyes: combined upper cheek and tear trough augmentation: a systematic approach utilizing two complementary hyaluronic acid fillers. *J Drugs Dermatol* 2012;11:1094-7.
  65. El Rayes EN. Suprachoroidal buckling. In: Oh H, Oshima Y, editors. *Microincision Vitrectomy Surgery*. Basel: S. KARGER AG; 2014. pp. 135-46.
  66. Worley B, Huang JW, Macdonald J. Approach to treatment of cicatricial ectropion: a systematic review and meta-analysis comparing surgical and minimally invasive options. *Arch Dermatol Res* 2020;312:165-72.
  67. Xi W, Han S, Feng S, et al. The Injection for the lower eyelid retraction: a mechanical analysis of the lifting effect of the hyaluronic acid. *Aesthetic Plast Surg* 2019;43:1310-7.
  68. Hilton S, Schrupf H, Bühren BA, Bölke E, Gerber PA. Hyaluronidase injection for the treatment of eyelid edema: a retrospective analysis of 20 patients. *Eur J Med Res* 2014;19:30.
  69. Choi SY, Ko EJ, Kim BJ, Song KY, Kim WS. Lump on the lower eyelid due to hyaluronic acid filler. *Clin Exp Dermatol* 2016;41:94-5.
  70. Skippen B, Baldelli I, Hartstein M, et al. Rehabilitation of the dysmorphic lower eyelid from hyaluronic acid filler: what to do after a good periocular treatment goes bad. *Aesthet Surg J* 2020;40:197-205.
  71. Hall MB, Roy S, Buckingham ED. Novel use of a volumizing hyaluronic acid filler for treatment of infraorbital hollows. *JAMA Facial Plast Surg* 2018;20:367-72.
  72. Mustaki H, Fiaschetti D, Goldberg RA. Filling the periorbital hollows with hyaluronic acid gel: Long-term review of outcomes and complications. *J Cosmet Dermatol* 2018;17:611-6.
  73. De Pasquale A, Russa G, Pulvirenti M, Di Rosa L. Hyaluronic acid filler injections for tear-trough deformity: injection technique and high-frequency ultrasound follow-up evaluation. *Aesthetic Plast Surg* 2013;37:587-91.
  74. Cho SY, Park JW, An H, et al. Physical properties of a novel small-particle hyaluronic acid filler: In vitro, in vivo, and clinical studies. *J Cosmet Dermatol* 2018;17:347-54.
  75. Romero R, Sanchez-Orgaz M, Granados M, et al. Use of hyaluronic acid gel in the management of cicatricial ectropion: results and complications. *Orbit* 2013;32:362-5.
  76. Meier JD, Glasgold RA, Glasgold MJ. 3D photography in the objective analysis of volume augmentation including fat augmentation and dermal fillers. *Facial Plast Surg Clin North Am* 2011;19:725-35, ix.
  77. Calvisi L, Diaspro A, Sito G. Tear trough. In: Acta Medica Griffin, editors. *Fillers - Illustrated Manual of Injection Techniques*. Cantù: Griffin Editore; 2020. pp. 37-54.
  78. CEBM News Release. Oxford Centre for Evidence-Based Medicine. Available from: <https://www.cebm.net/index.aspx?o=5653>. [Last accessed on 19 Oct 2020]
  79. Eaves F 3rd, Pusic AL. Why evidence-based medicine matters to aesthetic surgery. *Aesthet Surg J* 2012;32:117-9.

Review

Open Access



# Aging skin and non-surgical procedures: a basic science overview

Amy R. Vandiver<sup>1</sup>, Sara R. Hogan<sup>1,2</sup>

<sup>1</sup>Division of Dermatology, University of California Los Angeles, Los Angeles, CA 90095, USA.

<sup>2</sup>David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA.

**Correspondence to:** Dr. Amy R. Vandiver, Division of Dermatology, University of California Los Angeles, 200 Medical Plaza Suite 450, Los Angeles, CA 90095, USA. E-mail: avandiver@mednet.ucla.edu

**How to cite this article:** Vandiver AR, Hogan SR. Aging skin and non-surgical procedures: a basic science overview. *Plast Aesthet Res* 2020;7:63. <http://dx.doi.org/10.20517/2347-9264.2020.159>

**Received:** 1 Aug 2020 **First Decision:** 7 Aug 2020 **Revised:** 10 Sep 2020 **Accepted:** 12 Oct 2020 **Published:** 6 Nov 2020

**Academic Editor:** James E. Zins **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Skin aging is a major cosmetic concern and associated with extensive changes in skin function and structure. The understanding of the basic science underlying skin aging is rapidly progressing, anchored around nine fundamental hallmarks of aging defined in 2013. Here we present the evidence for the relevance of each hallmark of aging to skin aging, emphasizing the uniquely prominent roles of oxidative damage and the extracellular matrix in photoaging. We review the existing evidence for how established treatments of skin aging target each fundamental hallmark and discuss targets for potential future treatments.

**Keywords:** Aging, photoaging, intrinsic aging, antiaging, rejuvenation

## INTRODUCTION

Skin aging is associated with extensive changes in the structure and function of all aspects of the skin and serves as a major risk factor for multiple pathologies including atopy, impaired wound healing, infection and malignancy<sup>[1-5]</sup>. In addition to functional concerns, the aging of sun-exposed skin, with the face in particular, is a major cosmetic concern prompting patients to seek cosmetic procedures. While therapies to reduce or prevent aging of sun-exposed skin have been present for decades, our understanding of the basic science underlying aging is rapidly evolving, shedding light on the mechanisms of established treatments and identifying new treatment targets and methods. In this article, we will discuss the current



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



understanding of the hallmarks of aging as applied to skin aging, review how current treatments target this underlying biology and discuss future treatments identified by this emerging knowledge.

## SKIN AGING

The skin consists of two layers with distinct cell populations and underlying biology. The outermost layer, the epidermis, is a stratified epithelium consisting primarily of keratinocytes. The inner most layer of the epidermis consists of proliferating basal keratinocytes, above which are three differentiated layers: the stratum spinosum, stratum granulosum and stratum corneum. The outermost layer, the stratum corneum consists of anucleate corneocytes in a lipid-rich matrix. The epidermis is regenerated by epidermal stem cells, distinct populations of which are found throughout the basal layer of the epidermis and in the hair follicle<sup>[6,7]</sup>. Below the basal layer of the epidermis lies the dermis which consists of a collagen-rich extracellular matrix (ECM) supporting vasculature and adnexal structures. This matrix is generated by dermal fibroblasts, terminally differentiated cells of mesenchymal origin.

Aging of sun-exposed skin (photoaging) and sun-protected skin (intrinsic or chronological aging) are distinct processes with both common and unique manifestations and molecular mechanisms. Intrinsic aging is commonly associated with increased xerosis (dry skin), fine rhytids and laxity. Photoaging shares these features but also exhibits uneven pigmentation, deeper rhytids, telangiectasias and increased growth rate of malignant neoplasms. Histologically, photoaging demonstrates uneven thinning of the epidermal layer with thickening of the granular layer and more compact corneal layer, dermal ECM loss of collagen and elastin, as well as increased dermal inflammation<sup>[8-11]</sup>. Such histologic findings correlate with increased gene expression of matrix metalloproteinases and decreased gene expression of ECM components, particularly collagen and elastin. These changes are seen in multiple models of photoaging, and as such have been used as markers in many mechanistic and therapeutic studies<sup>[12-15]</sup>.

Investigation into the basic biology of aging has steadily grown since the 1980s. Original studies focused on identifying a single driving mechanism. However, in 2013, López-Otín *et al.*<sup>[16]</sup> proposed a distinct framework for studying aging biology focused on nine hallmarks of aging, which together contribute to age-related functional change. This marked a shift towards understanding aging not as a single process, but instead as a combination of biological changes. These hallmarks are broken into three categories: primary, antagonistic and integrative [Table 1]. The primary hallmarks of genomic instability, epigenetic change, loss of proteostasis and telomere attrition, are the foundational changes that initiate aging phenotypes. Antagonistic hallmarks occur in response to these alterations and include de-regulation of nutrient sensing, mitochondrial dysfunction and cellular senescence. Together, these all contribute to the integrative hallmarks - impaired intracellular communication and stem cell exhaustion - which most directly contribute to tissue aging phenotypes.

Since publication, López-Otín *et al.*<sup>[16]</sup>'s hallmarks of aging have been widely accepted and used extensively as a framework for aging research. It is increasingly recognized, however, that each tissue and cell population ages in a distinct manner, with differing hallmarks playing a more prominent role for each<sup>[17]</sup>. While photoaging shares the majority of the hallmarks of aging seen across cell types, we will discuss evidence that the central role of ultraviolet (UV) exposure and prominence of the ECM in aging biology make the damage and signaling associated hallmarks particularly relevant. Below, we will review each of the hallmarks of aging as applied to skin aging and discuss treatment modalities targeting each set of changes.

### Primary hallmarks of skin aging

#### *Genomic instability*

In photoaging, UV radiation plays a prominent role in inducing the primary hallmarks of aging, particularly genomic instability. UV radiation is linked to DNA damage *in vivo* and in cell culture. UVB



**Table 1. Hallmarks of aging**

	Molecular target	Treatment	Level of evidence
Primary hallmarks	DNA damage	Antioxidant supplementation	III
		Photodynamic therapy	I
	Epigenetic change	Antioxidant supplementation	Not examined
	Loss of protein homeostasis	Antioxidant supplementation	Not examined
		Laser resurfacing	V
Fractional radiofrequency microneedling		IV	
Antagonistic hallmarks	Telomere shortening	None	None
	Mitochondrial dysfunction	Coenzyme Q10 supplementation	III
		Nicotinamide supplementation	III
		Low level light treatment	III
	Impaired nutrient sensing		
	Increased mTOR	Rapamycin	VI
	Decreased AMPK	Metformin	None
	Integrative hallmarks	Decreased sirtuins	Resveratrol
Resveratrol			V
Senolytics in development			None
Senescent cells			
Altered intracellular signaling			
		Altered growth factors	Topical growth factors
Altered inflammatory signaling		Platelet-rich plasma	V
		Laser resurfacing	II
		Chemical peels	III
		Microdermabrasion	III
Altered ECM signaling		Fractional radiofrequency microneedling	IV
		Topical retinoids	I
	Exogenous filler	III	
	PDO and PLA threads	VI	
	Stem cell exhaustion	Mesenchymal stem cell transplantation	V

PDO: Polydioxanone; PLA: poly-lactic acid; ECM: extracellular matrix

exposure leads to the formation of cyclobutane dimers in the nuclear genome<sup>[18]</sup>. UVA exposure causes the generation of reactive oxidative species (ROS) and oxidative guanine damage<sup>[19]</sup>. Upon nucleic acid repair, both of these changes induce cytosine to thymidine nucleic acid sequence alterations, which, depending on location, leads to gene dysregulation. These UV-induced mutations in specific genes are linked to skin cancer and loss-of-cell function<sup>[20-23]</sup>. UVA exposure also leads to large modifications in the circular mitochondrial genome, which lacks many of the repair mechanisms that maintain the nuclear genome: photoaged skin is repeatedly observed to contain large deletions in the mitochondrial genome *in vivo*, and UVA directly induces these changes in cell culture<sup>[24-27]</sup>. Similar mutations are linked to mitochondrial dysfunction in other organ systems<sup>[28]</sup>.

### Epigenetic alterations

In addition to the sequence itself, expression of the nuclear genome is very closely regulated by the epigenome through modifications of the nucleotides and proteins involved in DNA packaging<sup>[29]</sup>. With both intrinsic aging and photoaging, epidermal tissue shows loss of methylation of cytosine bases, with a larger degree of loss in photoaged tissue<sup>[30,31]</sup>. Compared to sun-protected skin, sun-exposed skin also demonstrates changes in histone modifications, specifically increases in open areas of chromatin that are permissive to gene expression<sup>[32]</sup>. At least some of these changes are directly linked to UV-induced ROS,

which directly trigger de-methylation *in vitro*<sup>[33]</sup>. The loss of cytosine methylation and increase in histone acetylation are both linked to increased “transcriptional noise”, or low-level transcription of unnecessary genes thought to interfere with cell function in other systems<sup>[34,35]</sup>.

#### *Telomere attrition*

The nuclear genome, mitochondrial genome and epigenome are all clearly demonstrated to change in photoaged skin. It is less clear if changes in the length of telomeric end sequences are prominent in skin aging. Decreases in telomere length occur in dividing cells that lack the enzyme telomerase, and this has been shown to occur more with in association with increased levels of ROS in cultured fibroblasts<sup>[36]</sup>. Such decreases are strongly associated with aging phenotypes in mice<sup>[37]</sup>. However, the relationship between telomere length and human biological age is less direct<sup>[38]</sup>. For skin in particular, the relevance of shortened telomere sequences to epidermal and dermal aging phenotypes remains unclear. While some studies have documented significant decrease in telomere length in skin with age<sup>[39-41]</sup>, there appear to be equivalent levels of change in photoaged and intrinsic aged samples. In addition, studies focused on the epidermis suggest telomerase may be expressed in basal keratinocytes and increase with sun exposure and inflammation<sup>[42-44]</sup>, correlating with a low magnitude of telomere length decrease with age<sup>[43]</sup>.

#### *Loss of proteostasis*

Beyond the genome, aging is associated with notable loss of regulation of protein homeostasis - or “proteostasis”. In healthy cells, proteostasis is maintained by the proteasome, an enzyme complex that balances new protein synthesis with damaged protein degradation<sup>[45]</sup>. In photoaging, increased levels of ROS *in vivo* lead to dermal accumulation of oxidatively modified and damaged proteins and cellular dysfunction<sup>[46]</sup>. In concert, proteasome components decrease in photoaged and intrinsically aged skin<sup>[47,48]</sup>.

### **Targeting the primary hallmarks of aging**

UV-induced ROS play a clear role in generating many of the primary insults thought to initiate cutaneous aging phenotypes. The endogenous antioxidant system consists of non-enzymatic antioxidants, which acquire electrons to neutralize ROS, and enzymatic antioxidants, which deactivate ROS (A complete discussion of the antioxidant system in skin is available<sup>[49]</sup>). As such, classic antiaging treatments focus on preventing skin damage by decreasing levels of ROS through topical and oral supplementation of components of this system.

A wide range of endogenous non-enzymatic antioxidants are used in topical antiaging formulations. Examples include vitamin C, vitamin E, niacinamide, lycopene, carotenoids and polyphenols<sup>[49]</sup>. Resveratrol, a polyphenol, also increases synthesis of glutathione, an endogenous enzymatic antioxidant<sup>[50]</sup>. In cell culture models, supplementation with vitamin C directly prevents UV-induced DNA damage and increases expression of the proteasome to scavenge damaged proteins and supplementation with niacinamide enhances repair of UV-induced damage<sup>[51,52]</sup>. Clinically, topical application of vitamins C and E, polyphenols and resveratrol show efficacy in reducing acute UV-induced erythema and DNA damage markers<sup>[53-56]</sup>.

Oral antioxidant formulations are protective against acute UV-induced damage. Oral supplementation with multiple non-enzymatic antioxidants have been demonstrated to decrease UV-induced erythema, inflammatory markers and the incidence of actinic keratoses<sup>[57]</sup>. Systemic reviews, however, suggest that certain oral antioxidant formulations may be associated with increased mortality, likely given the beneficial role of an *appropriate* level of ROS in many body systems<sup>[58]</sup>.

Still, both topical and oral antioxidant formulations primarily show benefit for preventing - not reversing - photoaging damage. There is evidence that vitamin C supplementation *in vitro* increases collagen

synthesis and topical combinations result in modest photoaging benefits, but it is not known whether levels of existing damage are altered<sup>[59]</sup>. Furthermore, while ROS have been directly linked to nuclear and mitochondrial genome impairment, epigenetic change and protein oxidation, no study has demonstrated a reduction of these specific alterations after topical or oral treatment. The exact degree to which these changes are prevented or reversed remains unknown.

Destructive treatments targeting oxidatively damaged cells are shown to have clinical benefit for photoaging. Photodynamic therapy - in which aminolevulinic acid is activated by visible light to destroy rapidly proliferating cells - decreases damaged cells within the epidermis (as noted by p53 expression), promotes collagen synthesis and improves clinical photoaging grade<sup>[60]</sup>. Energy- and laser-based therapies such as radiofrequency microneedling, fractional non-ablative laser and fractional ablative laser, all induce broad destruction of tissue, including the destruction of cells containing oxidatively damaged DNA and proteins. While the level of oxidative damage post-treatment has not been specifically tested, these treatments are shown to induce the expression of specific heat shock proteins known to promote the clearance of damaged proteins<sup>[61-63]</sup>.

### **Antagonistic hallmarks of aging in photoaging**

#### *Mitochondrial dysfunction*

Mitochondria serve as the primary energy-generating powerhouses of the cell. The number, morphology and activity of mitochondria are closely regulated in numerous biological conditions<sup>[64]</sup>. Possibly related to the accumulation of mitochondrial DNA (mtDNA) alterations as discussed above, there is growing evidence of mitochondrial dysfunction involving altered oxidative phosphorylation in photoaged skin.

*In vivo* imaging of photoaged epidermis demonstrates a more fragmented mitochondrial network, which may correlate with altered aerobic function<sup>[65]</sup>. Cultured keratinocytes also show decreased electron transport chain (ETC) activity and thus decreased oxidative phosphorylation and a shift to anaerobic metabolism with photoaging<sup>[66]</sup>. A direct relationship between mitochondrial dysfunction and skin aging phenotypes is supported by multiple mouse models: in mouse models in which mitochondrial antioxidants or transcription factors are reduced, keratinocyte differentiation is impaired and increased senescence keratinocytes noted<sup>[67,68]</sup>, and in a model in which mtDNA is progressively decreased, dermal atrophy, increased MMP-1 expression and epidermal hyperplasia are noted<sup>[69]</sup>. *In vitro*, the restoration of ETC activity is shown to decrease MMP1 expression after UVA radiation, further suggesting a direct link between mitochondrial function and aging phenotypes<sup>[66]</sup>.

#### *Impaired nutrient sensing*

Closely linked to mitochondrial dysfunction and aerobic metabolism alteration, there is significant evidence that nutrient sensing is linked to photoaging. Nutrient sensing is the mechanism by which cells recognize and respond to energy substrates. The concept of impaired nutrient sensing stems from genetic evidence suggesting that those gene variants most linked to lifespan occur within pathways involved in sensing nutrient abundance<sup>[70]</sup>. Models suggest that upregulation of the protein mTOR kinase, which signals nutrient abundance, is associated with aging, and that proteins that signal nutrient scarcity, such as AMPK and sirtuins, are downregulated<sup>[71]</sup>. In mouse models of photoaging, mTOR components are increased and AMPK is decreased<sup>[72,73]</sup>. Similarly, sirtuins are noted to be decreased in fibroblasts isolated from both sun-exposed and photoaged individuals<sup>[74-76]</sup>. While these alterations infer that aged skin is signaling a state of nutrient excess, metabolomic profiling of epidermal samples suggests there is downregulation of anabolic biosynthetic pathways and upregulation of catabolic pathways, consistent with an overall impaired function of nutrient sensing<sup>[77,78]</sup>.

### *Accumulation of senescent cells*

Senescent cells are those that have permanently exited the replicative cell cycle, and accumulation of these cells has been observed with the aging of many tissues. Despite stopping replication, they are metabolically active and have a distinct secretory profile known as the senescence-associated secretory phenotype (SASP) which can have profound effects on surrounding cells<sup>[79,80]</sup>. The induction of senescence is strongly linked to DNA damage as well as epigenetic changes and loss of proteostasis, so it is not surprising that the presence of senescent cells is significantly increased in photoaged epidermis and dermis<sup>[81]</sup>. In addition, mTOR activity is linked to the induction of senescence in keratinocytes, indicating that the accumulation of senescent cells in photoaged skin is multifactorial<sup>[82]</sup>. The presence of senescent cells in photoaged skin has been linked to photoaging phenotypes in cell culture models: senescent fibroblasts promote ECM degradation through increased MMP-1 secretion and induce a proinflammatory environment, which can promote ECM degradation and keratinocyte tumorigenesis<sup>[79,83]</sup>. Also, the introduction of senescent fibroblasts into organotypic skin models induces thinning of the epidermis and decreased barrier function in the overlying epidermis<sup>[84]</sup>.

### **Targeting the antagonistic hallmarks of aging**

Given the evidence for mitochondrial dysfunction and impaired nutrient sensing in aging phenotypes, there is a move to target aging treatment and prevention at these deficits, both by improving mitochondrial function and reversing the signals of nutrient abundance. Mitochondrial dysfunction can be mitigated *in vivo* through supplementation with specific antioxidants. Supplementation with coenzyme Q10, which acts as a diffusible electron transporter in the mitochondrial transport chain, is shown to improve mitochondrial function in multiple oxidative systems *in vivo*<sup>[85,86]</sup> and restore oxidative metabolism in aged keratinocytes *in vitro*<sup>[66,87]</sup>. Nicotinamide, which acts as a precursor of the main oxidative substrate NAD<sup>+</sup>, is also shown to improve mitochondrial quality and promote keratinocyte regenerative capacity *in vitro*<sup>[88,89]</sup>.

In addition to working as an antioxidant, resveratrol exerts antiaging effects through benefits to mitochondrial function and modifications in nutrient sensing. Resveratrol promotes oxidative metabolism through multiple pathways. *In vivo* and *in vitro*, resveratrol activates the nutrient sensor AMPK and thereby activates sirtuin 1<sup>[90-92]</sup> to shift cells to a state of nutrient deficiency<sup>[93]</sup>. Through sirtuin 1, resveratrol directly increases the synthesis of new mitochondria in multiple tissues<sup>[94]</sup>. While this highlights resveratrol as an appealing option for topical therapy, current small trials of topical formulations have unclear evidence of benefit and the bioavailability of these formulations remains questionable<sup>[50,95,96]</sup>.

Low-level light therapy is promoted to treat photoaging through improving mitochondrial function. This treatment involves exposing target tissue to low levels of red or near infrared light and is rising in popularity as many devices can be used for home treatment<sup>[97]</sup>. *In vitro* studies have shown that specific wavelengths of red and near infrared light are absorbed by cytochrome oxidase c, an enzyme involved in the mitochondrial ETC, and that this absorption increases mitochondrial enzyme activity and energy production<sup>[98-100]</sup>. This increased mitochondrial activity is in turn shown to increase collagen production and growth factor secretion in cultured fibroblasts<sup>[101,102]</sup>, and multiple small trials of low-level light therapy demonstrate modest to moderate benefit for facial rhytids<sup>[103,104]</sup>.

Though not yet available for use on skin, multiple metabolic regulatory molecules studied for systemic antiaging have potential for use as topical therapies. Metformin, developed for use in diabetes mellitus, works as an AMPK activator to alter the cell metabolic profile. In fibroblast culture, metformin stimulates collagen production, and topical formulations promote wound healing and barrier integrity in mouse models<sup>[74,105]</sup>. Recent advances in topical formulations of metformin that permeate human skin suggest this may emerge as an antiaging treatment option<sup>[106]</sup>. Similarly, topical formulations of rapamycin, an mTOR inhibitor, reduce markers of cutaneous aging in human skin in addition to other aging models<sup>[107,108]</sup>.

Rapamycin was noted, however, to have negative effects on wound healing and thus requires further study before use for antiaging<sup>[74]</sup>.

Another future treatment strategy involves directly targeting senescent cells for removal. Due to their altered signaling profile, senescent cells can theoretically be targeted for destruction via compounds specific to their upregulated components. In transgenic mouse models, the selective apoptosis of cells entering senescence prevents many aging phenotypes but imparts minimal effect on dermal thickness and also delays wound healing<sup>[109,110]</sup>. Multiple compounds that target molecules upregulated in senescent cells, known as senolytics, including dasatinib, quercetin, navitoclax and piperlongumine show efficacy in clearing senescent fibroblasts and other cell populations in culture; however, none have been reported to have efficacy for skin aging phenotypes *in vivo* at this time<sup>[111-114]</sup>.

### **Integrative hallmarks of aging**

#### *Altered intracellular signaling*

The end points of the primary and antagonistic hallmarks of aging lead to the integrative hallmarks, specifically impaired intracellular communication and stem cell exhaustion.

The most prominent age-related change in intracellular communication is increased inflammation mediated by NF-kappaB (NF-κB), which leads to upregulation of IL-1B, tumor necrosis factor and interferon signaling, and overall decline in adaptive immune function<sup>[16]</sup>. In the epidermis, increased NF-κB signaling is inducible by UV exposure and promotes epidermal stem cell dysfunction<sup>[82,115]</sup>. When NF-κB is inhibited in mouse models, intrinsic aging phenotypes are reversed<sup>[116]</sup>. In addition, photoaged skin also exhibits decreases in other cytokines, such as TGF-B and fibroblast growth factor (FGF), which promote collagen synthesis and epidermal regeneration<sup>[117]</sup>.

In photoaging, the ECM plays a uniquely important role in mediating intracellular signaling. Photoaging is strongly associated with the degradation of collagen in the dermal ECM. This change is initiated by UV-induced increase in MMP secretion by basal keratinocytes and dermal fibroblasts, and is associated with the activation of AP-1 signaling<sup>[13]</sup>. Once degraded, collagen is present within the matrix, and *in vitro* studies suggest this amplifies intracellular aging. Dermal fibroblasts grown on synthetically-degraded collagen generate increased ROS, increased MMP secretion, and decreased collagen production<sup>[13]</sup>. MMP expression in co-culture fibroblasts also decreases the regenerative capacity and longevity of epidermal stem cells<sup>[118]</sup>.

#### *Stem cell exhaustion*

The final integrative hallmark of aging is stem cell exhaustion, which has a notable role in the epidermal photoaging phenotypes. Two distinct stem cell populations - epidermal and hair follicle stem cells - are thought to maintain the epidermis in different biological settings<sup>[6,7]</sup>. While decreased regeneration and epidermal thinning is noted with both murine and human aging, the specific changes to these stem cell populations is complex and continues to be investigated. *In vivo*, multiple studies have demonstrated consistent or increased numbers of stem cells in intrinsically aged human and mouse skin<sup>[119-121]</sup>; however, other studies have demonstrated decreased stem cell functional markers in intrinsically aged and photoaged skin, possibly partially linked to UV-induced basement membrane changes<sup>[122-124]</sup>. In photoaging, this is likely directly linked to many of the previously discussed UV-induced hallmarks, including the DNA-damage response, increased NF-kB signaling, metabolic dysregulation through mTOR upregulation, senescence and ECM degradation, emphasizing the inter-related nature of all aging hallmarks in cutaneous photoaging<sup>[82,114,116,118,121,125]</sup>.



### Targeting altered intracellular signaling and stem cell exhaustion

As evidence grows of photoaging-induced impairment in signaling molecules, various approaches have been tested to reverse this change. Researchers have attempted to directly supplement certain growth factors that decline with age. Multiple small trials show clinical benefit with topical formulations of growth factors and cytokines, including TGF-beta, platelet-derived growth factor (PDGF), FGF, IL-1 and TNF-alpha, in reducing fine lines and wrinkles and increasing dermal collagen<sup>[126-128]</sup>. The true efficacy of these topicals, however, is questionable. Growth factors and cytokines are large, hydrophilic molecules with greater than 15,000 Da molecular weight, and prior studies demonstrate that hydrophilic molecules greater than 500 Da have low penetration past the stratum corneum<sup>[129]</sup>.

Treatments that directly target ECM degradation and associated signaling amplification alteration are more effective. Topical retinoids are well established as clinically effective treatments for photoaging. Retinoids decrease AP-1 and NF-kB signaling and increase TGF-beta signaling to reduce expression of MMPs, and this in turn decreases degraded collagen levels associated with altered cell function<sup>[130,131]</sup>.

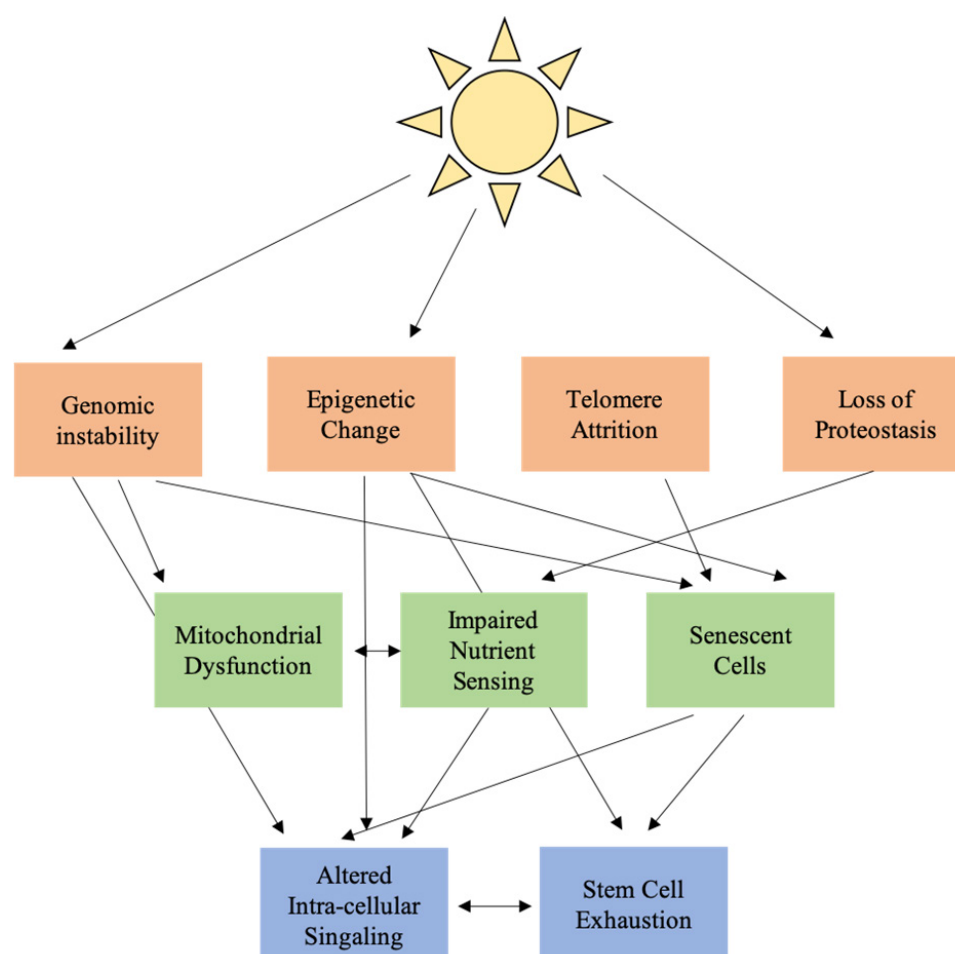
In multiple biological systems, dermal fillers that artificially restore the matrix on which fibroblasts live, are demonstrated to increase collagen synthesis. This is likely accomplished by interrupting the defective signaling associated with fragmented collagen. Hyaluronic acid fillers, in human and mouse models, restore the ECM via dermal fibroblast collagen synthesis. In mouse models, hyaluronic acid filler also stimulates collagen and elastin synthesis<sup>[132,133]</sup>. Calcium hydroxylapatite, a semi-permanent biostimulatory filler, is also shown in tissue culture to restore the contractile properties of photoaged dermal fibroblasts<sup>[134]</sup>.

Absorbable sutures are an emerging treatment that may also work through interruption of the altered signaling that is associated with fragmented collagen. Polydioxanone (PDO) and monofilament poly-lactic acid (PLA) absorbable sutures are posited to lift and tighten aging skin. In animal models, PDO and PLA threads on histology induce collagen-1 and -3 production two weeks after insertion. This benefit, however, sharply declines by 12 weeks post-insertion<sup>[135]</sup>. In another study, PDO sutures induced neocollagenesis, tissue contracture and improved vasculature four weeks after insertion, a change maintained at 48 weeks<sup>[136]</sup>. While molecular analysis has yet to be performed, it is possible that absorbable sutures induce physical contraction of skin and fibrosis to restore the stretch placed on fibroblasts, similar to an intact ECM.

Microdermabrasion, moderate to deep chemical peels, fractional radiofrequency microneedling, and fractional non-ablative and fractional ablative lasers, all demonstrate an induced wound repair response on histology<sup>[137-139]</sup>. Recent molecular analysis suggests that fractionated ablative CO<sub>2</sub> laser accomplishes this through alteration of inflammatory signal pathways, with damage-activating innate immune responses that in turn trigger the activation of retinoic acid-dependent pathways<sup>[140]</sup>. Interestingly, this process is mediated by NF-kB expression at significantly higher levels than those observed in chronically aged skin, indicating that the balance of signaling factors is crucial to regenerative responses.

Platelet-rich plasma (PRP) is an autologous blood product of concentrated platelets delivered via injection into target tissue. PRP is thought to contain over 800 bioactive molecules derived from platelet granules, including TGF-beta, epidermal growth factor, PDGF, FGF, IGF and angiogenic growth factors<sup>[141,142]</sup>. While demonstrated to have benefit for wound healing, arthritis, and more recently androgenetic alopecia, its use for photoaging is less well established. A systemic meta-analysis concluded modest clinical benefit of PRP for aging skin, mostly when used as an adjuvant following fractional and fully ablative laser resurfacing<sup>[143]</sup>.

Many of the treatments discussed above target photoaging-related stem cell exhaustion but do not directly restore the stem cell population that is depleted with aging. The direct delivery of stem cells to target tissue has been used in many models of regeneration. Transplanted epidermal stem cells exhibit



**Figure 1.** Interconnected hallmarks of aging in cutaneous photoaging

benefit the regeneration of epidermis lost in wound settings but have not been used for regeneration of intact photoaged epidermis<sup>[144]</sup>. Transplantation of an alternate cell population, that of adipose-derived mesenchymal stem cells, has benefit *in vivo* for both dermal and epidermal aging phenotypes<sup>[145]</sup>. The mechanism of action is unclear, but co-culture experiments demonstrate improved fibroblast synthetic function, suggesting the modification of the signaling milieu rather than direct stem cell regeneration<sup>[146]</sup>.

## CONCLUSION

Skin photoaging is associated with extensive functional and cosmetic changes. The classic hallmarks of systemic aging offer a framework for understanding the process of these changes on a molecular level [Figure 1]. In this framework, UV radiation works through ROS to induce primary changes in the nuclear genome, mitochondrial genome, epigenome and cell protein population. These primary changes lead to antagonistic changes in cell function, impaired mitochondrial function, dysregulated nutrient sensing and cellular senescence. Antagonistic changes result in altered cellular signaling and stem cell exhaustion, particularly increased low level NF- $\kappa$ B inflammatory signaling and decreased growth factor signaling, further amplified by changes in the ECM. Most current effective treatments for photoaging focus on the mitigation of ROS-associated damage through antioxidant supplementation and restoration of the intracellular signaling environment. Open areas for therapy include a focus on mitochondrial function and the process of nutrient sensing and clearance of senescent cells.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the review and performed data acquisition: Vandiver AR, Hogan SR

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

Both authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Yaar M, Eller MS, Gilchrist BA. Fifty years of skin aging. *J Invest Dermatol Symp Proc* 2002;7:51-8.
2. Ghadially R, Brown BE, Sequeira-Martin SM, Feingold KR, Elias PM. The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. *J Clin Invest* 1995;95:2281-90.
3. Rogers HW, Weinstock MA, Feldman SR, Coldiron BM. Incidence estimate of nonmelanoma skin cancer (keratinocyte carcinomas) in the U.S. population, 2012. *JAMA Dermatol* 2015;151:1081-6.
4. Sauder DN. Effect of age on epidermal immune function. *Dermatol Clin* 1986;4:447-54.
5. Gould L, Abadir P, Brem H, et al. Chronic wound repair and healing in older adults: current status and future research. *J Am Geriatr Soc* 2015;63:427-38.
6. Jones PH, Harper S, Watt FM. Stem cell patterning and fate in human epidermis. *Cell* 1995;80:83-93.
7. Taylor G, Lehrer MS, Jensen PJ, Sun T, Lavker RM. Involvement of follicular stem cells in forming not only the follicle but also the epidermis. *Cell* 2000;102:451-61.
8. Bhawan J, Andersen W, Lee J, Labadie R, Solares G. Photoaging versus intrinsic aging: a morphologic assessment of facial skin. *J Cutan Pathol* 1995;22:154-9.
9. Yaar M, Gilchrist BA. Skin aging: postulated mechanisms and consequent changes in structure and function. *Clin Geriatr Med* 2001;17:617-30, v.
10. Cinotti E, Bovi C, Tonini G, et al. Structural skin changes in elderly people investigated by reflectance confocal microscopy. *J Eur Acad Dermatol Venereol* 2020; doi: 10.1111/jdv.16466.
11. Bosset S, Bonnet-Duquennoy M, Barré P, et al. Photoageing shows histological features of chronic skin inflammation without clinical and molecular abnormalities. *Br J Dermatol* 2003;149:826-35.
12. Khorramizadeh MR, Tredget EE, Telasky C, Shen Q, Ghahary A. Aging differentially modulates the expression of collagen and collagenase in dermal fibroblasts. *Mol Cell Biochem* 1999;194:99-108.
13. Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ. Pathophysiology of premature skin aging induced by ultraviolet light. *N Engl J Med* 1997;337:1419-28.
14. Cho BA, Yoo SK, Seo JS. Signatures of photo-aging and intrinsic aging in skin were revealed by transcriptome network analysis. *Aging (Albany NY)* 2018;10:1609-26.
15. Lago JC, Puzzi MB. The effect of aging in primary human dermal fibroblasts. *PLoS One* 2019;14:e0219165.
16. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell* 2013;153:1194-217.
17. Spehar K, Pan A, Beerman I. Restoring aged stem cell functionality: Current progress and future directions. *Stem Cells* 2020;38:1060-77.
18. Besaratinia A, Synold TW, Chen HH, et al. DNA lesions induced by UV A1 and B radiation in human cells: comparative analyses in the overall genome and in the p53 tumor suppressor gene. *Proc Natl Acad Sci U S A* 2005;102:10058-63.

19. Kvam E, Tyrrell RM. Induction of oxidative DNA base damage in human skin cells by UV and near visible radiation. *Carcinogenesis* 1997;18:2379-84.
20. Pickering CR, Zhou JH, Lee JJ, et al. Mutational landscape of aggressive cutaneous squamous cell carcinoma. *Clin Cancer Res* 2014;20:6582-92.
21. Inman GJ, Wang J, Nagano A, et al. The genomic landscape of cutaneous SCC reveals drivers and a novel azathioprine associated mutational signature. *Nat Commun* 2018;9:3667.
22. Jayaraman SS, Rayhan DJ, Hazany S, Kolodney MS. Mutational landscape of basal cell carcinomas by whole-exome sequencing. *J Invest Dermatol* 2014;134:213-20.
23. Bonilla X, Parmentier L, King B, et al. Genomic analysis identifies new drivers and progression pathways in skin basal cell carcinoma. *Nat Genet* 2016;48:398-406.
24. Yang JH, Lee HC, Wei YH. Photoageing-associated mitochondrial DNA length mutations in human skin. *Arch Dermatol Res* 1995;287:641-8.
25. Berneburg M, Grether-Beck S, Kürten V, et al. Singlet oxygen mediates the UVA-induced generation of the photoaging-associated mitochondrial common deletion. *J Biol Chem* 1999;274:15345-9.
26. Krishnan KJ, Harbottle A, Birch-Machin MA. The use of a 3895 bp mitochondrial DNA deletion as a marker for sunlight exposure in human skin. *J Invest Dermatol* 2004;123:1020-4.
27. Krishnan KJ, Birch-Machin MA. The incidence of both tandem duplications and the common deletion in mtDNA from three distinct categories of sun-exposed human skin and in prolonged culture of fibroblasts. *J Invest Dermatol* 2006;126:408-15.
28. McKenzie D, Bua E, McKiernan S, Cao Z, Aiken JM, Wanagat J. Mitochondrial DNA deletion mutations: a causal role in sarcopenia. *Eur J Biochem* 2002;269:2010-5.
29. Sen P, Shah PP, Nativio R, Berger SL. Epigenetic mechanisms of longevity and aging. *Cell* 2016;166:822-39.
30. Vandiver AR, Irizarry RA, Hansen KD, et al. Age and sun exposure-related widespread genomic blocks of hypomethylation in nonmalignant skin. *Genome Biol* 2015;16:80.
31. Bormann F, Rodríguez-Paredes M, Hagemann S, et al. Reduced DNA methylation patterning and transcriptional connectivity define human skin aging. *Aging Cell* 2016;15:563-71.
32. Ding S, Chen J, Zeng Q, et al. Chronic sun exposure is associated with distinct histone acetylation changes in human skin. *Br J Dermatol* 2018;179:110-7.
33. Zhou X, Zhuang Z, Wang W, et al. OGG1 is essential in oxidative stress induced DNA demethylation. *Cell Signal* 2016;28:1163-71.
34. Huh I, Zeng J, Park T, Yi SV. DNA methylation and transcriptional noise. *Epigenetics Chromatin* 2013;6:9.
35. Cheung P, Vallania F, Warsinske HC, et al. Single-cell chromatin modification profiling reveals increased epigenetic variations with aging. *Cell* 2018;173:1385-97.e14.
36. Richter T, von Zglinicki T. A continuous correlation between oxidative stress and telomere shortening in fibroblasts. *Exp Gerontol* 2007;42:1039-42.
37. Armanios M, Alder JK, Parry EM, Karim B, Strong MA, Greider CW. Short telomeres are sufficient to cause the degenerative defects associated with aging. *Am J Hum Genet* 2009;85:823-32.
38. Boonekamp JJ, Simons MJ, Hemerik L, Verhulst S. Telomere length behaves as biomarker of somatic redundancy rather than biological age. *Aging Cell* 2013;12:330-2.
39. Butler MG, Tilburt J, Devries A, et al. Comparison of chromosome telomere integrity in multiple tissues from subjects at different ages. *Cancer Genetics and Cytogenetics* 1998;105:138-44.
40. Friedrich U, Griese E, Schwab M, Fritz P, Thon K, Klotz U. Telomere length in different tissues of elderly patients. *Mech Ageing Dev* 2000;119:89-99.
41. Sugimoto M, Yamashita R, Ueda M. Telomere length of the skin in association with chronological aging and photoaging. *J Dermatol Sci* 2006;43:43-7.
42. Taylor RS, Ramirez RD, Ogoshi M, Chaffins M, Piatyszek MA, Shay JW. Detection of telomerase activity in malignant and nonmalignant skin conditions. *J Invest Dermatol* 1996;106:759-65.
43. Kronic D, Moshir S, Greulich-Bode KM, et al. Tissue context-activated telomerase in human epidermis correlates with little age-dependent telomere loss. *Biochim Biophys Acta* 2009;1792:297-308.
44. Härle-Bachor C, Boukamp P. Telomerase activity in the regenerative basal layer of the epidermis in human skin and in immortal and carcinoma-derived skin keratinocytes. *Proc Natl Acad Sci U S A* 1996;93:6476-81.
45. Klaips CL, Jayaraj GG, Hartl FU. Pathways of cellular proteostasis in aging and disease. *J Cell Biol* 2018;217:51-63.
46. Sander CS, Chang H, Salzmann S, et al. Photoaging is associated with protein oxidation in human skin in vivo. *J Invest Dermatol* 2002;118:618-25.
47. Petropoulos I, Conconi M, Wang X, et al. Increase of oxidatively modified protein is associated with a decrease of proteasome activity and content in aging epidermal cells. *J Gerontol A Biol Sci Med Sci* 2000;55:B220-7.
48. Bulteau AL, Petropoulos I, Friguet B. Age-related alterations of proteasome structure and function in aging epidermis. *Exp Gerontol* 2000;35:767-77.
49. Chen L, Hu JY, Wang SQ. The role of antioxidants in photoprotection: a critical review. *J Am Acad Dermatol* 2012;67:1013-24.
50. Choi YJ. Shedding light on the effects of calorie restriction and its mimetics on skin biology. *Nutrients* 2020;12:E1529.
51. Singh B, Chatterjee A, Ronghe AM, Bhat NK, Bhat HK. Antioxidant-mediated up-regulation of OGG1 via NRF2 induction is associated with inhibition of oxidative DNA damage in estrogen-induced breast cancer. *BMC Cancer* 2013;13:253.

52. Kwak MK, Wakabayashi N, Greenlaw JL, Yamamoto M, Kensler TW. Antioxidants enhance mammalian proteasome expression through the Keap1-Nrf2 signaling pathway. *Mol Cell Biol* 2003;23:8786-94.
53. Wu Y, Zheng X, Xu XG, et al. Protective effects of a topical antioxidant complex containing vitamins C and E and ferulic acid against ultraviolet irradiation-induced photodamage in Chinese women. *J Drugs Dermatol* 2013;12:464-8.
54. Darr D, Dunston S, Faust H, Pinnell S. Effectiveness of antioxidants (vitamin C and E) with and without sunscreens as topical photoprotectants. *Acta Derm Venereol* 1996;76:264-8.
55. Elmets CA, Singh D, Tubesing K, Matsui M, Katiyar S, Mukhtar H. Cutaneous photoprotection from ultraviolet injury by green tea polyphenols. *J Am Acad Dermatol* 2001;44:425-32.
56. Afaq F, Adhami VM, Ahmad N. Prevention of short-term ultraviolet B radiation-mediated damages by resveratrol in SKH-1 hairless mice. *Toxicol Appl Pharmacol* 2003;186:28-37.
57. Parrado C, Philips N, Gilaberte Y, Juarranz A, González S. Oral photoprotection: effective agents and potential candidates. *Front Med (Lausanne)* 2018;5:188.
58. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Sao Paulo Med J* 2015;133:164-5.
59. Chung JH, Youn SH, Kwon OS, Cho KH, Youn JI, Eun HC. Regulations of collagen synthesis by ascorbic acid, transforming growth factor-beta and interferon-gamma in human dermal fibroblasts cultured in three-dimensional collagen gel are photoaging- and aging-independent. *J Dermatol Sci* 1997;15:188-200.
60. Orringer JS, Hammerberg C, Hamilton T, et al. Molecular effects of photodynamic therapy for photoaging. *Arch Dermatol* 2008;144:1296-302.
61. Manuskiatti W, Pattanaprichakul P, Inthasotti S, et al. Thermal response of in vivo human skin to fractional radiofrequency microneedle device. *Biomed Res Int* 2016;2016:6939018.
62. Sajjadi AY, Mitra K, Grace M. Expression of heat shock proteins 70 and 47 in tissues following short-pulse laser irradiation: assessment of thermal damage and healing. *Med Eng Phys* 2013;35:1406-14.
63. Mackanos MA, Contag CH. Pulse duration determines levels of Hsp70 induction in tissues following laser irradiation. *J Biomed Opt* 2011;16:078002.
64. McBride HM, Neuspiel M, Wasiak S. Mitochondria: more than just a powerhouse. *Curr Biol* 2006;16:R551-60.
65. Mellem D, Sattler M, Pagel-Wolff S, et al. Fragmentation of the mitochondrial network in skin in vivo. *PLoS One* 2017;12:e0174469.
66. Prah S, Kueper T, Biernoth T, et al. Aging skin is functionally anaerobic: importance of coenzyme Q10 for anti aging skin care. *Biofactors* 2008;32:245-55.
67. Hamanaka RB, Glasauer A, Hoover P, et al. Mitochondrial reactive oxygen species promote epidermal differentiation and hair follicle development. *Sci Signal* 2013;6:ra8.
68. Velarde MC, Flynn JM, Day NU, Melov S, Campisi J. Mitochondrial oxidative stress caused by Sod2 deficiency promotes cellular senescence and aging phenotypes in the skin. *Aging (Albany NY)* 2012;4:3-12.
69. Singh B, Schoeb TR, Bajpai P, Slominski A, Singh KK. Reversing wrinkled skin and hair loss in mice by restoring mitochondrial function. *Cell Death Dis* 2018;9:735.
70. Kenyon, CJ. The genetics of ageing. *Nature* 2010;464:504-12.
71. Barzilai N, Huffman DM, Muzumdar RH, Bartke A. The critical role of metabolic pathways in aging. *Diabetes* 2012;61:1315-22.
72. Choi YJ, Moon KM, Chung KW, et al. The underlying mechanism of proinflammatory NF- $\kappa$ B activation by the mTORC2/Akt/IKK $\alpha$  pathway during skin aging. *Oncotarget* 2016;7:52685-94.
73. Wu CL, Qiang L, Han W, Ming M, Viollet B, He YY. Role of AMPK in UVB-induced DNA damage repair and growth control. *Oncogene* 2013;32:2682-9.
74. Zhao P, Sui BD, Liu N, et al. Anti-aging pharmacology in cutaneous wound healing: effects of metformin, resveratrol, and rapamycin by local application. *Aging Cell* 2017;16:1083-93.
75. Golubtsova NN, Filippov FN, Gunin AG. Age-related changes in the content of sirtuin 1 in fibroblasts of human dermis. *Adv Gerontol* 2017;30:375-80.
76. Kalfalah F, Sobek S, Bornholz B, et al. Inadequate mito-biogenesis in primary dermal fibroblasts from old humans is associated with impairment of PGC1A-independent stimulation. *Exp Gerontol* 2014;56:59-68.
77. Kuehne A, Hildebrand J, Soehle J, et al. An integrative metabolomics and transcriptomics study to identify metabolic alterations in aged skin of humans in vivo. *BMC Genomics* 2017;18:169.
78. Randhawa M, Sangar V, Tucker-Samaras S, Southall M. Metabolic signature of sun exposed skin suggests catabolic pathway overweighs anabolic pathway. *PLoS One* 2014;9:e90367.
79. Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. *PLoS Biol* 2008;6:2853-68.
80. Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J. Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc Natl Acad Sci U S A* 2001;98:12072-7.
81. Ressler S, Bartkova J, Niederegger H, et al. p16INK4A is a robust in vivo biomarker of cellular aging in human skin. *Aging Cell* 2006;5:379-89.
82. Castilho RM, Squarize CH, Chodosh LA, Williams BO, Gutkind JS. mTOR mediates Wnt-induced epidermal stem cell exhaustion and aging. *Cell Stem Cell* 2009;5:279-89.
83. Malaquin N, Vercamer C, Bouali F, et al. Senescent fibroblasts enhance early skin carcinogenic events via a paracrine MMP-PAR-1 axis.



- PLoS One* 2013;8:e63607.
84. Weinmüller R, Zbiral B, Becirovic A, et al. Organotypic human skin culture models constructed with senescent fibroblasts show hallmarks of skin aging. *NPJ Aging Mech Dis* 2020;6:4.
  85. Spindler M, Beal MF, Henchcliffe C. Coenzyme Q10 effects in neurodegenerative disease. *Neuropsychiatr Dis Treat* 2009;5:597-610.
  86. Luo K, Yu JH, Quan Y, et al. Therapeutic potential of coenzyme Q<sub>10</sub> in mitochondrial dysfunction during tacrolimus-induced beta cell injury. *Sci Rep* 2019;9:7995.
  87. Knott A, Achterberg V, Smuda C, et al. Topical treatment with coenzyme Q10-containing formulas improves skin's Q10 level and provides antioxidative effects. *Biofactors* 2015;41:383-90.
  88. Tan CL, Chin T, Tan CYR, Rovito HA, Quek LS, et al. Nicotinamide metabolism modulates the proliferation/differentiation balance and senescence of human primary keratinocytes. *J Invest Dermatol* 2019;139:1638-47.e3.
  89. Kang HT, Hwang ES. Nicotinamide enhances mitochondria quality through autophagy activation in human cells. *Aging Cell* 2009;8:426-38.
  90. Beher D, Wu J, Cumine S, et al. Resveratrol is not a direct activator of SIRT1 enzyme activity. *Chem Biol Drug Des* 2009;74:619-24.
  91. Cantó C, Gerhart-Hines Z, Feige JN, et al. AMPK regulates energy expenditure by modulating NAD<sup>+</sup> metabolism and SIRT1 activity. *Nature* 2009;458:1056-60.
  92. Um JH, Park SJ, Kang H, et al. AMP-activated protein kinase-deficient mice are resistant to the metabolic effects of resveratrol. *Diabetes* 2010;59:554-63.
  93. Baur JA, Pearson KJ, Price NL, et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 2006;444:337-42.
  94. Csiszar A, Labinskyy N, Pinto JT, et al. Resveratrol induces mitochondrial biogenesis in endothelial cells. *Am J Physiol Heart Circ Physiol* 2009;297:H13-20.
  95. Hung CF, Lin YK, Huang ZR, Fang JY. Delivery of resveratrol, a red wine polyphenol, from solutions and hydrogels via the skin. *Biol Pharm Bull* 2008;31:955-62.
  96. Farris P, Krutmann J, Li YH, McDaniel D, Krol Y. Resveratrol: a unique antioxidant offering a multi-mechanistic approach for treating aging skin. *J Drugs Dermatol* 2013;12:1389-94.
  97. Dierickx CC, Anderson RR. Visible light treatment of photoaging. *Dermatol Ther* 2005;18:191-208.
  98. Karu TI, Pyatibrat LV, Kolyakov SF, Afanasyeva NI. Absorption measurements of a cell monolayer relevant to phototherapy: reduction of cytochrome c oxidase under near IR radiation. *J Photochem Photobiol B* 2005;81:98-106.
  99. Karu T, Pyatibrat L, Kalendo G. Irradiation with He-Ne laser increases ATP level in cells cultivated in vitro. *J Photochem Photobiol B* 1995;27:219-23.
  100. Greco M, Guida G, Perlino E, Marra E, Quagliarile E. Increase in RNA and protein synthesis by mitochondria irradiated with helium-neon laser. *Biochem Biophys Res Commun* 1989;163:1428-34.
  101. Yu W, Naim JO, Lanzafame RJ. The effect of laser irradiation on the release of bFGF from 3T3 fibroblasts. *Photochem Photobiol* 1994;59:167-70.
  102. Barolet D, Roberge CJ, Auger FA, Boucher A, Germain L. Regulation of skin collagen metabolism in vitro using a pulsed 660 nm LED light source: clinical correlation with a single-blinded study. *J Invest Dermatol* 2009;129:2751-9.
  103. Sadick NS. A study to determine the efficacy of a novel handheld light-emitting diode device in the treatment of photoaged skin. *J Cosmet Dermatol* 2008;7:263-7.
  104. Russell BA, Kellett N, Reilly LR. A study to determine the efficacy of combination LED light therapy (633 nm and 830 nm) in facial skin rejuvenation. *J Cosmet Laser Ther* 2005;7:196-200.
  105. Soydas T, Yaprak Sarac E, Cinar S, et al. The protective effects of metformin in an in vitro model of aging 3T3 fibroblast under the high glucose conditions. *J Physiol Biochem* 2018;74:273-81.
  106. Rostamkalaei SS, Akbari J, Saeedi M, Morteza-Semnani K, Nokhodchi A. Topical gel of metformin solid lipid nanoparticles: a hopeful promise as a dermal delivery system. *Colloids Surf B Biointerfaces* 2019;175:150-7.
  107. Chung CL, Lawrence I, Hoffman M, et al. Topical rapamycin reduces markers of senescence and aging in human skin: an exploratory, prospective, randomized trial. *Geroscience* 2019;41:861-9.
  108. Blagosklonny MV. Rapamycin for longevity: opinion article. *Aging (Albany NY)* 2019;11:8048-67.
  109. Baker DJ, Wijshake T, Tchkonja T, et al. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. *Nature* 2011;479:232-6.
  110. Baker DJ, Childs BG, Durik M, et al. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature* 2016;530:184-9.
  111. Wang Y, Chang J, Liu X, Zhang X, Zhang S, Zhang X, Zhou D, Zheng G. Discovery of piperlongumine as a potential novel lead for the development of senolytic agents. *Aging (Albany NY)* 2016;8:2915-26.
  112. Zhu Y, Tchkonja T, Fuhrmann-Stroissnigg H, et al. Identification of a novel senolytic agent, navitoclax, targeting the Bcl-2 family of anti-apoptotic factors. *Aging Cell* 2016;15:428-35.
  113. Zhu Y, Tchkonja T, Pirtskhalava T, Gower AC, Ding H, et al. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell* 2015;14:644-58.
  114. Toutfaire M, Bauwens E, Debacq-Chainiaux F. The impact of cellular senescence in skin ageing: A notion of mosaic and therapeutic strategies. *Biochem Pharmacol* 2017;142:1-12.
  115. Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF- $\kappa$ B activity. *Annu Rev Immunol* 2000;18:621-63.
  116. Adler AS, Sinha S, Kawahara TL, Zhang JY, Segal E, et al. Motif module map reveals enforcement of aging by continual NF- $\kappa$ B activity. *Genes Dev* 2007;21:3244-57.

117. Han KH, Choi HR, Won CH, et al. Alteration of the TGF-beta/SMAD pathway in intrinsically and UV-induced skin aging. *Mech Ageing Dev* 2005;126:560-7.
118. Muffler S, Stark HJ, Amoros M, et al. A stable niche supports long-term maintenance of human epidermal stem cells in organotypic cultures. *Stem Cells* 2008;26:2506-15.
119. Giangreco A, Qin M, Pintar JE, Watt FM. Epidermal stem cells are retained in vivo throughout skin aging. *Aging Cell* 2008;7:250-9.
120. Rittié L, Stoll SW, Kang S, Voorhees JJ, Fisher GJ. Hedgehog signaling maintains hair follicle stem cell phenotype in young and aged human skin. *Aging Cell* 2009;8:738-51.
121. Doles J, Storer M, Cozzuto L, Roma G, Keyes WM. Age-associated inflammation inhibits epidermal stem cell function. *Genes Dev* 2012;26:2144-53.
122. Giangreco A, Goldie SJ, Failla V, Saintigny G, Watt FM. Human skin aging is associated with reduced expression of the stem cell markers beta1 integrin and MCSP. *J Invest Dermatol* 2010;130:604-8.
123. Iriyama S, Yasuda M, Nishikawa S, Takai E, Hosoi J, Amano S. Decrease of laminin-511 in the basement membrane due to photoaging reduces epidermal stem/progenitor cells. *Sci Rep* 2020;10:12592.
124. Kwon OS, Yoo HG, Han JH, Lee SR, Chung JH, et al. Photoaging-associated changes in epidermal proliferative cell fractions in vivo. *Arch Dermatol Res* 2008;300:47-52.
125. Gannon HS, Donehower LA, Lyle S, Jones SN. Mdm2-p53 signaling regulates epidermal stem cell senescence and premature aging phenotypes in mouse skin. *Dev Biol* 2011;353:1-9.
126. Fitzpatrick RE, Rostan EF. Reversal of photodamage with topical growth factors: a pilot study. *J Cosmet Laser Ther* 2003;5:25-34.
127. Ehrlich M, Rao J, Pabby A, Goldman MP. Improvement in the appearance of wrinkles with topical transforming growth factor beta(1) and l-ascorbic acid. *Dermatol Surg* 2006;32:618-25.
128. Gold MH, Goldman MP, Biron J. Efficacy of novel skin cream containing mixture of human growth factors and cytokines for skin rejuvenation. *J Drugs Dermatol* 2007;6:197-201.
129. Bos JD, Meinardi MM. The 500 Dalton rule for the skin penetration of chemical compounds and drugs. *Exp Dermatol* 2000;9:165-9.
130. Griffiths CE, Russman AN, Majmudar G, Singer RS, Hamilton TA, Voorhees JJ. Restoration of collagen formation in photodamaged human skin by tretinoin (retinoic acid). *N Engl J Med* 1993;329:530-5.
131. Weinstein GD, Nigra TP, Pochi PE, et al. Topical tretinoin for treatment of photodamaged skin. A multicenter study. *Arch Dermatol* 1991;127:659-65.
132. Fan Y, Choi TH, Chung JH, Jeon YK, Kim S. Hyaluronic acid-cross-linked filler stimulates collagen type 1 and elastic fiber synthesis in skin through the TGF-β/Smad signaling pathway in a nude mouse model. *J Plast Reconstr Aesthet Surg* 2019;72:1355-62.
133. Quan T, Wang F, Shao Y, et al. Enhancing structural support of the dermal microenvironment activates fibroblasts, endothelial cells, and keratinocytes in aged human skin in vivo. *J Invest Dermatol* 2013;133:658-67.
134. Courderot-Masuyer C, Robin S, Tauzin H, Humbert P. Evaluation of lifting and antiwrinkle effects of calcium hydroxylapatite filler. In vitro quantification of contractile forces of human wrinkle and normal aged fibroblasts treated with calcium hydroxylapatite. *J Cosmet Dermatol* 2016;15:260-8.
135. Shin JJ, Park TJ, Kim BY, et al. Comparative effects of various absorbable threads in a rat model. *J Cosmet Laser Ther* 2019;21:158-62.
136. Yoon JH, Kim SS, Oh SM, Kim BC, Jung W. Tissue changes over time after polydioxanone thread insertion: An animal study with pigs. *J Cosmet Dermatol* 2019;18:885-91.
137. Freedman BM, Rueda-Pedraza E, Waddell SP. The epidermal and dermal changes associated with microdermabrasion. *Dermatol Surg* 2001;27:1031-3;1033-4.
138. Abdel-Motaleb AA, Abu-Dief EE, Hussein MR. Dermal morphological changes following salicylic acid peeling and microdermabrasion. *J Cosmet Dermatol* 2017;16:e9-14.
139. Rosenberg GJ, Brito MA Jr, Aportella R, Kapoor S. Long-term histologic effects of the CO2 laser. *Plast Reconstr Surg* 1999;104:2239-44;2245-6.
140. Kim D, Chen R, Sheu M, et al. Noncoding dsRNA induces retinoic acid synthesis to stimulate hair follicle regeneration via TLR3. *Nat Commun* 2019;10:2811.
141. Macaulay IC, Carr P, Gusnanto A, Ouwehand WH, Fitzgerald D, Watkins NA. Platelet genomics and proteomics in human health and disease. *J Clin Invest* 2005;115:3370-7.
142. Pavlovic V, Ciric M, Jovanovic V, Stojanovic P. Platelet Rich Plasma: a short overview of certain bioactive components. *Open Med (Wars)* 2016;11:242-7.
143. Maisel-Campbell AL, Ismail A, Reynolds KA, et al. A systematic review of the safety and effectiveness of platelet-rich plasma (PRP) for skin aging. *Arch Dermatol Res* 2020;312:301-15.
144. Yang R, Liu F, Wang J, Chen X, Xie J, Xiong K. Epidermal stem cells in wound healing and their clinical applications. *Stem Cell Res Ther* 2019;10:229.
145. Charles-de-Sá L, Gontijo-de-Amorim NF, Rigotti G, et al. Photoaged skin therapy with adipose-derived stem cells. *Plast Reconstr Surg* 2020;145:1037e-49.
146. Son WC, Yun JW, Kim BH. Adipose-derived mesenchymal stem cells reduce MMP-1 expression in UV-irradiated human dermal fibroblasts: therapeutic potential in skin wrinkling. *Biosci Biotechnol Biochem* 2015;79:919-25.

Review

Open Access



# Microsurgical salvage of complex dorsal shearing injuries of the hand and wrist

Praveen G. Murthy<sup>1,2,\*</sup>, Adam B. Strohl<sup>1,2,3,\*</sup>

<sup>1</sup>Philadelphia Hand to Shoulder Center, Philadelphia, PA 19107, USA.

<sup>2</sup>Department of Orthopaedics, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA.

<sup>3</sup>Department of General Surgery - Plastic Surgery, Thomas Jefferson University Hospital, Philadelphia, PA 19107, USA.

\*Both authors contributed equally to the article.

**Correspondence to:** Dr. Adam B. Strohl, Philadelphia Hand to Shoulder Center, 834 Chestnut Street Suite G-114, Philadelphia, PA 19107, USA. E-mail: abstrohl@handcenters.com

**How to cite this article:** Murthy PG, Strohl AB. Microsurgical salvage of complex dorsal shearing injuries of the hand and wrist. *Plast Aesthet Res* 2020;7:64. <http://dx.doi.org/10.20517/2347-9264.2020.72>

**Received:** 12 Apr 2020 **First Decision:** 9 Sep 2020 **Revised:** 24 Sep 2020 **Accepted:** 23 Oct 2020 **Published:** 13 Nov 2020

**Academic Editor:** Alessandro Thione **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Dorsal shearing injuries of the hand and wrist can be seen with high-energy motor vehicle accidents and present challenging problems to the reconstructive surgeon. At the time of initial injury, care must be taken to adequately debride the mangled extremity and extensive wounds. Following debridement, a series of decisions must be made regarding bony stabilization, extensor tendon reconstruction, and soft tissue coverage. These reconstructions often require staged procedures, and appropriate planning is warranted from the start. Reasonable function of the hand can be expected from the patient following such injuries.

**Keywords:** Dorsal shear, hand, wrist, trauma, extensor, free flap, mangle

## INTRODUCTION

Mangle injuries of the upper extremity are devastating injuries involving a combination of multiple tissue layers and structures that are critical for hand function. Among these, traumatic dorsal shearing injuries of the hand and wrist pose unique challenges with regards to soft tissue and bony reconstruction<sup>[1,2]</sup>.

Dorsal shearing injuries generally involve the skin and soft tissue envelope covering the dorsal hand and wrist, the extensor tendons, and often the bony structures that comprise the many complex articulations of



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



the wrist and hand. Such injuries can lead to significant problems with bony stability and extensor tendon function.

There is limited information in the current literature to guide management of complex dorsal hand and wrist injuries. In this article, we discuss the challenges associated with treating these injuries, provide an algorithm for management, and review a series of representative cases presenting to our tertiary referral center.

## INITIAL EVALUATION

Dorsal shearing hand injuries are often associated with high energy trauma. It is imperative first to evaluate the patient using standard advanced trauma life support (ATLS) protocol with our trauma colleagues, including complete primary and secondary surveys, prior to addressing the more obvious injury to the hand. If and when the patient is deemed stable from a systemic perspective, focused evaluation of the hand is appropriate.

A thorough history is obtained if possible, including the timing of injury; where the injury occurred, with particular attention to the degree of contamination of the wound; and the mechanism of injury. Such details provide important information regarding the zone of injury and the quality of the underlying soft tissues for subsequent repair or reconstruction. Gross contamination can lead to infection and doom any achievements in reconstruction. Associated crush forces and thermal burns can be expected to cause secondary injuries in the time period following injury, thereby expanding the zone of obvious injury.

While detailed examination of the injured extremity in the emergency department is often difficult, a preliminary evaluation should focus primarily on two questions: (1) Are the fingers vascularized? (2) Are the fingers sensate?

Dorsal shearing injuries, by definition, spare the critical vascular and nervous structures that travel on the volar aspect of the hand and wrist. Unlike with many mangling injuries to the hand, microsurgical repair of vessels and nerves is generally not required to restore functionality in these cases. Once the patient is deemed to have a dorsal shearing injury with a vascularized and sensate hand, the goals of treatment become simplified. Formal assessment of the degree injury to each of the dorsal tissue layers may be performed in the operating room.

Preoperative radiographic examination of the forearm, wrist, and hand is essential to determine the nature of the skeletal injury and prompt fixation planning. The addition of embedded hardware with likely exposure within the wound will prompt additional coverage concerns. Any open fractures identified by exam and/or radiographs should be treated with prompt administration of broad-spectrum intravenous antibiotics.

The goals of management of complex dorsal hand injuries are as follows: (1) debridement; (2) bony stabilization; (3) stable soft tissue coverage; and (4) extensor tendon reconstruction.

## DEBRIDEMENT

Thorough, careful excisional debridement is perhaps the most important step in ensuring a favorable result<sup>[1]</sup>. Wound infection after mangling injuries can be devastating and can negate any benefit gained by surgical reconstruction. As such, any reconstructive procedures should be delayed until the wound bed is clean and free of any devitalized tissue. If the patient is hemodynamically stable, debridement can be done without tourniquet to allow for visualizing of bleeding surfaces, signifying viability. No attempt should be



**Figure 1.** Negative pressure wound therapy can be used despite difficult-to-seal webspaces with the use of a sterile surgical glove

made at this step to minimize the wound dimensions and/or depth. This often requires multiple trips to the operating room for serial debridement, particularly in the case of farming injuries or motor vehicle accidents with significant environmental debris, as the extent of contamination and devitalization may not be immediately evident at the index procedure.

If serial debridement is deemed necessary, provisional skeletal stabilization can be achieved via external fixation. Negative pressure wound therapy is also beneficial when definitive wound coverage must be delayed. We use a “glove technique” [Figure 1] in order to facilitate vacuum-assisted closure in the setting of a complex and irregular soft tissue injury of the hand. A sterile, surgical glove can be placed over the sponge dressing and then sealed at the wrist level before applying the negative pressure via hose. This technique is ideal for “sealing” the webspaces from subsequent leak in the interoperative periods.

## BONY STABILIZATION

Once the wound bed is deemed appropriate to proceed with reconstruction, the first goal of management is skeletal stabilization. Internal fixation of fractures may be pursued where able; however, dorsal shearing injuries of the wrist and hand can be associated with significant bone loss and comminuted articular fracture dislocations that are not amenable to anatomic reduction and stable internal fixation.

External fixation or spanning bridge plate technique may be used for definitive stabilization when applicable, as in cases with high energy distal radius fractures or radiocarpal dislocations with intact carpal bones. When using an external fixator, hardware should be placed as far from the zone of injury as possible without compromising the stability of the construct. One should be mindful of causing additional injury to nerves and to vessels that may allow for later anastomosis with free tissue transfer. Percutaneous wire fixation can be used but may lack the necessary stability in the setting of significant comminution and/or bone loss. In cases with significant bone loss and/or complex articular fracture dislocations involving multiple articulations in the wrist and hand, acute total wrist arthrodesis may be the most reliable solution.

If the metacarpophalangeal joints have been destroyed, reconstruction with arthroplasties can be considered. A cement spacer can be used to maintain the joint space if immediate arthroplasty is not performed in the acute setting. The metacarpophalangeal joint has been successfully reconstructed with the transfer of a vascularized toe joint<sup>[3,4]</sup>. Reconstruction of multiple joints may limit this option in such dorsal shearing injuries.



## SOFT TISSUE COVERAGE

In the context of complex dorsal shearing injuries with extensor tendon disruption, the restoration of a gliding bed for reconstructed extensor tendons is critical. Vascularized soft tissue coverage is imperative in these cases, as non-vascularized skin grafts and skin substitutes will invariably result in extensor tendon adhesions. Several options for vascularized soft tissue coverage of the dorsal hand have been described in the literature, including pedicled and free fasciocutaneous flaps<sup>[5-9]</sup>, fascial flaps<sup>[10,11]</sup>, and muscle flaps<sup>[12-15]</sup>.

Pedicled fasciocutaneous flaps, including distally-based reverse radial<sup>[6]</sup> and ulnar artery<sup>[7]</sup> flaps, are technically less demanding than free flaps, but they sacrifice a major supply of blood flow to an already comprised area, and may be insufficient for coverage of larger defects. The reverse posterior interosseous artery flap<sup>[8]</sup> is similarly limited in its dimensions. Additionally, perforator-based fasciocutaneous flaps such as the radial artery perforator flap negate the sacrifice of a major artery to the hand but have a higher complication rate and are limited to 8 cm × 10 cm dimension<sup>[16,17]</sup>.

Several free fasciocutaneous flaps have been described with a common favorite being the free anterolateral thigh flap<sup>[9]</sup>. This is an ideal option for coverage of large dorsal hand defects. In particular, we recommend utilizing a fasciocutaneous flap when a secondary operation requiring flap elevation is likely, as in the case of delayed bone, joint, and/or tendon reconstruction.

Various muscle flaps are also well-suited for dorsal hand coverage, including the latissimus dorsi, rectus abdominus, and serratus anterior<sup>[12-15]</sup>. A muscle flap carries the advantage of more robust vascularity, but it can create a less favorable environment for reoperation. In our experience, healed muscle flaps can be more difficult to elevate than fasciocutaneous flaps in this area. As such, a muscle flap is more ideal when secondary surgery is not anticipated, or significant dead space is created by bone loss. Staged tendon reconstruction with silastic tendon implants can be performed without flap elevation and is not a contraindication to utilizing a muscle flap.

More recently, several authors have described single-stage reconstruction of dorsal hand defects with composite cutaneotendinous flaps, including the dorsalis pedis cutaneotendinous flap<sup>[18]</sup> and the composite anterolateral thigh flap taken with fascia lata<sup>[19-21]</sup>. Composite flaps including bone have also been described, such as the free serratus anterior fascial flap with vascularized scapula for combined soft tissue and bony defects of the dorsal hand<sup>[22]</sup>. The primary disadvantage of composite flaps, particularly the cutaneotendinous dorsalis pedis flap or anterolateral thigh fascia lata flap, is the chance of considerable donor site morbidity. Additionally, a considerable amount of planning is needed to ensure correct orientation, placement, and size of the chimeric flap. Reconstructive surgeons around the world have also had success with “successive flap” or “daisy-chain” reconstruction, utilizing multiple free vascularized flaps connected via flow-through anastomosis at the terminal end<sup>[23]</sup>. In this fashion, dorsal soft tissue coverage could be supplied by previously described flaps and could then be connected to a free vascularized toe joint.

## EXTENSOR TENDON RECONSTRUCTION

The primary challenge in extensor tendon reconstruction in the context of complex dorsal shearing injuries is the loss of a gliding plane for tendon excursion. One critical element of restoring a gliding plane involves the introduction of vascularized soft tissue coverage, as detailed in the previous section. The second element involves restoration of a tendon pseudosheath. While primary extensor tendon reconstruction in dorsal hand injuries has been described, the results have been mixed<sup>[24,25]</sup>. We prefer staged extensor tendon reconstruction using silicone tendon implants (often referred to somewhat inaccurately as “rods”), in order to ensure that tendon grafting is ultimately performed in a healthy wound bed that will allow for optimal tendon gliding.

Staged tendon reconstruction using a silicone implant was first described by Hunter in 1965<sup>[26]</sup>. His technique is well-known and commonly used for staged flexor tendon reconstruction<sup>[27]</sup>, although, in fact, the first case demonstrating the utility of a silastic implant for tendon surgery was performed in 1960 and involved reconstruction of the extensor tendon to the index finger<sup>[26]</sup>.

The surgical technique is relatively straightforward<sup>[27]</sup>. In the first stage, silicone tendon implants are placed beneath the flap and secured to the extensor tendon stumps distally using 4-0 Prolene suture, outside of the zone of injury. To reconstruct four tendons, two such implants can be passed and split into a Y configuration distally to attach to two extensor tendons each or four separate units can be used. The proximal ends of the implants are trimmed at the appropriate level and left freestanding in a subcutaneous pocket in the distal forearm.

In the second stage, tendon grafts are harvested, secured to the proximal ends of the silicone implants, and then passed from proximal to distal through the new pseudosheath by pulling the implants distally. To reconstruct four tendons, two tendon grafts are harvested, passed, and similarly split into a Y configuration distally. The distal junctures are performed via Pulvertaft weave using non-absorbable suture. Proximally, the tendon grafts may be powered either by the extensor digitorum communis if sufficient proximal tendon remains, or by the flexor carpi radialis via tendon transfer. The proximal tendon juncture is performed similarly via Pulvertaft weave. The overlying flap is then sutured back into place, and early short-arc extensor tendon transfer/reconstruction protocols are begun postoperatively.

If staged reconstruction is not chosen, one could consider immediate tendon transfer for restoration of extensor function. Free tendon donor grafts can be used as immediate interposition grafts to restore anatomical continuity or in conjunction with tendon transfers such as flexor carpi ulnaris or flexor carpi radialis, especially if the wrist has been fused. A side-to-side tenodesis of injured extensor slips to adjacent non-injured slips can be effective to restore finger extension. A potential pitfall of this technique is adhesions of the tendon grafts or transfers beneath the healing flap. Finally, one may consider tenodesis of the distal extensor tendons such as extensor pollicis longus (EPL) or extensor digitorum communis to the metacarpal or radius for passive opening of the hand, as described in the spinal cord and brachial plexus populations.

### **Postoperative rehabilitation**

Preserving motion throughout the phases is paramount to ultimate success of the reconstruction. Joints that are not directly injured cannot be neglected during the perioperative periods or else additional therapy, and even surgery, may be indicated to address stiffness. We advocate passive range of motion of uninvolved joints until active modalities can be resumed or restored by reconstruction. This is particularly important in the week or so immediately after flap reconstruction.

Once the soft tissue reconstruction is stable, we begin formal hand therapy, typically as early as Week 2, including such passive range of motion and appropriate splinting. Digits that are not directly involved can begin active range of motion and joint mobilization therapies. Joint arthroplasties are typically splinted in extension for four weeks while the proximal interphalangeal and distal interphalangeal joints can receive passive and/or active range of motion exercises as well as tendon glides, as indicated by the extensor status. Therapists can also focus on edema control and scar management throughout maturation of the reconstruction. For those patients requiring second stage extensor reconstruction, we perform this no sooner than eight weeks after the first stage with stable equilibrium of the soft tissue envelope.



**Figure 2.** Following debridement, extensive destruction of the soft tissue envelope, extensor tendons (extensor digitorum communis, extensor indicis, and extensor digiti minimi), and metacarpophalangeal joints is noted

## CASE EXAMPLES

### Case 1

A 19-year-old male was transferred to our trauma center after a motor vehicle accident. He was an unrestrained driver and was ejected from the car, sustaining multiple injuries including a dorsal shearing injury of the left hand. Focused examination of the extremity revealed that the fingers were well-perfused and sensate. He was able to flex but unable to extend the fingers except the thumb.

He was taken to the operating room for exploration and debridement [Figure 2]. He was found to have significant skin and soft tissue loss; segmental loss of the extensor tendons to all fingers and laceration of the wrist extensors; open arthrotomies of the midcarpal and carpometacarpal joints; open fracture dislocations of the metacarpophalangeal (MP) joints of the index through small fingers; open arthrotomy long finger proximal interphalangeal joint; and dorsal radial sensory nerve loss.

At the index procedure, he underwent thorough excisional debridement and vacuum-assisted closure. He returned to the operating room every 2-3 days for seven subsequent debridements. After this time, the wound bed was deemed appropriate for definitive soft tissue coverage. He underwent free anterolateral thigh flap from the right thigh to the left hand and wrist, along with placement of antibiotic cement spacers in the MP joints of the index through small fingers [Figure 3]. Fasciocutaneous flap was used in this case to allow ease of lifting the flap for the planned, secondary procedures.

Four months later, he underwent silastic MP arthroplasty of the index through small fingers and first stage extensor tendon reconstruction of index through long fingers with placement of silastic tendon implants (Wright Medical Technology, Memphis, TN), as described above [Figure 4]. Three months after this, he

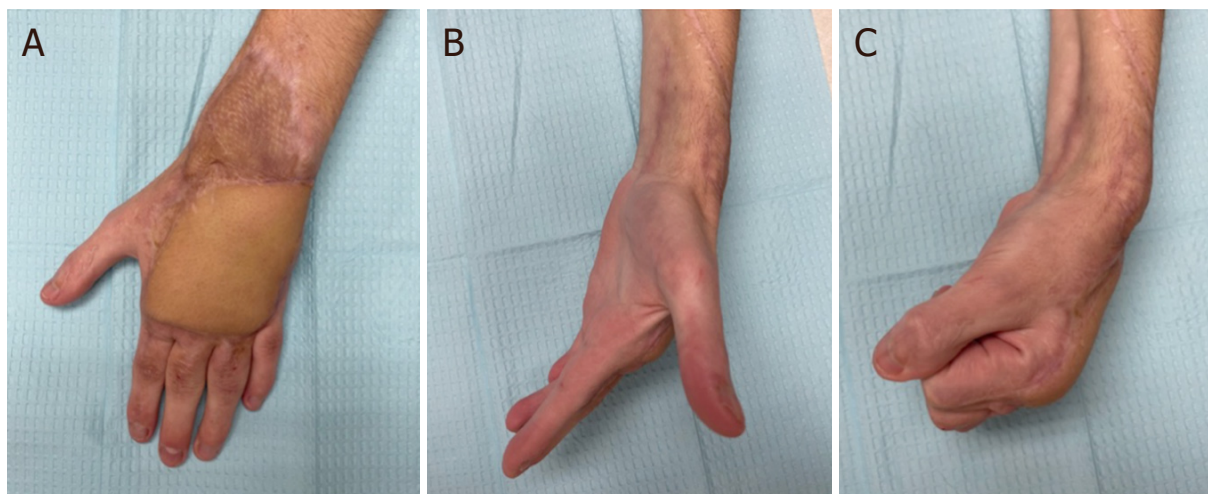




**Figure 3.** Free antero-lateral thigh flap with split thickness skin graft as well as cement spacers to the metacarpophalangeal joints of index through small fingers. Seen in the photograph is the implantable Synovis Flow Coupler (Baxter, Birmingham, AL) device for postoperative monitoring purposes



**Figure 4.** Staged extensor tendon reconstruction with silastic tendon implant placement and silastic arthroplasties (Integra LifeSciences, Princeton, NJ) of the index through small finger metacarpophalangeal joints



**Figure 5.** Appearance of reconstructed hand following suction lipectomy of the ALT flap for contour improvement and regular sessions of hand therapy (A-C). Patient has pain-free, functional arc of flexion and extension of the digits with aesthetically pleasing result

underwent second stage extensor tendon reconstruction. Bilateral palmaris longus tendon grafts were harvested. These were passed from proximal to distal into the pseudosheath using the silicone implants, then split into a Y configuration distally, and sutured to each of the extensor tendons distally via Pulvertaft weave.

Finally, six months later, he underwent suction lipectomy for re-contouring of the left anterolateral thigh flap. At latest follow up, the flap was healthy, and he had reasonable function of the hand and was able to grip [Figure 5]. He had active extension of the digits enough to allow for typing and video-gaming. He could flex his fingers to touch the palm but not the distal palmar crease, and he was able to grasp objects. He returned to driving and working as a store clerk.

## Case 2

A 47-year-old male was transferred to our trauma center after a rollover motor vehicle accident. He sustained an isolated dorsal shearing injury of the left wrist and hand with heavy, gross contamination of road and field debris. Focused examination of the extremity revealed that the fingers were well-perfused.

He was taken to the operating room for exploration and debridement [Figure 6]. He was found to have significant skin and soft tissue loss; segmental loss of the extensor tendons to all fingers; and open coronal plane fractures of the distal radius, distal ulna, scaphoid, lunate, capitate, hamate, and long and ring finger metacarpals.

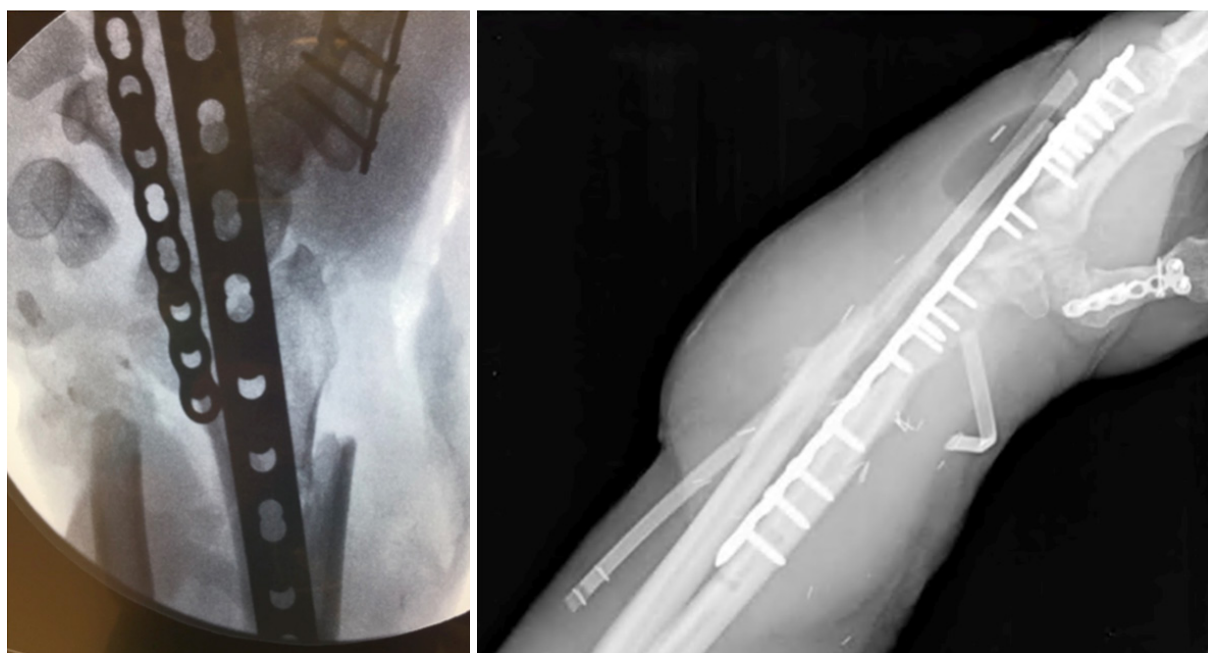
At the index procedure, he underwent thorough excisional debridement, placement of antibiotic cement beads, and vacuum-assisted closure. Skeletal stabilization was achieved via external fixation, spanning from the radial diaphysis to the index metacarpal. He was brought back to the OR every 2-3 days for a total of three subsequent debridements. After this time, the wound was felt to be appropriate to proceed with definitive bony stabilization and soft tissue reconstruction.

For bony stabilization, he underwent total wrist arthrodesis using a Synthes 3.5-mm metaphyseal locking compression plate (DePuy Synthes, Raynham, MA) spanning from the distal radius to the third metacarpal, with additional stabilization using a Synthes 2.7-mm reconstruction plate from the distal radius to the fourth metacarpal [Figure 7] For soft tissue reconstruction, he underwent free latissimus dorsi muscle flap





**Figure 6.** Following thorough extensive debridement of non-vitalized tissues and bone resulting in large wound with exposed bony defects, carpal instability, and extensor tendon loss



**Figure 7.** Arthrodesis of carpometacarpal, midcarpal, and radiocarpal joints with dual plate fixation for control of unstable carpal bones and distal radial fracture. Hardware seen on thumb metacarpal was present from previous injury

that was transferred from the right side to the left dorsal wrist and hand and covered with a split thickness skin graft [Figure 8].

At the same time, he underwent first stage extensor tendon reconstruction of the EPL and common extensors to the index through small fingers, using three silicone tendon implants (one secured to the distal



**Figure 8.** Free latissimus dorsi muscle flap to the defect followed by skin-thickness skin grafting of the muscle belly



**Figure 9.** First stage extensor tendon reconstruction with silastic hunter tendon implants (Wright Medical Technology, Memphis, TN) to the extensor hood mechanisms distally





**Figure 10.** Four months postoperative follow-up showing atrophy of the latissimus muscle with improved contouring and use of the reconstructed hand for activities of daily living

EPL stump, one split to the index and long extensors, and one split to the ring and small extensors) [Figure 9]. These were placed directly on the hardware beneath the muscle flap. The flap contoured nicely to the dorsal of the hand and wrist, and the arthrodesis achieved bony union by three months [Figure 10]. At latest follow up, the second stage extensor tendon reconstruction has been delayed secondary to unplanned incarceration of the patient.

## CONCLUSION

Dorsal shearing injuries to the hand and wrist can be devastating to hand function given their destruction of the soft tissue envelope, extensor tendon, and underlying bony skeleton. Additionally, these injuries pose unique and challenging problems to treating surgeons charged with reconstructing these deficits. Appropriate management begins with stabilization of the traumatic patient followed by thorough and often repeated debridements of the wound. Once ready for reconstruction, goals of reconstruction include bony stabilization, extensor tendon reconstruction, and stable soft tissue coverage. The reconstructive surgeon should assess coverage options based on planned, future surgeries such as staged tendon reconstruction.

Throughout the perioperative period, passive and/or active range of motion modalities are indicated for those joints that are deemed stable and appropriate. This is particularly important if staged debridements or surgeries are required. Extended periods of immobility through multiple stages of surgery can negatively affect eventual outcomes of mobility and function. Once healed, reasonable return of function to a previously mangled hand and wrist can be expected for the patient.

## DECLARATIONS

### Authors' contributions

Contributed to literature review and writing manuscript: Murthy PG

Contributed surgical cases and photos, contributed to literature review and to writing and editing manuscript: Strohl AB

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

Both authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Bakri K, Moran SL. Initial assessment and management of complex forearm defects. *Hand Clin* 2007;23:255-68, vii.
2. Reid DC. Hand injuries requiring skin replacement and restoration of tendon function. *Br J Plast Surg* 1974;27:5-18.
3. Dautel G. Vascularized toe joint transfers to the hand for PIP or MCP reconstruction. *Hand Surg Rehabil* 2018;37:329-36.
4. Pappalardo M, Laurence VG, Lin YT. Chimeric free vascularized metatarsophalangeal joint with toe fillet flap: a technique for reconstruction of the posttraumatic metacarpophalangeal joint with concomitant soft tissue defect. *J Hand Surg Am* 2018;43:193.e1-6.
5. Soutar DS, Tanner NS. The radial forearm flap in the management of soft tissue injuries of the hand. *Br J Plast Surg* 1984;37:18-26.
6. Mandrekas AD, Zambacos GJ. Reverse-flow radial forearm flap for reconstruction of the hand. *Ann Plast Surg* 1994;33:536-43.
7. Grobbelaar AO, Harrison DH. The distally based ulnar artery island flap in hand reconstruction. *J Hand Surg Br* 1997;22:204-11.
8. Zancolli EA, Angrigiani C. Posterior interosseous island forearm flap. *J Hand Surg Br* 1988;13:130-5.
9. Adani R, Tarallo L, Marcoccio I, Cipriani R, Gelati C, Innocenti M. Hand reconstruction using the thin anterolateral thigh flap. *Plast Reconstr Surg* 2005;116:467-73; discussion 474-7.
10. Tuncer S, Sezgin B, Sencan A, Sari A. Free serratus fascia flap for reconstruction of soft tissue defects involving the distal upper and lower extremity. *Ann Plast Surg* 2020;84:672-8.
11. Hirase Y, Kojima T, Bang HH. Double-layered free temporal fascia flap as a two-layered tendon-gliding surface. *Plast Reconstr Surg* 1991;88:707-12.
12. Logan SE, Alpert BS, Buncke HJ. Free serratus anterior muscle transplantation for hand reconstruction. *Br J Plast Surg* 1988;41:639-43.
13. Brody GA, Buncke HJ, Alpert BS, Hing DN. Serratus anterior muscle transplantation for treatment of soft tissue defects in the hand. *J Hand Surg Am* 1990;15:322-7.
14. Kim JT, Kim SK. Hand resurfacing with the superthin latissimus dorsi perforator-based free flap. *Plast Reconstr Surg* 2003;111:366-70.
15. Kim SW, Lee HJ, Kim JT, Kim YH. Multiple-digit resurfacing using a thin latissimus dorsi perforator flap. *J Plast Reconstr Aesthet Surg* 2014;67:74-80.
16. Mateev MA, Beermanov KA, Subanova LK, Novikova TV, Shaltakova G. Shape-modified method using the radial forearm perforator flap for reconstruction of soft-tissue defects of the scalp. *J Reconstr Microsurg* 2005;21:21-4.
17. Ho AM, Chang J. Radial artery perforator flap. *J Hand Surg Am* 2010;35:308-11.
18. Caroli A, Adani R, Castagnetti C, Pancaldi G, Squarzina PB. Dorsalis pedis flap with vascularized extensor tendons for dorsal hand reconstruction. *Plast Reconstr Surg* 1993;92:1326-30.
19. Adani R, Marcoccio I, Tarallo L. Flap coverage of dorsum of hand associated with extensor tendons injuries: a completely vascularized single-stage reconstruction. *Microsurgery* 2003;23:32-9.
20. Cui MY, Shen H. Anterolateral thigh free flap for simultaneous reconstruction of digital extensor tendon and defect of the dorsal hand: a

- case report. *Chin J Traumatol* 2016;19:309-10.
21. di Summa PG, Sapino G, Cherubino M, et al. Reconstruction of complex soft tissue defects including tendons with anterolateral thigh flap extended to fascia lata: long term recovery and functional outcomes. *Microsurgery* 2019;39:405-15.
22. Kitazawa T, Shiba M, Tsunekawa K. Free serratus anterior fascial flap combined with vascularized scapular bone for reconstruction of dorsal hand and finger defects. *Case Reports Plast Surg Hand Surg* 2017;5:1-8.
23. Koshima I. A new classification of free combined or connected tissue transfers: introduction to the concept of bridge, siamese, chimeric, mosaic, and chain-circle flaps. *Acta Med Okayama* 2001;55:329-32.
24. Scheker LR, Langley SJ, Martin DL, Julliard KN. Primary extensor tendon reconstruction in dorsal hand defects requiring free flaps. *J Hand Surg Br* 1993;18:568-75.
25. Ozbaydar M, Orman O, Ozel O, Altan E. Multiple extensor tendons reconstruction with hamstring tendon grafts and flap coverage for severe dorsal hand injuries. *Hand Surg Rehabil* 2017;36:410-5.
26. Hunter J. Artificial tendons. Early development and application. *Am J Surg* 1965;109:325-38.
27. Schneider LH. Staged flexor tendon reconstruction using the method of Hunter. *Clin Orthop Relat Res* 1982;171:164-71.



Review

Open Access



# Strategies for innervation of the neophallus

Rayisa Hontscharuk<sup>1</sup>, Charalampos Siotos<sup>1</sup>, Loren S. Schechter<sup>1,2,3</sup>

<sup>1</sup>Department of Plastic and Reconstructive Surgery, Rush University Medical Center, Chicago, IL 60612, USA.

<sup>2</sup>Department of Plastic and Reconstructive Surgery, University of Illinois at Chicago, Chicago, IL 60612, USA.

<sup>3</sup>The Center for Gender Confirmation Surgery, Weiss Memorial Hospital, Chicago, IL 60640, USA.

**Correspondence to:** Dr. Loren S. Schechter, The Center for Gender Confirmation Surgery, Weiss Memorial Hospital, 9000 Waukegan Rd Suite 210, Morton Grove, IL 60053, USA. E-mail: lss@univplastics.com

**How to cite this article:** Hontscharuk R, Siotos C, Schechter LS. Strategies for innervation of the neophallus. *Plast Aesthet Res* 2020;7:65. <http://dx.doi.org/10.20517/2347-9264.2020.124>

**Received:** 25 May 2020 **First Decision:** 7 Sep 2020 **Revised:** 15 Sep 2020 **Accepted:** 23 Sep 2020 **Published:** 13 Nov 2020

**Academic Editor:** Marlon E. Buncamper **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

A fundamental goal of phalloplasty includes the construction of a sensate neophallus. Both tactile and erogenous sensation are important for protective sensation (including retention of implantable penile prosthesis) as well as sexual satisfaction. This article will describe the sensory innervation of flaps commonly used for phalloplasty including the radial forearm flap, anterolateral thigh flap, and musculocutaneous latissimus dorsi flap. The sensory innervation of the perineum and external genitalia will be reviewed as a basis for selecting recipient nerves. Additionally, surgical techniques, such as neurotomy, will be discussed. Finally, outcome data, although limited, will be assessed.

**Keywords:** Phalloplasty, sensation, radial forearm flap, anterolateral thigh flap

## INTRODUCTION

Recent studies estimate that approximately 25 million individuals worldwide, including 1 million people in the United States, identify as transgender<sup>[1-3]</sup>. Over the past several years, the demand for gender affirmation surgery has steadily increased<sup>[4]</sup>. The American Society of Plastic Surgeons reported that a total of 2,885 feminizing procedures and 6,691 masculinizing procedures were performed in 2018, representing an increase of 109% and 392%, respectively, since 2015<sup>[5]</sup>. This includes an increase in the number of transgender men seeking consultation for “bottom” surgery; approximately 40% of whom ultimately undergoing a phalloplasty procedure<sup>[6]</sup>.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Table 1. Phalloplasty flap options**

Free flap options	Pedicled flap options
Radial forearm flap <sup>[7,8,10,11,20-23,24-28,68]</sup>	Anterolateral thigh flap <sup>[7,20,21,23,34,35,68]</sup>
Anterolateral thigh flap <sup>[7,21]</sup>	Abdominal flaps <sup>[7,21]</sup>
Osteocutaneous fibula flap <sup>[7,27,42,70]</sup>	Groin flap <sup>[7]</sup>
Musculocutaneous latissimus dorsi flap <sup>[40,41]</sup>	Gracilis flap <sup>[7]</sup>
Lateral arm flap <sup>[10,66]</sup>	Superficial circumflex iliac artery perforator flap <sup>[45-47]</sup>

Individuals who have undergone phalloplasty procedures express high rates of satisfaction<sup>[7]</sup> and improved quality of life<sup>[8,9]</sup>. However, phalloplasty remains a complex procedure with no “one size fits all” approach. A variety of techniques are used to create an aesthetic and functional neophallus, with the most common being the radial forearm flap (RFF) and the anterolateral thigh flap (ALT). The goals of phalloplasty are well described; among these goals include the construction of a sensate neophallus, capable of providing both protective and erogenous sensation<sup>[10,11]</sup>. An individualized approach, utilizing a shared decision-making model, is recommended.

Restoring genital sensibility is highly desired by patients, and a sensate neophallus is a major determinant of postoperative satisfaction<sup>[10,12]</sup>. Tactile sensation is important in reducing the risk of inadvertent injuries as well as providing protection for subsequent implantable penile prostheses<sup>[13]</sup>. Erogenous sensation is *sine qua non* for sexual satisfaction and orgasm<sup>[14]</sup>. Microneurosurgical techniques used to restore sensation include coaptation of recipient flap nerves, such as the lateral and/or medial antebrachial cutaneous nerves or lateral femoral cutaneous nerves, to donor nerves in the groin and/or existing genitalia, such as the ilioinguinal, genitofemoral, and/or branches of the dorsal pudendal or dorsal clitoral nerves<sup>[15]</sup>.

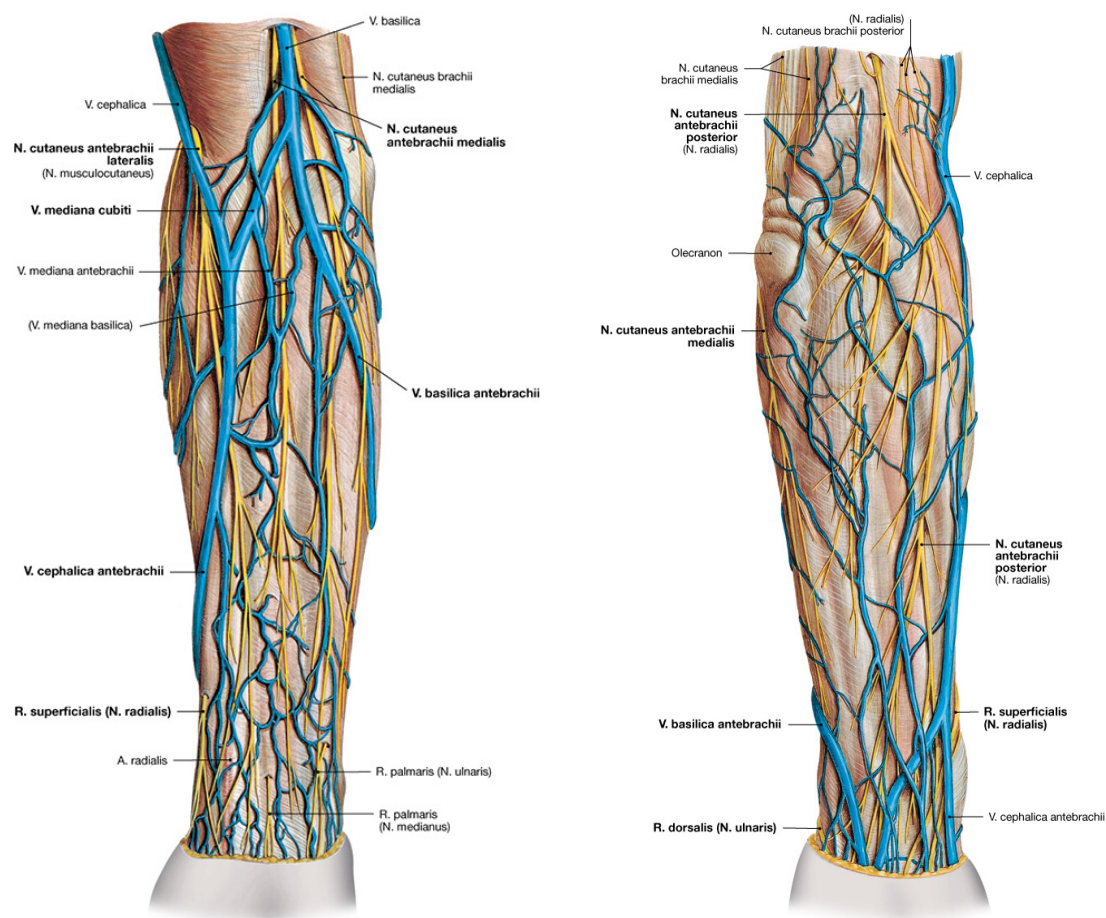
The ideal approach to achieving sensation in the neophallus is yet to be identified. This review discusses common flaps used in phalloplasty procedures as well as strategies to optimize flap innervation. Relevant genital anatomy, nerve coaptation techniques, and outcomes data are reviewed.

## CURRENT PHALLOPLASTY FLAP OPTIONS

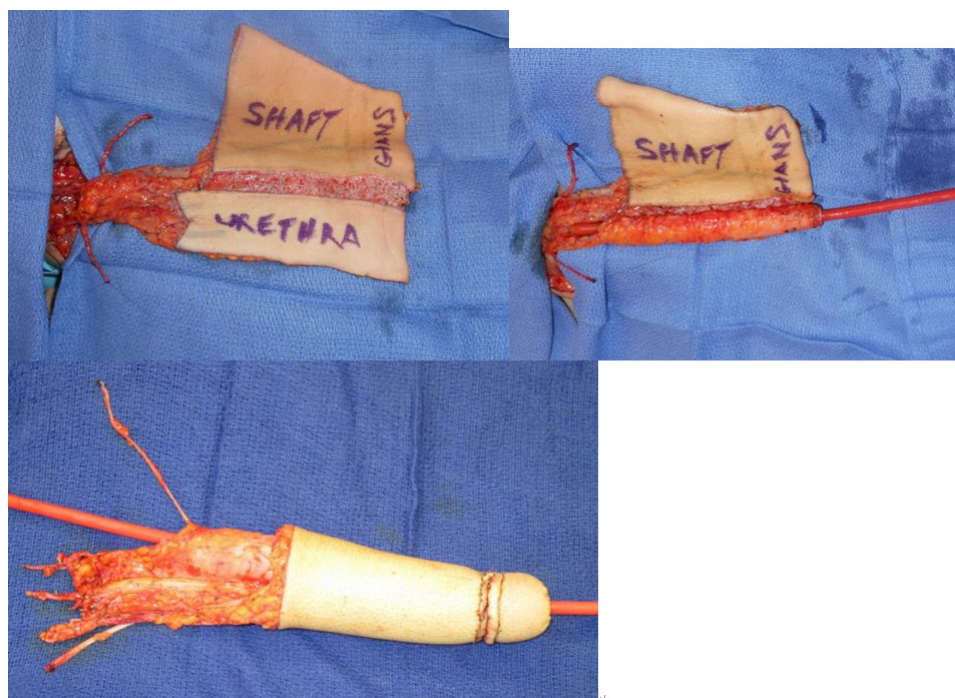
The goals of phalloplasty include the provision of both tactile (protective) and erogenous sensation to facilitate orgasm and enhance sexual satisfaction. A variety of flap options are available [Table 1], with the most common being the RFF and ALT flaps.

The RFF is based on the radial artery, venae comitantes, and cephalic vein and can be harvested with the lateral and/or medial antebrachial cutaneous nerves [Figure 1]. The medial antebrachial cutaneous nerve originates from the medial cord of the brachial plexus. It travels through the arm, medial to the brachial artery. Distally, it becomes more superficial, running adjacent to the basilic vein. In the elbow, it divides into anterior and posterior branches. The anterior branch passes between the medial epicondyle and the biceps tendon, then travels superficially over the flexor carpi ulnaris at the level of the wrist. The posterior branch courses anterior to the elbow, then posteriorly over the flexor muscles<sup>[16,17]</sup>. The lateral antebrachial cutaneous nerve is the terminal branch of the musculocutaneous nerve. In the forearm, it lies in the subcutaneous fat, lateral to the biceps tendon. It then travels distally with the cephalic vein towards the 1st and 2nd extensor compartments of the wrist<sup>[18,19]</sup>.

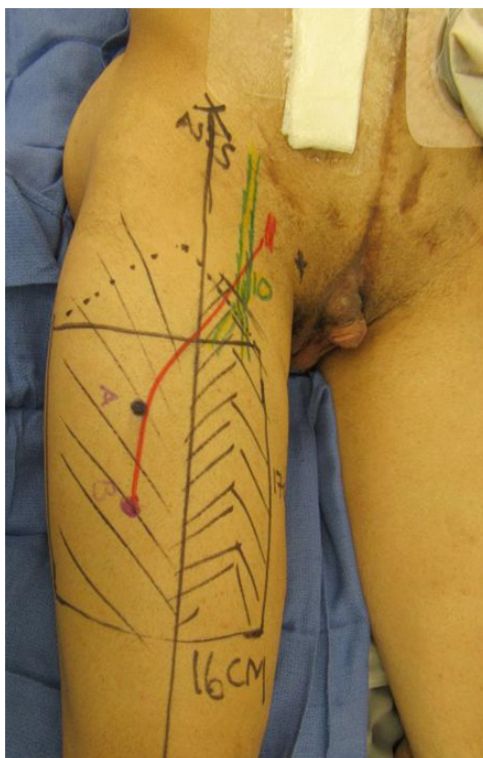
The RFF's reliable neurovascular anatomy, including its innervation density, and its thin subcutaneous fat layer (helpful for flap shaping and a double tube procedure) make it a frequent “first-choice” option for phalloplasty [Figure 2]<sup>[7,10,20-26]</sup>. However, color mismatch, atrophy over time, and a conspicuous donor site may lead some individuals to select other flap options<sup>[7,10,20,21,24,27,28]</sup>. Morrison *et al.*<sup>[7]</sup> found that 78.1% of patients undergoing RFF phalloplasty were satisfied with the procedure. Within this group, 98.1% of



**Figure 1.** Superficial veins and cutaneous nerves of the forearm (volar and dorsal surface).



**Figure 2.** Design of the neophallus using a radial forearm free flap: tube within a tube procedure



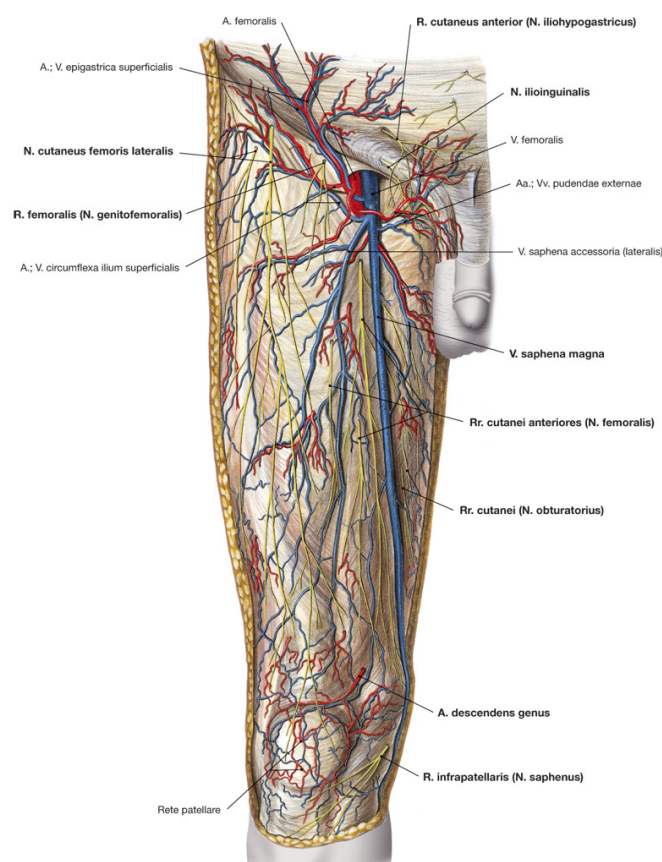
**Figure 3.** Preoperative marking for an Anterolateral thigh flap phalloplasty in a patient with exstrophy

patients reported tactile sensation, and 71.4% of patients reported the return of erogenous sensation. Monstrey *et al.*<sup>[24]</sup> found that all patients regained tactile sensitivity 1 year following RFF phalloplasty. In addition, > 80% of patients reported sexual satisfaction, and 100% of patients were able to reach orgasm following surgery. Selvaggi *et al.*<sup>[29]</sup> found that pressure thresholds over the glans, corona, and shaft of the neophallus correlated well with cisgender penile controls. Also, Selvaggi *et al.*<sup>[29]</sup> found that 100% of patients who underwent RFF phalloplasty were able to experience orgasm following surgery. This is consistent with other reports confirming erogenous sensation in the neophallus following RFF phalloplasty<sup>[8,30,31]</sup>.

The ALT flap, another frequent choice for phalloplasty<sup>[7]</sup>, is based on the descending branch of the lateral circumflex femoral artery [Figure 3]. The flap can be harvested with the lateral femoral cutaneous nerve (LFCN), which provides sensation to the inferolateral thigh [Figure 4]. The LFCN emerges from the deep fascia, approximately 10 cm distal to the ASIS. It travels within the deep subcutaneous tissue and divides into three branches in the middle third of the thigh. The anterior branch is the largest (1 to 2 mm in diameter) and courses inferiorly along the vascular axis of the ALT flap.

Other sensory nerves, the superior perforator nerve, and the median perforator nerve arise from the lateral musculocutaneous nerve (the terminal branch of the femoral nerve) and lie within the anatomical boundaries of the ALT flap<sup>[32,33]</sup>. In the proximal portion of the flap, the superior and median perforator nerves are superficial. In the distal flap, the perforator nerves branch freely and repeatedly, gradually disappearing. Ribuffo *et al.*<sup>[32]</sup> theorized that the superior and median perforator nerves can be included in the flap, in addition to the LFCN, to further optimize sensory reinnervation. The LFCN (classically used as the sole innervating nerve of the sensate ALT flap) has been shown to provide sensation to 100% of the ALT skin flap territory, whereas the median perforator nerve and superior perforator nerve supply sensation to approximately 60% and 25% of the medial aspect of the flap, respectively<sup>[32]</sup>. Insufficient data currently exist to recommend the routine inclusion of these nerves in the ALT flap harvest.





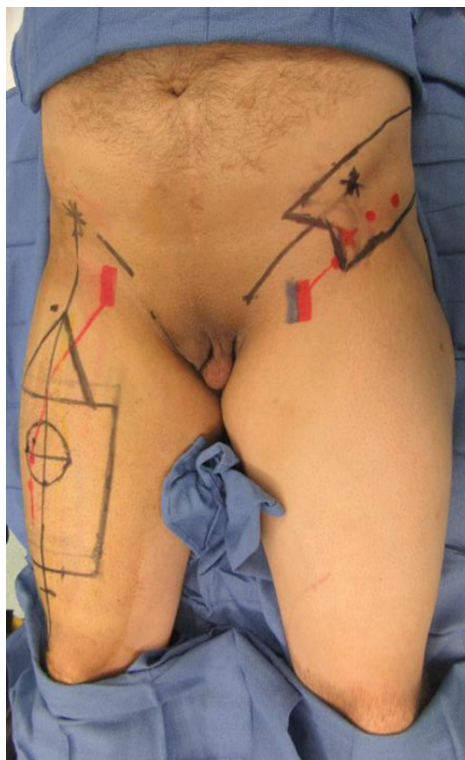
Paulsen/Waschke: Sobotta – Atlas der Anatomie, 24. A. 2017 © Elsevier GmbH

**Figure 4.** Superficial nerves and blood vessels of the anterior thigh

The ALT flap offers a good color match, a less conspicuous donor site as compared to the RFF, and may be performed as a pedicled procedure<sup>[7,11,20,21,34,35]</sup>. The ALT flap may offer an advantage over the RFF by providing additional bulk, however, in many individuals, the amount of subcutaneous fat precludes its use in a double tube procedure<sup>[20,21]</sup>. Additionally, flap defatting should be undertaken with caution due to the risk of injury to the LFCN and subsequent loss of sensation in the flap<sup>[20]</sup>. When comparing a one-stage ALT flap to a one-stage RFF (both performed with urethral lengthening and vaginectomy), the ALT flap was found to have a higher rate of postoperative complications<sup>[36]</sup>. Despite this, overall patient satisfaction remains high (reported at 100%)<sup>[7]</sup>. Following coaptation of the LFCN to the dorsal clitoral nerve, patients achieved tactile and erogenous sensation and reported satisfactory sexual function following surgery<sup>[7,34,37]</sup>. In a recent literature review, 75% of patients undergoing ALT phalloplasty reported tactile sensation, and 60% of patients undergoing reported erogenous sensation<sup>[7]</sup>. In a case study, Rubino *et al.*<sup>[34]</sup> reported normal ranges of vibration and thermal sensation in the neophallus when compared to the left thenar eminence. The patient in this case report also experienced erogenous stimuli during penetrative intercourse.

The musculocutaneous latissimus dorsi (MLD) flap is another option, although not commonly used in North America. Its vascular supply is based upon the thoracodorsal system. While the thoracodorsal nerve can be harvested with the flap, this is primarily a motor nerve, supplying the latissimus dorsi muscle rather than the overlying skin. The thoracodorsal (or middle or long subscapular) nerve arises as a single nerve from the posterior cord of the brachial plexus. It descends through the axilla, posterior to the axillary vein, and then runs parallel to the vascular pedicle of the latissimus dorsi muscle<sup>[38,39]</sup>.





**Figure 5.** Preoperative markings for phalloplasty using a SCIAP flap for urethral reconstruction combined with an anterolateral thigh flap. SCIAP: superficial circumflex iliac artery perforator

Potential advantages of the MLD flap include its reliable vascular pedicle, large surface area, and minimal donor site morbidity. Proponents note maintenance of flap bulk which may enhance retention of subsequent prosthetic devices<sup>[40,41]</sup>. Overall, 93.8% of patients report being satisfied with their phallus following surgery<sup>[7]</sup>. The primary disadvantage of the MLD flap was the lack of orgasmic sensibility<sup>[40]</sup>. A literature review reported sensory function in 17 patients, yet 100% of patients reported tactile sensation following the procedure<sup>[7]</sup>. No studies examining erogenous sensation have been published.

The osteocutaneous fibula flap (OCFF) was first described in 1993 as a phalloplasty option to achieve neophallic rigidity without the use of a prosthesis<sup>[7]</sup>. The flap is based on the peroneal artery and may be harvested with the sural nerves<sup>[7,42]</sup>. The sural (or short saphenous) nerve arises from the medial sural cutaneous nerve between the two head of the gastrocnemius muscle. It travels superficial to the muscle until the middle of the leg, where it joins the sural communicating branch of the common fibular nerve. It then travels to the lateral malleolus, providing sensory innervation of the posterior-lateral leg<sup>[43,44]</sup>. Tactile and erogenous sensation have been reported following OCFF phalloplasty<sup>[7,42]</sup>, and patients reported feeling “good to very good” with sexual intercourse following this procedure<sup>[42]</sup>.

The superficial circumflex iliac artery perforator flap (SCIAP), often used in conjunction with other flaps, is another option for phalloplasty procedures [Figure 5]<sup>[45-47]</sup>. The SCIAP flap is based on perforating vessels from the superficial circumflex iliac artery, a branch of the external iliac/superficial femoral artery. It is not commonly used as a sensate flap in phalloplasty procedures. However, Iida *et al.*<sup>[48]</sup> describe the use of a sensate SCIAP flap in head and neck reconstruction using the lateral cutaneous branch of an intercostal nerve. Potential advantages of this flap include a concealed donor site, minimal donor-site morbidity, and the ability to thin the flap at the time of harvest<sup>[48,49]</sup>.

Before the advent of microsurgery, pedicled abdominal flaps, such as the suprapubic phalloplasty, were more commonly utilized. Abdominal flaps are based upon the epigastric vessels<sup>[7,50]</sup>, and generally performed as a two-stage procedure; tubularization of the abdominal skin forms the shaft, and a skin graft is used for subsequent urethral reconstruction<sup>[7]</sup>. These flaps are considered non-sensate, as no direct nerve coaptations are performed. In general, abdominal flaps have fallen out of favor due to higher complication rates, lack of sensation, less favorable aesthetics results, and their general use as shaft only procedures<sup>[7,37]</sup>. However, 95% of patients report being satisfied with abdominal-based phalloplasty procedures<sup>[50]</sup>.

## SENSORY INNERVATION OF THE VULVA AND CLITORIS

Understanding the innervation of the vulva and clitoris is integral to achieving successful phallic reconstruction. The ilioinguinal nerve, with the iliohypogastric nerve, arises as a single trunk from the first spinal lumbar nerve [Figure 4]. On the lateral border of the iliopsoas, the two nerves separate. The ilioinguinal nerve courses between the transverse and internal oblique abdominis muscle before entering the inguinal canal. The ilioinguinal nerve provides sensory innervation to the skin overlying the inguinal ligament, the pubis, the superomedial region of the thigh, and the labia majora or scrotum<sup>[51,52]</sup>.

The iliohypogastric nerve initially travels on the anterior surface of the quadratus lumborum muscle. It then travels in the plane between the transversus abdominis and internal oblique muscles. The lateral cutaneous branch provides sensation to the posterolateral gluteal skin. The anterior cutaneous branch courses through the internal oblique muscle in a downward and medial fashion before entering the external oblique muscle. The anterior cutaneous branch provides sensation to the skin in the pubic region<sup>[44]</sup>.

The genitofemoral nerve arises from the first and second spinal lumbar roots. As it descends into the true pelvis, the nerve branches into a genital and femoral branch at the medial aspect of the inguinal ligament. The former accompanies the round ligament or spermatic cord and supplies sensation to the skin of the mons pubis and the labia majora or the upper part of the scrotum, respectively. The femoral branch provides sensation to the anterolateral thigh<sup>[52]</sup>.

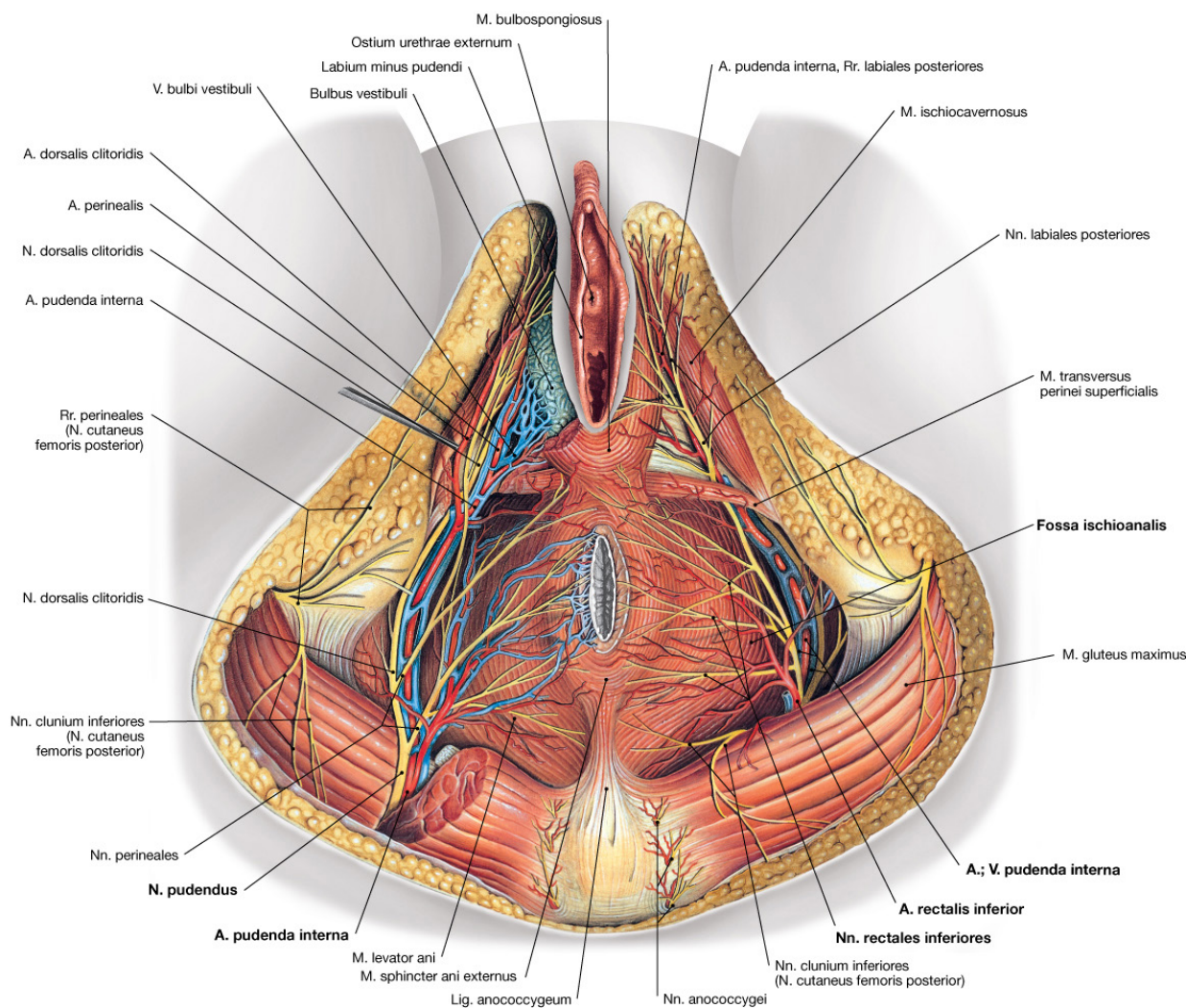
Somatic innervation to the lower urogenital system is provided by the pudendal nerve [Figure 6]. The pudendal nerve travels between the piriformis and coccygeus muscles. It then exits the pelvic cavity through the greater sciatic foramen. At the level of the ischium, it courses inferomedial to the sacrospinous ligament to re-enter the true pelvis via the lesser sciatic foramen. It then enters the pudendal canal<sup>[53]</sup>.

The pudendal nerve travels on the dorsal surface of the cavernous bodies of the clitoris, before innervating the glans clitoris [Figure 6]. The right and left dorsal clitoral nerves initially exist as two separate bundles that fan out laterally on the clitoral bodies<sup>[54]</sup>. Proximally, the dorsal nerves of the clitoris extend around the tunica of the clitoral bodies. There is extensive innervation at the 11 and 1 o'clock positions, with a relative lack of nerves along the dorsal midline of the clitoral body (12 o'clock position)<sup>[55-57]</sup>.

## MECHANISMS AND ASSESSMENTS OF FLAP REINNERVATION

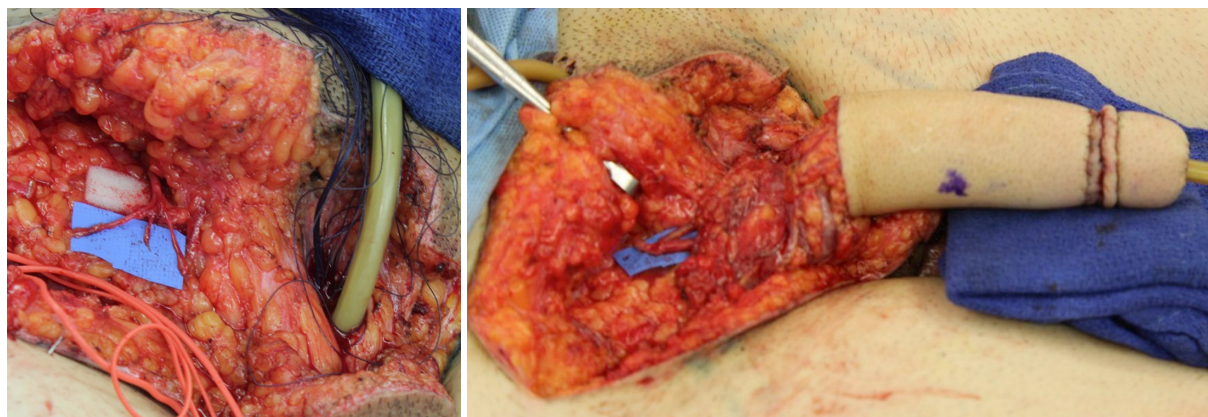
Flaps achieve sensation through two mechanisms<sup>[58]</sup>. The first mechanism is referred to as the “peripheral way” and describes sensory recovery when a nerve coaptation is not performed. For example, tactile and temperature sensation may be restored as nerves propagate from the wound margin and/or the recipient bed<sup>[59]</sup>. The second pathway, called the “central way” is based on nerve coaptations between donor and recipient nerves. This technique typically results in improved sensory recovery and forms the basis of reinnervation strategies for phalloplasty procedures [Figure 7].

The ability to achieve tactile and/or erogenous sensation is affected by both the accuracy of nerve coaptation and the nerve's regeneration capacity. Nerve coaptation techniques have not been systematically



Paulsen/Waschke: Sobotta – Atlas der Anatomie, 24. A. 2017 © Elsevier GmbH

**Figure 6.** Nerves and blood vessels of the female perineum



**Figure 7.** Urethral inset, vascular anastomoses, and neuroraphies of the neophallus using a radial forearm free flap



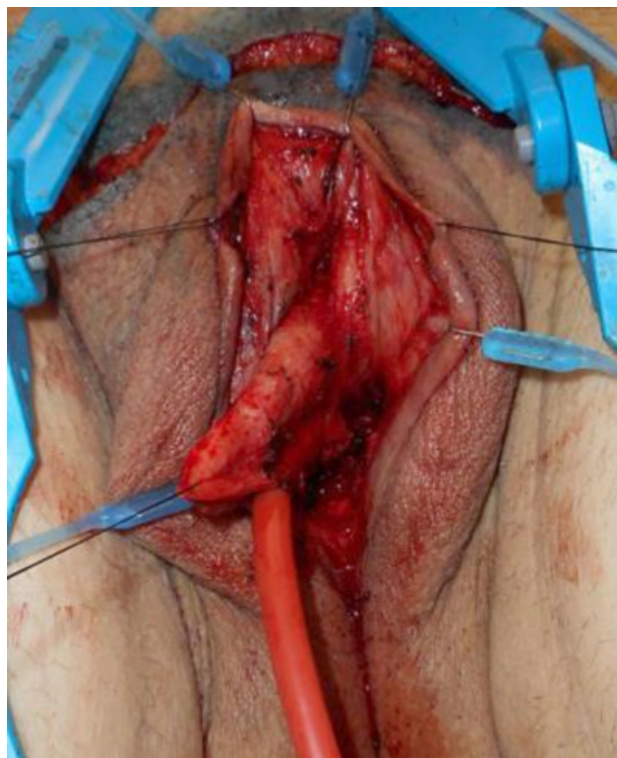
studied in the field of gender affirmation surgery. A suture-only epineurial nerve repair remains the mainstay of care. An end-to-end repair is usually performed, but end-to-side and epineurial sleeve repairs have been described<sup>[60]</sup>. When a tension-free coaptation is not possible, synthetic or biological conduits may be used<sup>[61]</sup>. In the field of peripheral nerve surgery, connector-assisted coaptations may decrease operative time, improve sensory outcomes, and decrease pain<sup>[62]</sup>. Multiple pharmacologic agents, such as Acetyl-L-carnitine, testosterone, and various growth factors have been investigated as a means to enhance nerve regeneration<sup>[63,64]</sup>. New approaches and techniques are under investigation, and the role of molecular, stem cell-based or gene therapies in nerve regeneration may play an important role in the future<sup>[65]</sup>.

The restoration of protective or tactile sensation is important in helping to avoid incidental injuries to the neophallus, but also in facilitating retention of an erectile prosthesis<sup>[24,29,58]</sup>. In addition, consistent with reports of penile reconstruction in cisgender men<sup>[11]</sup>, restoration of tactile sensation contributes to the orgasm experience in transgender men<sup>[29]</sup>. To achieve tactile or protective sensation, the recipient flap sensory nerve is typically coapted to the donor ilioinguinal nerve<sup>[15,22,24,25,28-30]</sup>.

There is no consistent method of reporting or assessing tactile sensation following phalloplasty procedures. In general, tactile sensation returns ~ 1 year following surgery<sup>[15,22]</sup>, however, Fang *et al.*<sup>[28]</sup> reported sensory recovery approximately 6 months following surgery. Selvaggi *et al.*<sup>[29]</sup> reported pressure thresholds over the glans and corona of the neophallus (RFF) comparable to penile controls. Vibratory thresholds were consistent with the restoration of tactile sensitivity. However, these thresholds were high in comparison to penile controls, indicating that sensation was low following this procedure. Kim *et al.*<sup>[22]</sup> reported outcomes of 40 transgender men undergoing phalloplasty with a radial forearm osteocutaneous flap. In their series, the medial antebrachial cutaneous nerve was coapted to the ilioinguinal nerve, and the lateral antebrachial cutaneous nerve was coapted to the deep pudendal or dorsal clitoral nerve. Sensation was assessed using the Zachary and Holmes scheme (ranging from S0 to S4). At a 12-month follow-up, all patients recovered tactile sensation greater than S2. This indicated a minimum recovery of pain and some touch sensibility. Monstrey *et al.*<sup>[24]</sup> used identical techniques in their cohort of 287 - of mostly transgender male-patients. After a year, all patients had regained tactile sensitivity.

A common strategy designed to provide erogenous sensation in phalloplasty procedures involves the coaptation of a flap sensory nerve to the dorsal pudendal nerve or the dorsal clitoral nerve<sup>[15,22,24,29]</sup>. Coaptations using the medial antebrachial cutaneous nerve or the lateral antebrachial cutaneous nerve are the two most commonly reported techniques<sup>[15]</sup>. It is theorized that nerve coaptations to the dorsal clitoral nerve allow the patient to reach orgasm from direct erogenous stimulation following the clitoral nerve pathway<sup>[29]</sup>. Oftentimes, the ilioinguinal nerve is used as a donor nerve to achieve tactile or protective sensibility and one branch of the dorsal clitoral nerve is used as a donor nerve to achieve erogenous sensation<sup>[15,24,29]</sup>. This technique leaves one dorsal clitoral nerve intact and untouched. The denuded glans clitoris is then buried at the base of the neophallus. Morrison *et al.*<sup>[15]</sup> postulate that even with two independent nerve coaptations, the sensation of the reconstructed neophallus differs from that of cisgender men; however, no direct comparisons have been reported. Some authors advocate using only the dorsal clitoral nerves for the restoration of both tactile and erogenous sensation<sup>[8,10,23,26,31,34,42,66]</sup>. This follows the reconstructive concepts employed in cisgender men undergoing penile reconstruction<sup>[11,35,58,67-69]</sup>.

Despite few studies using validated measures to assess tactile sensation, reports of sensation, independent of flap type, are reported in the majority of transgender men (83%-100%) following phalloplasty<sup>[8,26,31,34,42,66]</sup>. Rubino *et al.*<sup>[34]</sup> reported on the use of an ALT flap in one transgender man. In this case, the lateral femoral cutaneous nerve was coapted, end-to-side, to one of the dorsal nerves of the clitoris. Six months after the procedure, tactile sensation along the shaft of the neophallus was reported. Sensory threshold measurements for temperature and vibration were all within the normal range, and the patient experienced



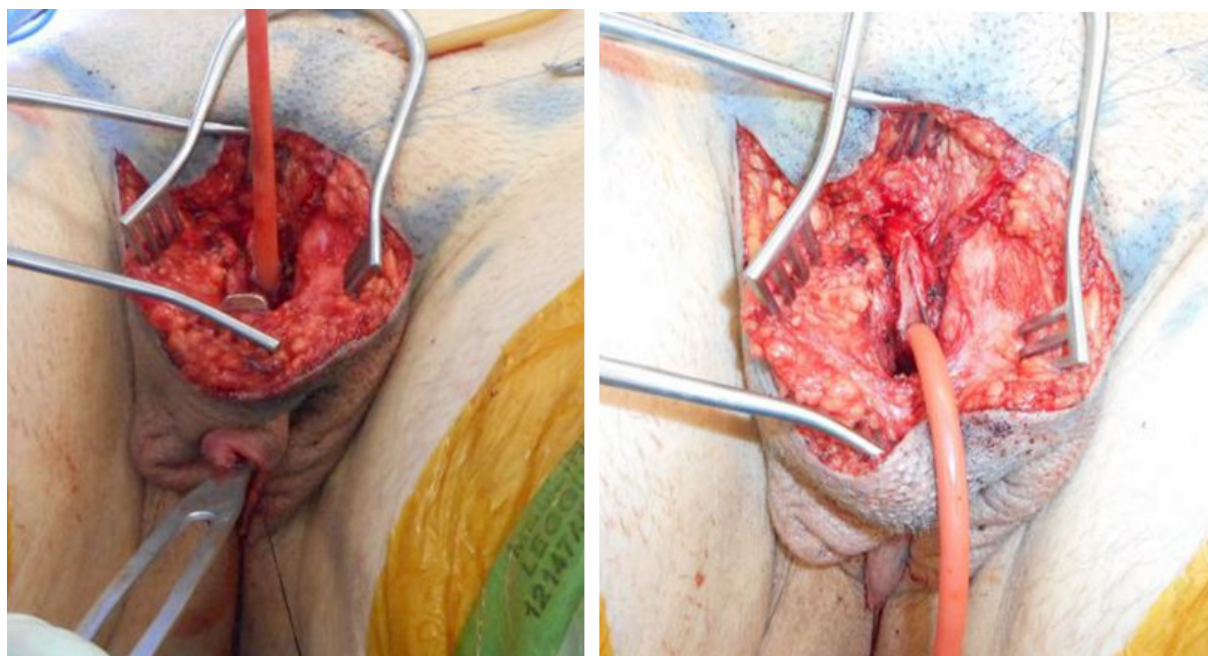
**Figure 8.** The clitoris de-epithelialized during the phalloplasty procedure

erogenous stimulation during intercourse. In their series, Ascha *et al.*<sup>[23]</sup> described outcomes of 64 transgender men undergoing an ALT flap with coaptation of the lateral femoral cutaneous nerve to the dorsal clitoral nerve, and 149 transgender men undergoing RFF with coaptation of the dorsal clitoral nerve to branches of the antebrachial cutaneous nerves. At 6 months follow up, 1 patient (1.6%) in the ALT group and 2 (1.3%) patients in the RFF group had not gained any sensation. Papadopoulos *et al.*<sup>[42]</sup> reported on the outcomes of 32 osteocutaneous fibula flap phalloplasties involving the coaptation of sural nerve branches or subcutaneous nerve branches to the dorsal clitoral nerves. They report “acceptable” tactile and erogenous sensation in all patients at 6 months following the procedure. In one study of 56 RFF phalloplasties<sup>[28]</sup>, nerve coaptations were performed to the ilioinguinal and iliohypogastric nerves. The authors report the return of only tactile sensation, with minimal erogenous sensation noted.

Many surgeons postulate that preservation of the clitoris allows and preserves erogenous sensibility and hence, orgasmic function following phalloplasty<sup>[15]</sup>. As such, one branch of the dorsal clitoral nerve is typically left intact to maintain clitoral sensation<sup>[10,15]</sup>. The denuded or de-epithelialized clitoris is buried at the base of the neophallus to provide an additional source of erogenous sensation [Figures 8 and 9]<sup>[7,8,10,15,28,29,42,70]</sup>. Monstrey *et al.*<sup>[24]</sup> describe burying the de-epithelialized clitoris above the pubic symphysis. With this technique, it is thought that manipulation of the base of the neophallus during penetrative intercourse or masturbation allows for the stimulation of the buried clitoris. Other authors describe incorporating the clitoral hood into the neoscrotum to preserve as much original erogenous sensitivity as possible<sup>[29,30]</sup>.

Other authors describe placement of the clitoris on the shaft of the neophallus<sup>[22,71]</sup> or at the junction of the neoscrotum and the inner thigh so as to increase stimulation during penetrative intercourse<sup>[72]</sup>. Garaffa *et al.*<sup>[25]</sup> report offering two options for clitoral management during phalloplasty procedures. One option was to leave the clitoris exteriorized so as facilitate manipulation, and the second option entails burying the de-epithelialized clitoris beneath the skin. Patients may select the first option due to a concern that clitoral





**Figure 9.** The clitoral-urethral construct transferred subcutaneously into position at the pubic symphysis

transposition may lead to a loss of pre-existing erogenous sensation. Clitoral transposition has not been found to result in a loss of ability to achieve orgasm with direct stimulation, and in these individuals, erogenous sensation remains consolidated outside of the neophallus<sup>[8]</sup>. Some surgeons postulate that placing the clitoris more superficially, just below the skin surface, rather than deeper, on the pubic symphysis, leaves it more accessible for direct stimulation and may enhance postoperative sexual function<sup>[8]</sup>. Despite this, no studies have compared the various techniques of clitoral placement.

Some patients report experiencing erogenous sensibility on the shaft of the neophallus during self-stimulation or penetrative intercourse<sup>[8,10,30,34,42]</sup>. Some transgender men report masturbating their neophallus regardless of whether or not they have undergone clitoral transposition and also report the ability to achieve orgasm with stimulation of the neophallus itself<sup>[8]</sup>. These findings are based on subjective patient reports rather than validated instruments. As such, it is difficult to discern the mechanism by which orgasmic function is attained. Future research is required to evaluate outcomes and elucidate the role by which various nerve coaptations result in orgasmic capability<sup>[15]</sup>.

## DISCUSSION

Current innervation strategies are based on the premise that nerve coaptations to the dorsal pudendal or dorsal clitoral nerves and the ilioinguinal nerves will elicit erogenous and tactile sensation, respectively<sup>[29]</sup>. However, the ability to compare techniques is limited by a lack of standardized measures<sup>[15]</sup>. In a recent literature review<sup>[15]</sup>, recovered sensibility, whether tactile or erogenous, was found to be similar regardless of the choice of donor nerve. This finding is consistent with patient reports<sup>[8,10,22-24,26,29,31,34,42,66]</sup>. These findings suggest that the choice of donor nerve may also have little impact on post-surgical tactile or erogenous sensory outcomes<sup>[15]</sup>. This has led some surgeons to leave the dorsal clitoral nerves untouched, and simply bury the denuded clitoris within the base of the flap. While this technique may not lead to erogenous sensation along the shaft of the neophallus, it may reduce the possibility of a loss of erogenous sensation<sup>[28]</sup>. Conversely, other surgeons may advocate the importance of coaptation to the dorsal clitoral nerve so as to provide erogenous sensation to the neophallic shaft<sup>[8,10,30,34,42]</sup>. Doria-Medina *et al.*<sup>[30]</sup> reported functional

neuroimaging (MRI) findings following RFF phalloplasty in a transgender man after coaptation of the LABC and MABC to the dorsal clitoral and ilioinguinal nerves, respectively. Bilateral cortical activation was identified in areas suspected to play a role in the conscious perception of sexual stimuli. Their findings suggest somatotopic arrangements in the groin and clitoris after stimulating the neophallus. While these data are indicative of successful nerve coaptation, no significant activation in the insular region was noted. This is the area that typically activates when stimuli are perceived as sexual. Also, stroking of the neophallus and groin led to diffuse and non-assessable findings on fMRI. These findings could not be further analyzed, making it difficult to understand the extent by which erogenous sensation contributes to these findings.

The “sensory upgrading phenomenon” offers additional thoughts as to the selection of donor and recipient nerves<sup>[58,69]</sup>. This concept is based on the finding that neurotized free flaps have better sensitivity at their new location as compared to their native site. This suggests that the sensory representation of the donor nerve in the cerebral cortex plays a more important role than the recipient nerve itself<sup>[58,69]</sup>. This is supported by findings in cisgender men which demonstrate decreased two-point discrimination in the non-operated donor site forearms as compared to the reinnervated radial forearm free flaps used for phalloplasty<sup>[58]</sup>. It is also consistent with the hypothesis proposed by Morrison *et al.*<sup>[15]</sup> which states that erogenous sensation is more dependent on the cortical interpretation of neural input rather than the choice of the recipient nerve. In other words, perhaps the choice of donor nerve is more important than the choice of recipient nerve. Regardless, many factors likely play a role in sensory recovery<sup>[58,69]</sup>, and most flaps report overall good sensory outcomes. Flap choice may play a less important role than previously thought. An additional hypothesis proposed by Gilbert *et al.*<sup>[71]</sup> is the potential for “dissociated sensibility”. This phenomenon is explained as the ability of the brain to interpret other peripheral stimuli as erogenous in nature. The authors hypothesize that the “cortex feels what it wants to feel” despite a scarcity of somatosensory input. They cite this as a possible mechanism for achieving erogenous sensation following phalloplasty procedures. This concept is also alluded to by Hage and De Graaf<sup>[31]</sup> when they hypothesized that cortical control exerts a “tremendous” influence regarding erogenous stimulation. These authors state that nerve coaptation should not be expected to result in erogenous phallic sensibility. Since four of their eleven patients reported erogenous sensation in some parts of their neophallus, they postulated that cortical control must play an important role in contributing to erogenous sensation. Further studies may help to delineate how these factors contribute to the sexual experience following phalloplasty.

Additionally, preservation and placement of the clitoris such that it is readily amenable to stimulation may be important in orgasmic potential following phalloplasty. Although studies comparing clitoral placement are lacking, the majority of studies report that placing the denuded clitoris at the base of the neophallus helps to consolidate the orgasm experience within the neophallus<sup>[7,8,10,15,28,29,42,70]</sup>. Therefore, a hybrid approach, combining tactile or protective sensation through coaptation to the ilioinguinal nerve and achieving erotic sensation by either coaptation to the dorsal clitoral nerve or preservation of the denuded clitoris may offer the most sensible approach.

Currently, no validated outcome instruments exist by which to evaluate erogenous sensation in transgender men following phalloplasty<sup>[15]</sup>. Erogenous sensory outcomes have relied on patient reports, such as the ability to achieve orgasm<sup>[8,22,29]</sup>. Selvaggi *et al.*<sup>[29]</sup> noted that 100% of patients were able to experience orgasm following RFF phalloplasty. These findings have been corroborated by other surgeons<sup>[8,15,22,24]</sup>. Monstrey *et al.*<sup>[24]</sup> reported > 80% improvement in sexuality and greater sexual satisfaction following RFF phalloplasty. Patients reported improved sexual satisfaction and greater ease in reaching orgasm. While the ability to reach orgasm may be correlated with direct stimulation of the clitoral nerve pathway, the presence of tactile sensation may facilitate orgasmic potential. Garcia *et al.*<sup>[8]</sup> cite patient reports identifying “purely psychological” pleasure and enhanced orgasm quality derived from self-stimulation of the phallus. 100% of patients in the RFF phalloplasty cohort and 90% of patients in the suprapubic phalloplasty cohort reported:

(1) masturbating their neophallus; and (2) achieving orgasm with stimulation of the phallus alone. This was independent of the treatment of the clitoris and supports the concept that additional factors contribute to the ability to achieve orgasm. One potential contributing factor is the improvement that occurs in one's identity following phalloplasty<sup>[12]</sup>. van de Grift *et al.*<sup>[12]</sup> report the development of positive self-esteem and affirmation of a masculine identity following phalloplasty. This may facilitate engagement in and, enjoyment of, sexual activity including increased sexual initiative and pleasure.

## CONCLUSION

While there is no consensus as to the optimal flap or the optimal pattern of innervation, tactile, and erogenous sensation following phalloplasty is an important goal. Understanding the relevant flap and donor site anatomy is important in optimizing outcomes, but there are likely multiple factors that contribute to both sexual satisfaction and orgasmic potential. Additional studies comparing various nerve coaptation strategies will help elucidate these issues.

## DECLARATIONS

### Authors' contributions

Literature review, writing: Hontscharuk R, Siotos C

Generation of an idea, editing, writing, photo contributor: Schechter LS

### Availability of data and materials

All referenced publications can be searched and found on PubMed. These papers are available to all readers through published journal websites.

### Financial support and sponsorship

None.

### Conflicts of interest

Hontscharuk R and Siotos C have no potential conflicts; Schechter LS receives royalties from textbooks from Elsevier and Springer publishers.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Consent for publication has been obtained from the publisher of the anatomy textbook.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Meervijk EL, Sevelius JM. Transgender population size in the United States: a meta-regression of population-based probability samples. *Am J Public Health* 2017;107:e1-8.
2. Winter S, Diamond M, Green J, et al. Transgender people: health at the margins of society. *Lancet* 2016;388:390-400.
3. Tollinche LE, Walters CB, Radix A, et al. The perioperative care of the transgender patient. *Anesth Analg* 2018;127:359-66.
4. Tran BNN, Epstein S, Singhal D, et al. Gender affirmation surgery: a synopsis using American College of Surgeons National Surgery Quality Improvement Program and National Inpatient Sample Databases. *Ann Plast Surg* 2018;80:S229-35.
5. American Society of Plastic Surgeons. Plastic Surgery Statistics. Available from: <https://www.plasticsurgery.org/news/plastic-surgery-statistics>. [Last accessed on 12 Oct 2020]
6. Al-Tamimi M, Pigot GL, Elfering L, et al. Genital gender-affirming surgery in transgender men in the Netherlands from 1989 to 2018: the evolution of surgical care. *Plast Reconstr Surg* 2020;145:153e-61.

7. Morrison SD, Shakir A, Vyas KS, et al. Phalloplasty: a review of techniques and outcomes. *Plast Reconstr Surg* 2016;138:594-615.
8. Garcia MM, Christopher NA, De Luca F, Spilotros M, Ralph DJ. Overall satisfaction, sexual function, and the durability of neophallus dimensions following staged female to male genital gender confirming surgery: the Institute of Urology, London U.K. experience. *Transl Androl Urol* 2014;3:156-62.
9. Al-Tamimi M, Pigot GL, van der Sluis WB, et al. The surgical techniques and outcomes of secondary phalloplasty after metoidioplasty in transgender men: an international, multi-center case series. *J Sex Med* 2019;16:1849-59.
10. Hage JJ, Bloem JJ, Suliman HM. Review of the literature on techniques for phalloplasty with emphasis on the applicability in female-to-male transsexuals. *J Urol* 1993;150:1093-8.
11. Cheng KX, Hwang WY, Eid AE, et al. Analysis of 136 cases of reconstructed penis using various methods. *Plast Reconstr Surg* 1995;95:1070-80; discussion 1081-4.
12. van de Grift TC, Pigot GLS, Kreukels BPC, Bouman MB, Mullender MG. Transmen's experienced sexuality and genital gender-affirming surgery: findings from a clinical follow-up study. *J Sex Marital Ther* 2019;45:201-5.
13. Overgoor ML, de Jong TP, Kon M. Restoring tactile and erogenous penile sensation in low-spinal-lesion patients: procedural and technical aspects following 43 TOMAX nerve transfer procedures. *Plast Reconstr Surg* 2014;134:294e-301.
14. Alwaal A, Breyer BN, Lue TF. Normal male sexual function: emphasis on orgasm and ejaculation. *Fertil Steril* 2015;104:1051-60.
15. Morrison SD, Massie JP, Dellon AL. Genital sensibility in the neophallus: getting a sense of the current literature and techniques. *J Reconstr Microsurg* 2019;35:129-37.
16. Masear VR, Meyer RD, Pichora DR. Surgical anatomy of the medial antebrachial cutaneous nerve. *J Hand Surg Am* 1989;14:267-71.
17. Race CM, Saldana MJ. Anatomic course of the medial cutaneous nerves of the arm. *J Hand Surg Am* 1991;16:48-52.
18. Beldner S, Zlotolow DA, Melone CP Jr, Agnes AM, Jones MH. Anatomy of the lateral antebrachial cutaneous and superficial radial nerves in the forearm: a cadaveric and clinical study. *J Hand Surg Am* 2005;30:1226-30.
19. Bourne MH, Wood MB, Carmichael SW. Locating the lateral antebrachial cutaneous nerve. *J Hand Surg Am* 1987;12:697-9.
20. Safa B, Lin WC, Salim AM, Deschamps-Braly JC, Poh MM. Current concepts in masculinizing gender surgery. *Plast Reconstr Surg* 2019;143:857e-71.
21. Frey JD, Poudrier G, Chiodo MV, Hazen A. An update on genital reconstruction options for the female-to-male transgender patient: a review of the literature. *Plast Reconstr Surg* 2017;139:728-37.
22. Kim SK, Lee KC, Kwon YS, Cha BH. Phalloplasty using radial forearm osteocutaneous free flaps in female-to-male transsexuals. *J Plast Reconstr Aesthet Surg* 2009;62:309-17.
23. Ascha M, Massie JP, Morrison SD, Crane CN, Chen ML. Outcomes of single stage phalloplasty by pedicled anterolateral thigh flap versus radial forearm free flap in gender confirming surgery. *J Urol* 2018;199:206-14.
24. Monstrey S, Hoebeke P, Selvaggi G, et al. Penile reconstruction: is the radial forearm flap really the standard technique? *Plast Reconstr Surg* 2009;124:510-8.
25. Garaffa G, Christopher NA, Ralph DJ. Total phallic reconstruction in female-to-male transsexuals. *Eur Urol* 2010;57:715-22.
26. Leriche A, Timsit MO, Morel-Journel N, et al. Long-term outcome of forearm free-flap phalloplasty in the treatment of transsexualism. *BJU Int* 2008;101:1297-300.
27. Babaei A, Safarinejad MR, Farrokhi F, Iran-Pour E. Penile reconstruction: evaluation of the most accepted techniques. *Urol J* 2010;7:71-8.
28. Fang RH, Lin JT, Ma S. Phalloplasty for female transsexuals with sensate free forearm flap. *Microsurgery* 1994;15:349-52.
29. Selvaggi G, Monstrey S, Ceulemans P, et al. Genital sensitivity after sex reassignment surgery in transsexual patients. *Ann Plast Surg* 2007;58:427-33.
30. Doria-Medina R, Carlsson Å, Jönsson EH, et al. fMRI after phalloplasty with nerve anastomosis in a trans-man patient. *Plast Reconstr Surg Glob Open* 2017;5:e1353.
31. Hage JJ, De Graaf FH. Addressing the ideal requirements by free flap phalloplasty: some reflections on refinements of technique. *Microsurgery* 1993;14:592-8.
32. Ribuffo D, Cigna E, Gargano F, Spalvieri C, Scuderi N. The innervated anterolateral thigh flap: anatomical study and clinical implications. *Plast Reconstr Surg* 2005;115:464-70.
33. Gilroy A, MacPherson B, Ross L. Superficial Nerves & Vessels of the Lower Limb. *Atlas of Anatomy*, 2nd ed. Thieme, New York; 2012. pp. 458-9.
34. Rubino C, Figus A, Dessy LA, et al. Innervated island pedicled anterolateral thigh flap for neo-phallic reconstruction in female-to-male transsexuals. *J Plast Reconstr Aesthet Surg* 2009;62:e45-9.
35. Morrison SD, Son J, Song J, et al. Modification of the tube-in-tube pedicled anterolateral thigh flap for total phalloplasty: the mushroom flap. *Ann Plast Surg* 2014;72 Suppl 1:S22-6.
36. Felici N, Felici A. A new phalloplasty technique: the free anterolateral thigh flap phalloplasty. *J Plast Reconstr Aesthet Surg* 2006;59:153-7.
37. Heston AL, Esmonde NO, Dugi DD 3rd, Berli JU. Phalloplasty: techniques and outcomes. *Transl Androl Urol* 2019;8:254-65.
38. Tubbs RS, Loukas M, Shahid K, et al. Anatomy and quantitation of the subscapular nerves. *Clin Anat* 2007;20:656-9.
39. Chu B, Bordoni B. Anatomy, Thorax, Thoracodorsal Nerves. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK539761/>. [Last accessed on 12 Oct 2020]
40. Perovic SV, Djinic R, Bumbasirevic M, Djordjevic M, Vukovic P. Total phalloplasty using a musculocutaneous latissimus dorsi flap. *BJU Int* 2007;100:899-905; discussion 905.
41. Dennis M, Granger A, Ortiz A, et al. The anatomy of the musculocutaneous latissimus dorsi flap for neophalloplasty. *Clin Anat* 2018;31:152-9.

42. Papadopoulos NA, Schaff J, Biemer E. The use of free prelaminate and sensate osteofasciocutaneous fibular flap in phalloplasty. *Injury* 2008;39 Suppl 3:S62-7.
43. Popieluszko P, Mizia E, Henry BM, et al. The surgical anatomy of the sural nerve: an ultrasound study. *Clin Anat* 2018;31450-5.
44. Standring S, Gray H. Gray's anatomy: the anatomical basis of clinical practice. Edinburgh: Churchill Livingstone/Elsevier; 2008.
45. Al-Tamimi M, Pigot GL, Ronkes B, et al. The first experience of using the pedicled labia minora flap for urethral lengthening in transgender men undergoing anterolateral thigh and superficial circumflex iliac artery perforator flap phalloplasty: a multicenter study on clinical outcomes. *Urology* 2020;138:179-87.
46. Veerman H, de Rooij FPW, Al-Tamimi M, et al. Functional outcomes and urological complications after genital gender affirming surgery with urethral lengthening in transgender men. *J Urol* 2020;204:104-9.
47. D'Arpa S, Claes K, Lumen N, et al. Urethral reconstruction in anterolateral thigh flap phalloplasty: a 93-case experience. *Plast Reconstr Surg* 2019;143:382e-92.
48. Iida T, Mihara M, Yoshimatsu H, Narushima M, Koshima I. Versatility of the superficial circumflex iliac artery perforator flap in head and neck reconstruction. *Ann Plast Surg* 2014;72:332-6.
49. Koshima I, Nanba Y, Tsutsui T, et al. Superficial circumflex iliac artery perforator flap for reconstruction of limb defects. *Plast Reconstr Surg* 2004;113:233-40.
50. Terrier JÉ, Courtois F, Ruffion A, Morel Journal N. Surgical outcomes and patients' satisfaction with suprapubic phalloplasty. *J Sex Med* 2014;11:288-98.
51. Ndiaye A, Diop M, Ndiaye JM, et al. Emergence and distribution of the ilioinguinal nerve in the inguinal region: applications to the ilioinguinal anaesthetic block (about 100 dissections). *Surg Radiol Anat* 2010;32:55-62.
52. Hollinshead WH. Anatomy for Surgeons, Volume 2: The Back And Limbs. New York: Harper and Row; 1969.
53. Kaur J, Singh P. Pudendal nerve entrapment syndrome. 2020 Mar 5. Treasure Island (FL): StatPearls Publishing; 2020.
54. Baskin LS. Anatomical studies of the female genitalia: surgical reconstructive implications. *J Pediatr Endocrinol Metab* 2004;17:581-7.
55. Yucel S, De Souza A Jr, Baskin LS. Neuroanatomy of the human female lower urogenital tract. *J Urol* 2004;172:191-5.
56. Baskin LS, Erol A, Li YW, et al. Anatomical studies of the human clitoris. *J Urol* 1999;162:1015-20.
57. Tajkarimi K, Burnett AL. The role of genital nerve afferents in the physiology of the sexual response and pelvic floor function. *J Sex Med* 2011;8:1299-312.
58. Ma S, Cheng K, Liu Y. Sensibility following innervated free radial forearm flap for penile reconstruction. *Plast Reconstr Surg* 2011;127:235-41.
59. Waris T, Rechart L, Kyösola K. Reinnervation of human skin grafts: a histochemical study. *Plast Reconstr Surg* 1983;72:439-47.
60. Siemionow M, Brzezicki G. Chapter 8: current techniques and concepts in peripheral nerve repair. *Int Rev Neurobiol* 2009;87:141-72.
61. Griffin JW, Hogan MV, Chhabra AB, Deal DN. Peripheral nerve repair and reconstruction. *J Bone Joint Surg Am* 2013;95:2144-51.
62. Ducic I, Safa B, DeVinney E. Refinements of nerve repair with connector-assisted coaptation. *Microsurgery* 2017;37:256-63.
63. Panagopoulos GN, Megaloikonomos PD, Mavrogenis AF. The present and future for peripheral nerve regeneration. *Orthopedics* 2017;40:e141-56.
64. Pabari A, Lloyd-Hughes H, Seifalian AM, Mosahebi A. Nerve conduits for peripheral nerve surgery. *Plast Reconstr Surg* 2014;133:1420-30.
65. Lin MY, Manzano G, Gupta R. Nerve allografts and conduits in peripheral nerve repair. *Hand Clin* 2013;29:331-48.
66. Khouri RK, Young VL, Casoli VM. Long-term results of total penile reconstruction with a prefabricated lateral arm free flap. *J Urol* 1998;160:383-8.
67. Ramesh S, Serjius A, Wong TB, Jagjeet S, John R. Two stage penile reconstruction with free prefabricated sensate radial forearm osteocutaneous flap. *Med J Malaysia* 2008;63:343-5.
68. Cheng KX, Zhang RH, Zhou S, et al. Cheng's method for reconstruction of a functionally sensitive penis. *Plast Reconstr Surg* 1997;99:87-91; discussion 92.
69. Ma S, Liu Y, Chang T, Cheng K. Long-term follow-up of sensation recovery of the penis reconstructed by Cheng's method. *Plast Reconstr Surg* 2011;127:1546-52.
70. Schaff J, Papadopoulos NA. A new protocol for complete phalloplasty with free sensate and prelaminate osteofasciocutaneous flaps: experience in 37 patients. *Microsurgery* 2009;29:413-9.
71. Gilbert DA, Williams MW, Horton CE, et al. Phallic reinnervation via the pudendal nerve. *J Urol* 1988;140:295-9.
72. Fang RH, Kao YS, Ma S, Lin JT. Phalloplasty in female-to-male transsexuals using free radial osteocutaneous flap: a series of 22 cases. *Br J Plast Surg* 1999;52:217-22.



Review

Open Access



# Review on the treatment of scars

Daniel J. Callaghan

Colorado Dermatology Specialists, Denver, CO 80237, USA.

**Correspondence to:** Dr. Daniel J. Callaghan, Colorado Dermatology Specialists, 3540 S Poplar St Suite 300, Denver, CO 80237, USA. E-mail: danieljcallaghan3@gmail.com

**How to cite this article:** Callaghan DJ. Review on the treatment of scars. *Plast Aesthet Res* 2020;7:66.  
<http://dx.doi.org/10.20517/2347-9264.2020.166>

**Received:** 16 Aug 2020 **First Decision:** 15 Sep 2020 **Revised:** 29 Sep 2020 **Accepted:** 26 Oct 2020 **Published:** 18 Nov 2020

**Academic Editor:** James E. Zins **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Scarring is a major concern for patients. From acne scarring to surgical scars, scars can have a dramatically negative effect on one's self-esteem and are a common complaint for which patients seek treatment. This review will focus on the treatment of acne scarring including ice pick, boxcar and rolling scars, and also the treatment of surgical scars including atrophic and hypertrophic scars.

**Keywords:** Scarring, acne scarring, surgical scars

## INTRODUCTION

Scarring is a condition that aesthetic physicians are frequently called upon to improve. The treatment of scars can be a rewarding albeit frustrating endeavor. Scars or scarring come in a number of varieties, and treatment must be tailored specifically for each patient. This chapter will focus on the treatment of acne scars and surgical scars as these are most routinely encountered in practice. Acne can produce ice pick, rolling or boxcar scars and treatment can vary widely from the use of fillers, trichloroacetic acid (TCA) or energy-based devices. Similarly, surgical scars can be treated with a number of modalities from injectables such as intralesional triamcinolone or 5-fluorouracil (5-FU) to resurfacing technologies.

## ACNE SCARS

When it comes to facial rejuvenation, the treatment of acne scars is one of the things that can make the most dramatic improvement. While acne, and thereby acne scarring, generally occurs in one's teens or



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



twenties, patients can come in requesting treatment of acne scarring at any age. Acne scarring is generally classified as ice pick, rolling or boxcar, and the treatments of each subtype can vary. That said, patients typically have a variety of these subtypes at any given time, and this must be taken into account when deciding on the preferred treatment approach. One challenge in determining the optimal approach for treating acne scars is that there is a dearth of high-quality studies. The studies that exist are often small and underpowered, biased, without uniform baseline variables or outcomes or without long-term follow-up<sup>[1]</sup>.

### Ice pick scars

Ice pick scars are deep but narrow (< 2 mm) scars that look like they could have been created by an ice pick. Due to the depth of the scars, which can extend into the dermis, they are often more resistant to the typical treatment modalities used for rolling or boxcar scars. Although they have less treatment options in general, the ones that they have can provide superb results.

Punch excision is an excellent treatment option for ice pick scars. Although this is essentially trading a scar for a scar, the scars created by the punch excision itself often heal to the point they are difficult to see<sup>[2]</sup>. For the best cosmetic outcome, scars should be at least 4-5 mm apart to be treated at the same time. Otherwise, there will be too much tension on the skin surface for them to heal optimally. If scars are within 4-5 mm of one another, then waiting 4 weeks between treatments will provide the best long-term results<sup>[3]</sup>.

The use of TCA, particularly with the CROSS technique (chemical reconstruction of skin scars), has more recently emerged as a treatment option for ice pick scars. The CROSS technique involves using an instrument such as a syringe needle or a sharpened wooden applicator that is dipped into high-concentration TCA and then applied directly onto the scar. The desired endpoint is a white frosting of the scar. TCA creates coagulative necrosis of the epidermis, thereby increasing collagen production which ultimately results in improvement of the scar<sup>[2]</sup>.

In a study of 30 patients treated with the CROSS technique utilizing 100% TCA every two weeks for a total of 4 sessions, Khunger *et al.*<sup>[4]</sup> found that 73% of patients achieved excellent improvement in ice pick scars, whereas 20% achieved good improvement. Side effects of this technique include hypopigmentation which is largely transient, a burning or tingling sensation at the time of treatment and erythema or edema<sup>[4]</sup>. The CROSS technique has also been described using other chemicals such as 88% phenol with similar results as TCA<sup>[5]</sup>.

Although energy-based devices often provide less-than-satisfactory results for the treatment of ice pick scars, Ramesh *et al.*<sup>[6]</sup> found that ice pick scars responded better than rolling or boxcar scars to a fractional radiofrequency (FRF) device. Conversely, other studies found the opposite result<sup>[6,7]</sup>.

### Rolling scars

Rolling scars are typically  $\geq 4$  mm in diameter and have soft, irregular walls which gives them a rolling appearance. These are caused by bands that tether the subcutis to the dermis. As such, the treatment of these scars generally targets these bands to improve their appearance.

Subcision has been a longstanding technique to target and release these bands. In this method, an instrument, such as a needle, is inserted into the subcutaneous plane and fanned back and further in an effort to sever these bands. Blunt blade subcision has also been used, in which a blunt blade is inserted in a single puncture site and is able to safely treat a wider area. A study by Barikbin *et al.*<sup>[8]</sup> involving 18 patients with mainly rolling scars found that this method led to marked improvement in 50% of patients, while 33% had moderate improvement and 17% mild improvement<sup>[8]</sup>.

Fillers have also been used to treat rolling scars. Sapra *et al.*<sup>[9]</sup> looked into the use of poly-L-lactic acid for the treatment of rolling scars in 22 patients and found that 68.2% of patients had a satisfactory response as judged by blinded-evaluators. Hyaluronic acid and calcium hydroxyapatite have also been used to treat acne scars with success<sup>[10,11]</sup>.

Resurfacing is also used to treat rolling scars but will be discussed further below. Although it is not directly targeting the bands tethering the scars down, it can be effective in many circumstances.

### Boxcar scars

Boxcar scars are wider than ice pick scars (1-4 mm in diameter) which gives them a U-shaped appearance. Their sharply demarcated edges are in contrast to the soft edges of rolling scars and can extend 0.1-0.5 mm into the dermis. Although boxcar scars are indeed a distinct form of acne scar, they are seldomly studied in isolation but rather are most often grouped together with the treatment of other types of scars.

In general, boxcar scars are treated with resurfacing, which can be performed with anything from a chemical peel or microneedling to a number of different energy-based devices. The aggressiveness of the treatment is often correlated to the results obtainable, but also must be weighed against the risks as well as the acceptable downtime for the patient.

Microneedling can be performed either with a dermaroller or a microneedling pen, and can be performed alone or with the use of a variety of topical applications to the pores created by microneedling such as platelet rich plasma (PRP). Alam *et al.*<sup>[12]</sup> performed a randomized, split-face study with a dermaroller on a number of morphologic acne scar types and found that after 3 treatments there was improvement in scarring, with a mean difference of 3.4 based on the quantitative global scarring grading system ( $P = 0.03$ )<sup>[12]</sup>. A separate blinded, randomized controlled trial involving 42 patients comparing microneedling to a non-ablative fractional erbium 1,340-nm laser found that both were effective and that there was no statistically significant difference between the two ( $P = 0.264$ ). Microneedling had fewer side effects and less downtime<sup>[13]</sup>. One study found microneedling combined with the use of PRP to be more effective than microneedling alone; however, an alternative study demonstrated no difference in these outcomes<sup>[14,15]</sup>.

Of the energy-based devices, fully ablative lasers typically offer the best cosmetic outcomes, but at the cost of the longest downtime and greatest risk for adverse events. Walia and Alster<sup>[16]</sup> demonstrated a 75% improvement in atrophic acne scars at 18 months after high-energy CO<sub>2</sub> laser treatment<sup>[16]</sup>. However, erythema lasting on average 3.5 months and a 36% incidence of hyperpigmentation help explain why this is not a commonly used modality to treat acne scars.

Fractional ablative lasers have helped to fill this void. They are effective but have a more acceptable recovery and side effect profile than fully ablative lasers. Bjørn *et al.*<sup>[17]</sup> found that a fractional CO<sub>2</sub> laser improved acne scarring with minor postoperative adverse effects, and that a treatment interval of either 1 month or 3 months did not influence the final outcome. Cho *et al.*<sup>[18]</sup> compared the efficacy of fractional CO<sub>2</sub> to non-ablative fractional laser (NAFL) treatment with the 1,550-nm erbium:glass laser. They found that while the fractional CO<sub>2</sub> laser demonstrated greater improvement, it was not statistically significant as there were only 8 patients in the study<sup>[18]</sup>. This improvement came at the cost of greater adverse effects including erythema and crusting.

NAFL are a mainstay in the treatment of acne scarring. With a lower downtime than ablative fractional lasers, patients often prefer them even if they may require more treatment sessions to achieve equal results. Sardana *et al.*<sup>[19]</sup> found that boxcar scars were most responsive to treatment with the 1,540-nm erbium:glass laser, demonstrating a 52.9% improvement compared to rolling scars which had a 43.1% improvement.

Boxcar scars had a statistically significant improvement after four sessions ( $P < 0.05$ ). Ice pick scars showed the lowest improvement rate of only 25.9%, although this was not statistically significant ( $P = 0.09$ )<sup>[19]</sup>.

Radiofrequency devices can be monopolar, bipolar or fractional. Of these, FRF devices seem to provide the best results, with an expected improvement of 25% to 75% after 3 to 4 sessions. Although adverse effects are limited, the procedure itself can be associated with a significant amount of pain, even with nerve blocks or topical anesthesia<sup>[20,21]</sup>.

### Erythematous scars

Beyond treating the textural changes of acne scarring, a typical complaint is post-inflammatory erythema. Although with time, this typically resolves on its own, but it can take months if not years. Vascular lasers such as the 595-nm pulsed dye laser (PDL) or 532-nm potassium titanyl phosphate (KTP) laser are widely used to treat this erythema because of their consistent and reliable results with minimal adverse effects.

## SURGICAL SCARS

Physicians must be well-versed in the treatment of surgical scars. Every patient heals differently, and even the most precise surgical technique can lead to scarring. The treatment of an unfortunately placed or unsightly scar can be the most immediate thing a patient can do to improve his or her appearance, as the scar is often the first thing one's eye is attracted to upon seeing a person.

There are a number of things to consider when treating a surgical scar, including the timing of when interventions should be implemented, and what specific interventions should take place. Scars can manifest in a number of ways, and may be erythematous, raised or depressed.

Perhaps the earliest question that physicians or surgeons face in the management of scars is what should patients do in the immediate aftermath of surgery. Beyond appropriate wound care and timely suture removal, patients frequently inquire about the benefit of silicone gel sheeting. Although there have been a number of studies published touting the effects of silicone gel sheeting not only for preventing hypertrophic scars but also to treat those that are already present, a systematic review involving 20 trials and 873 patients found the evidence to be weak and heavily susceptible to bias<sup>[22]</sup>.

### Hypertrophic scars

Hypertrophic scars are commonly treated with a number of modalities including intralesional kenalog (ILK), 5-FU or laser treatments. ILK has long been considered the first line treatment of hypertrophic scars. ILK suppresses inflammation, causes vasoconstriction which reduces the delivery of oxygen and nutrients to the scar and also has an antimitotic effect, inhibiting the growth of keratinocytes and fibroblasts. Additionally, it reduces plasma protease inhibitors which degrade collagen through collagenase<sup>[23]</sup>. The concentration used needs to be carefully considered for each individual scar and is dependent on the size and location of the scar. It is prudent to start with a lower dose with the expectation that multiple treatments may be necessary rather than risk using a higher dose which may lead to atrophy and pigmentary changes. It is much easier to treat conservatively than to have to treat additional complications down the line.

5-FU is a well-established albeit less commonly used technique for the treatment of hypertrophic scars. 5-FU is an antimetabolic agent that has been demonstrated to inhibit fibroblast proliferation and decrease collagen synthesis<sup>[24]</sup>. It can be used alone or in combination with ILK, and has been shown to decrease the risk of side effects of ILK when used in combination<sup>[25,26]</sup>. 5-FU should not be used in patients who have an infection, are pregnant or have anemia, leukopenia or bone marrow suppression. 5-FU is typically injected at a dose of 50 mg/mL for a maximum dose ranging from 50-150 mg. It can be diluted with ILK, which is typically diluted to a dose of 2 to 10 mg/mL dependent on the size and location of the scar. Care must be taken to avoid overtreating the scars, which can lead to atrophy, being more challenging to treat.

Both ablative and non-ablative resurfacing are also popular techniques in treating hypertrophic scars. More recently, these have been combined with laser-assisted drug delivery with corticosteroids or 5-FU<sup>[27,28]</sup>. The use of 5-FU has been demonstrated to be as effective but with fewer side effects than the use of corticosteroids in laser-assisted drug delivery<sup>[28]</sup>.

Dermabrasion is one of the oldest methods used to revise scars. It can be done manually with sandpaper, or mechanically with a rotating wire brush or diamond fraise. Dermabrasion, particularly mechanical dermabrasion, is extremely operator dependent and carries a number of risks including making the scar worse. A randomized controlled trial comparing fractional ablative resurfacing to dermabrasion found that while both were effective, laser resurfacing was safer and showed quicker clinical recovery<sup>[29]</sup>. Conversely, in a randomized, blinded, split-scar study involving 14 patients, manual dermabrasion with sterilized sandpaper was demonstrated to be an effective but safe, simple and cost-effective treatment option for surgical scars<sup>[30]</sup>. Mechanical dermabrasion has fallen out of favor due to the risks associated with aerosolization of blood.

### Atrophic scars

Atrophic surgical scars show a different set of challenges than hypertrophic scars and can generally be more difficult to treat.

Fractional laser therapy with either non-ablative or fully ablative lasers has been shown to improve the color, texture, thickness and patient satisfaction of atrophic surgical scars<sup>[31-33]</sup>. These lasers are effective because they stimulate neocollagenesis and dermal remodeling.

The use of fillers has been shown to improve the appearance of atrophic surgical scars. Both hyaluronic acid and calcium hydroxyapatite have been shown to be safe and effective with the additional benefit of having an immediate improvement<sup>[34]</sup>. One downside to the use of fillers is that the results are not permanent.

### Pigmentary changes

Pigmentary changes can affect both hypertrophic and atrophic scars. The most common color change is typically erythema, resulting from the healing process that stimulates neovascularization. Scars can also be hyper- or hypopigmented. Although the previously mentioned techniques to treat scar texture may provide the added benefit of improving such pigmentary changes, in many cases this must be addressed separately.

Erythematous scars tend to be relatively receptive to treatment. PDL has long been used to treat erythematous surgical scars. It has been shown to be effective at both short and long pulse durations<sup>[35]</sup>. Although improvement in erythema should be the main objective when treating surgical scars with PDL, it has been shown to improve texture as well<sup>[36]</sup>. The 532-nm KTP laser is also well-established for the treatment of scars and has been demonstrated to be comparable in safety and efficacy to PDL<sup>[37]</sup>.

Hypopigmented scars can be challenging to treat; however, the combination of fractional resurfacing with the use of topical tretinoin, pimecrolimus or bimatoprost has been shown to be effective at re-pigmenting the scar<sup>[38]</sup>. Similarly, laser-assisted drug delivery of bimatoprost has been shown to be effective at repigmenting hypopigmented scars<sup>[39]</sup>.

### EMERGING TECHNOLOGIES

Although this chapter focused on the most commonly used techniques to treat scars, the field of medicine is always working on emerging technologies that may one day complement or replace standard therapies. One such technology that may play a role in the management of scarring is laser speckle contrast imaging (LSCI). LSCI illuminates tissue with coherent laser light and then detects backscatter from the tissue



which ultimately can be used to detect blood flow<sup>[40]</sup>. This is relevant to scarring because adequate tissue perfusion is necessary for the healing process to take place. This technology has been studied in patients with systemic sclerosis and can detect a reduction of blood perfusion in areas affected by Raynaud's phenomenon. It also has been used to demonstrate that a decrease in blood perfusion is found in patients with microangiopathy<sup>[41]</sup>. LSCI has also been used to help evaluate burn wounds which is important, because it can detect the severity of partial-thickness wounds, which in turn influences treatment<sup>[42]</sup>.

## CONCLUSION

Scarring, regardless of etiology, is a challenging but treatable condition that can make a significant difference in the lives of patients. Although energy-based devices are the workhorses of many treatment regimens, they are not absolutely necessary, and any physician can be equipped to manage them. A thorough understanding of different types of scars is crucial to tailor a treatment course for individual patients. The treatment of acne scars differs depending on whether they are ice pick, rolling or boxcar scars. Surgical scars may be raised or depressed, or suffer from pigmentary changes, and treatments vary for each. As our understanding of the formation and maturation of scars continues to develop, new technologies will likely emerge to target scars or even inhibit their formation altogether.

## DECLARATIONS

### Authors' contributions

Contribute solely to the article: Callaghan DJ

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

The author declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Abdel Hay R, Shalaby K, Zaher H, et al. Interventions for acne scars. *Cochrane Database Syst Rev* 2016;4:CD011946.
2. Levy LL, Zeichner JA. Management of acne scarring, part II: a comparative review of non-laser-based, minimally invasive approaches. *Am J Clin Dermatol* 2012;13:331-40.
3. Jacob CI, Dover JS, Kaminer MS. Acne scarring: a classification system and review of treatment options. *J Am Acad Dermatol* 2001;45:109-17.
4. Khunger N, Bhardwaj D, Khunger M. Evaluation of CROSS technique with 100% TCA in the management of ice pick acne scars in darker skin types. *J Cosmet Dermatol* 2011;10:51-7.
5. Dalpizzol M, Weber MB, Mattiazzi AP, Manzoni AP. Comparative study of the use of trichloroacetic acid and phenolic acid in the treatment of atrophic-type acne scars. *Dermatol Surg* 2016;42:377-83.
6. Ramesh M, Gopal M, Kumar S, Talwar A. Novel technology in the treatment of acne scars: the matrix-tunable radiofrequency technology.

- J Cutan Aesthet Surg* 2010;3:97-101.
7. Peterson JD, Palm MD, Kiripolsky MG, Guiha IC, Goldman MP. Evaluation of the effect of fractional laser with radiofrequency and fractionated radiofrequency on the improvement of acne scars. *Dermatol Surg* 2011;37:1260-7.
8. Barikbin B, Akbari Z, Yousefi M, Dowlati Y. Blunt blade dubcision: an evolution in the treatment of atrophic acne scars. *Dermatol Surg* 2017;43 Suppl 1:S57-63.
9. Sapra S, Stewart JA, Mraud K, Schupp R. A canadian study of the use of poly-L-lactic acid dermal implant for the treatment of hill and valley acne scarring. *Dermatol Surg* 2015;41:587-94.
10. Goodman GJ, Van Den Broek A. The modified tower vertical filler technique for the treatment of post-acne scarring. *Australas J Dermatol* 2016;57:19-23.
11. Goldberg DJ, Amin S, Hussain M. Acne scar correction using calcium hydroxylapatite in a carrier-based gel. *J Cosmet Laser Ther* 2006;8:134-6.
12. Alam M, Han S, Pongprutthipan M, et al. Efficacy of a needling device for the treatment of acne scars: a randomized clinical trial. *JAMA Dermatol* 2014;150:844-9.
13. Cachafeiro T, Escobar G, Maldonado G, Cestari T, Corleta O. Comparison of nonablative fractional erbium laser 1,340 nm and microneedling for the treatment of atrophic acne scars: a randomized clinical trial. *Dermatol Surg* 2016;42:232-41.
14. Asif M, Kanodia S, Singh K. Combined autologous platelet-rich plasma with microneedling verses microneedling with distilled water in the treatment of atrophic acne scars: a concurrent split-face study. *J Cosmet Dermatol* 2016;15:434-43.
15. Ibrahim MK, Ibrahim SM, Salem AM. Skin microneedling plus platelet-rich plasma versus skin microneedling alone in the treatment of atrophic post acne scars: a split face comparative study. *J Dermatolog Treat* 2018;29:281-6.
16. Walia S, Alster TS. Prolonged clinical and histologic effects from CO2 laser resurfacing of atrophic acne scars. *Dermatol Surg* 1999;25:926-30.
17. Bjørn M, Stausbøl-Grøn B, Braae Olesen A, Hedelund L. Treatment of acne scars with fractional CO2 laser at 1-month versus 3-month intervals: an intra-individual randomized controlled trial. *Lasers Surg Med* 2014;46:89-93.
18. Cho SB, Lee SJ, Cho S, et al. Non-ablative 1550-nm erbium-glass and ablative 10 600-nm carbon dioxide fractional lasers for acne scars: a randomized split-face study with blinded response evaluation. *J Eur Acad Dermatol Venereol* 2010;24:921-5.
19. Sardana K, Manjhi M, Garg VK, Sagar V. Which type of atrophic acne scar (ice-pick, boxcar, or rolling) responds to nonablative fractional laser therapy? *Dermatol Surg* 2014;40:288-300.
20. Simmons BJ, Griffith RD, Falto-Aizpurua LA, Nouri K. Use of radiofrequency in cosmetic dermatology: focus on nonablative treatment of acne scars. *Clin Cosmet Investig Dermatol* 2014;7:335-9.
21. Boen M, Jacob C. A review and update of treatment options using the acne scar classification system. *Dermatol Surg* 2019;45:411-22.
22. O'Brien L, Jones DJ. Silicone gel sheeting for preventing and treating hypertrophic and keloid scars. *Cochrane Database Syst Rev* 2013;2013:CD003826.
23. Morelli Coppola M, Salzillo R, Segreto F, Persichetti P. Triamcinolone acetonide intralesional injection for the treatment of keloid scars: patient selection and perspectives. *Clin Cosmet Investig Dermatol* 2018;11:387-96.
24. Bulstrode NW, Mudera V, McGrouther DA, Grobbelaar AO, Cambrey AD. 5-fluorouracil selectively inhibits collagen synthesis. *Plast Reconstr Surg* 2005;116:209-21; discussion 222-3.
25. Darougheh A, Asilian A, Shariati F. Intralesional triamcinolone alone or in combination with 5-fluorouracil for the treatment of keloid and hypertrophic scars. *Clin Exp Dermatol* 2009;34:219-23.
26. Ren Y, Zhou X, Wei Z, Lin W, Fan B, Feng S. Efficacy and safety of triamcinolone acetonide alone and in combination with 5-fluorouracil for treating hypertrophic scars and keloids: a systematic review and meta-analysis. *Int Wound J* 2017;14:480-7.
27. Waibel JS, Wulkan AJ, Shumaker PR. Treatment of hypertrophic scars using laser and laser assisted corticosteroid delivery. *Lasers Surg Med* 2013;45:135-40.
28. Waibel JS, Wulkan AJ, Rudnick A, Daoud A. Treatment of hypertrophic scars using laser-assisted corticosteroid versus laser-assisted 5-fluorouracil delivery. *Dermatol Surg* 2019;45:423-30.
29. Christophel JJ, Elm C, Endrizzi BT, Hilger PA, Zelickson B. A randomized controlled trial of fractional laser therapy and dermabrasion for scar resurfacing. *Dermatol Surg* 2012;38:595-602.
30. Poulos E, Taylor C, Solish N. Effectiveness of dermasanding (manual dermabrasion) on the appearance of surgical scars: a prospective, randomized, blinded study. *J Am Acad Dermatol* 2003;48:897-900.
31. Cohen JL. Minimizing skin cancer surgical scars using ablative fractional Er: Yag laser treatment. *J Drugs Dermatol* 2013;12:1171-3.
32. Gokalp H. Evaluation of nonablative fractional laser treatment in scar reduction. *Lasers Med Sci* 2017;32:1629-35.
33. Tidwell WJ, Owen CE, Kulp-Shorten C, Maity A, McCall M, Brown TS. Fractionated Er: YAG laser versus fully ablative Er: YAG laser for scar revision: results of a split scar, double blinded, prospective trial. *Lasers Surg Med* 2016;48:837-43.
34. Kasper DA, Cohen JL, Saxena A, Morganroth GS. Fillers for postsurgical depressed scars after skin cancer reconstruction. *J Drugs Dermatol* 2008;7:486-7.
35. Nouri K, Elsaie ML, Vejjabhinanta V, et al. Comparison of the effects of short- and long-pulse durations when using a 585-nm pulsed dye laser in the treatment of new surgical scars. *Lasers Med Sci* 2010;25:121-6.
36. Alster T, Williams C. Treatment of keloid sternotomy scars with 585 nm flashlamp-pumped pulsed-dye laser. *Lancet* 1995;345:1198-200.
37. Keaney TC, Tanzi E, Alster T. Comparison of 532 nm potassium titanyl phosphate laser and 595 nm pulsed dye laser in the treatment of erythematous surgical scars: a randomized, controlled, open-label study. *Dermatol Surg* 2016;42:70-6.
38. Massaki AB, Fabi SG, Fitzpatrick R. Repigmentation of hypopigmented scars using an erbium-doped 1,550-nm fractionated laser and

- topical bimatoprost. *Dermatol Surg* 2012;38:995-1001.
39. Waibel JS, Rudnick A, Arheart KL, Nagrai N, Gonzalez A, Gianatasio C. Re-pigmentation of hypopigmentation: fractional lasers vs laser-assisted delivery of bimatoprost vs epidermal melanocyte harvesting system. *J Drugs Dermatol* 2019;18:1090-6.
  40. Heeman W, Steenbergen W, van Dam G, Boerma EC. Clinical applications of laser speckle contrast imaging: a review. *J Biomed Opt* 2019;24:1-11.
  41. Ruaro B, Sulli A, Pizzorni C, Paolino S, Smith V, Cutolo M. Correlations between skin blood perfusion values and nailfold capillaroscopy scores in systemic sclerosis patients. *Microvasc Res* 2016;105:119-24.
  42. Stewart CJ, Frank R, Forrester KR, Tulip J, Lindsay R, Bray RC. A comparison of two laser-based methods for determination of burn scar perfusion: laser Doppler versus laser speckle imaging. *Burns* 2005;31:744-52.

Review

Open Access



# Laser Resurfacing for the Management of Periorbital Scarring

Nathan Pirakitikulr<sup>1</sup>, John J. Martin<sup>2</sup>, Sara T. Wester<sup>1</sup>

<sup>1</sup>Division of Oculofacial Plastic and Reconstructive Surgery, Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami-Miller School of Medicine, Miami, FL 33136, USA.

<sup>2</sup>Private practice, Coral Gables, FL 33134, USA.

**Correspondence to:** Dr. Sara T. Wester, Division of Oculofacial Plastic and Reconstructive Surgery, Department of Ophthalmology, Bascom Palmer Eye Institute, University of Miami-Miller School of Medicine, 900 NW 17th St, Miami, FL 33136, USA.  
E-mail: swester2@med.miami.edu

**How to cite this article:** Pirakitikulr N, Martin JJ, Wester ST. Laser resurfacing for the management of periorbital scarring. *Plast Aesthet Res* 2020;7:67. <http://dx.doi.org/10.20517/2347-9264.2020.77>

**Received:** 13 Apr 2020 **First Decision:** 27 Jul 2020 **Revised:** 28 Aug 2020 **Accepted:** 9 Nov 2020 **Published:** 20 Nov 2020

**Academic Editor:** Antonino Araco **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Laser (light amplification by the stimulated emission of radiation) skin resurfacing is currently one of the most widely adopted technologies in facial rejuvenation. While most often used for aesthetic purposes, lasers also have applications in the management of scars. Since the introduction of the CO<sub>2</sub> laser for skin rejuvenation in the 1990s, the last three decades have seen significant growth in the number of laser devices available to the physician. More recently, promising alternatives to light-based resurfacing technologies have emerged that include radiofrequency and intense focused ultrasound. To help the physician navigate the most current laser technologies as they apply to periocular scars, this review discusses the available treatment modalities, pre-treatment assessment of periorbital scars, treatment selection, and reported outcomes and complications. The recommendations described herein are based on published literature and the authors' experience in an academic oculoplastics practice.

**Keywords:** Periorbital scarring, ectropion, laser resurfacing, laser assisted drug delivery

## INTRODUCTION

Laser skin resurfacing is an important adjunct in the management of many types of periorbital scars. Skin in this region is prone to photoaging, telangiectasias, erythema, and hypertrophy. Scars arising from prior surgery, trauma, or inflammation are highly visible and may ultimately compromise the mechanical



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Table 1. Properties of lasers used for periorbital skin resurfacing**

		Ablative			Non-ablative					
		CO <sub>2</sub>	Er: YAG	Er:YSGG	Er:Glass	Nd:YAG	Alex	Ruby	PDL	KTP
Wavelength (nm)		10,600	2,940	2,790	1,540	1,064	755	694	585-595	532
Fractional		x	x	x	x	x		x	x	x
Depth (mm)		2.0	1.0	1.0	1.4	1.0	0.7	0.7	1.2	0.8
Chromophore	Hemoglobin		x	x	x	x	x		x	x
	Melanin						x	x		x
	Water	x	x	x	x					
Emission	Continuous	x	x	x	x					
	Long-Pulsed	x	x	x	x		x	x	x	x
	Q-switched					x	x	x		
	Picosecond					x	x			

IPL: intense pulsed light; KTP: potassium titanyl phosphate; PDL: pulsed dye laser

function of the eyelids, thereby causing damage to the ocular surface. Initially, periorbital scars are most often managed conservatively with mechanical massage or medically with topical and intralesional corticosteroids and antimetabolites such as 5-fluorouracil<sup>[1]</sup>. Lasers can be used as an alternative or in combination with some of these therapies<sup>[2]</sup>. Lasers can help soften scar tissue through controlled thermal damage to the skin to promote collagen remodeling<sup>[3,4]</sup>. In addition, lasers can aid in topical drug delivery by increasing skin permeability, which helps distribute and increase the penetrance of topically applied medications<sup>[4]</sup>. By selectively targeting specific chromophores, lasers can also be used to address dyspigmentation<sup>[5]</sup>. Complications are rare with proper preoperative assessment and technique, but the susceptibility of the eye to laser damage warrants special precautions. In this review, we present a general approach to treating periorbital scars with laser. Recommendations are based on the authors' clinical practice and a review of the PubMed-indexed literature published within the last 30 years. Sources include systematic reviews, meta-analyses, and clinical trials, which are cited accordingly throughout the text.

## PRINCIPLES OF PERI-OCULAR LASER SKIN RESURFACING

A wide range of lasers has been used to treat the periorbital tissue [Table 1]<sup>[6,7]</sup>. Lasers used for skin resurfacing are defined by their lasing medium and emission wavelength, and further categorized based on whether the superficial epidermis is removed during treatment. Ablative lasers, which include CO<sub>2</sub>, Erbium:YAG, and Erbium:yttrium-scandium-gallium-garnet (Er:YSGG) lasers, were the first lasers to come to market and target both the dermis and the overlying epidermis. These lasers can be very effective; however, they also carry a greater risk of causing scarring and hyperpigmentation, particularly in patients with higher Fitzpatrick skin types. In contrast, non-ablative lasers do not cause thermal damage to the overlying epidermis. Examples include Erbium:glass, diode, Nd:YAG, alexandrite, ruby, pulsed dye (PDL), and potassium titanyl phosphate.

Both ablative and non-ablative lasers can be fractionated. Fractionation divides a single laser beam into thousands of microscopic beams of light that generate columns of treated tissue and leave intervening skin untouched. This allows treatment depth to be safely increased and creates deeper channels for topical drug delivery, as discussed below<sup>[8]</sup>. With the thermal energy distributed over a larger surface area, there is also a lower risk of overtreatment<sup>[9]</sup>. While maintaining similar efficacy, fractionation has made ablative lasers in particular much safer because, by leaving small areas of tissue untreated, areas of ablated epidermis re-epithelialize more rapidly<sup>[10]</sup>.

Other light-based therapies such as intense pulsed light (IPL) or BroadBand Light (BBL)<sup>TM</sup> emit a spectrum of light rather than a single wavelength. These are also used for non-ablative skin treatment in the



**Table 2. Depth of periorbital skin**

	Epidermis (μm)	Dermis (μm)
Forehead	202	969
Glabella	144	325
Eyelid	130	215
Cheek	145	909

periocular region, but care must be taken with these light-based therapies as the risk of ocular damage is high without proper precautions<sup>[11]</sup>.

Light emitted from all lasers can be delivered in a continuous wave form or, more commonly, as long-pulsed, nanosecond (also referred to as Q-switched) or picosecond pulses. By selecting a wavelength that is preferentially absorbed by a target chromophore, applying enough energy to cause thermal destruction, and setting a pulse duration shorter than the target's thermal relaxation time, tissues can be targeted at precise depths for treatment with minimal surrounding damage<sup>[12]</sup>.

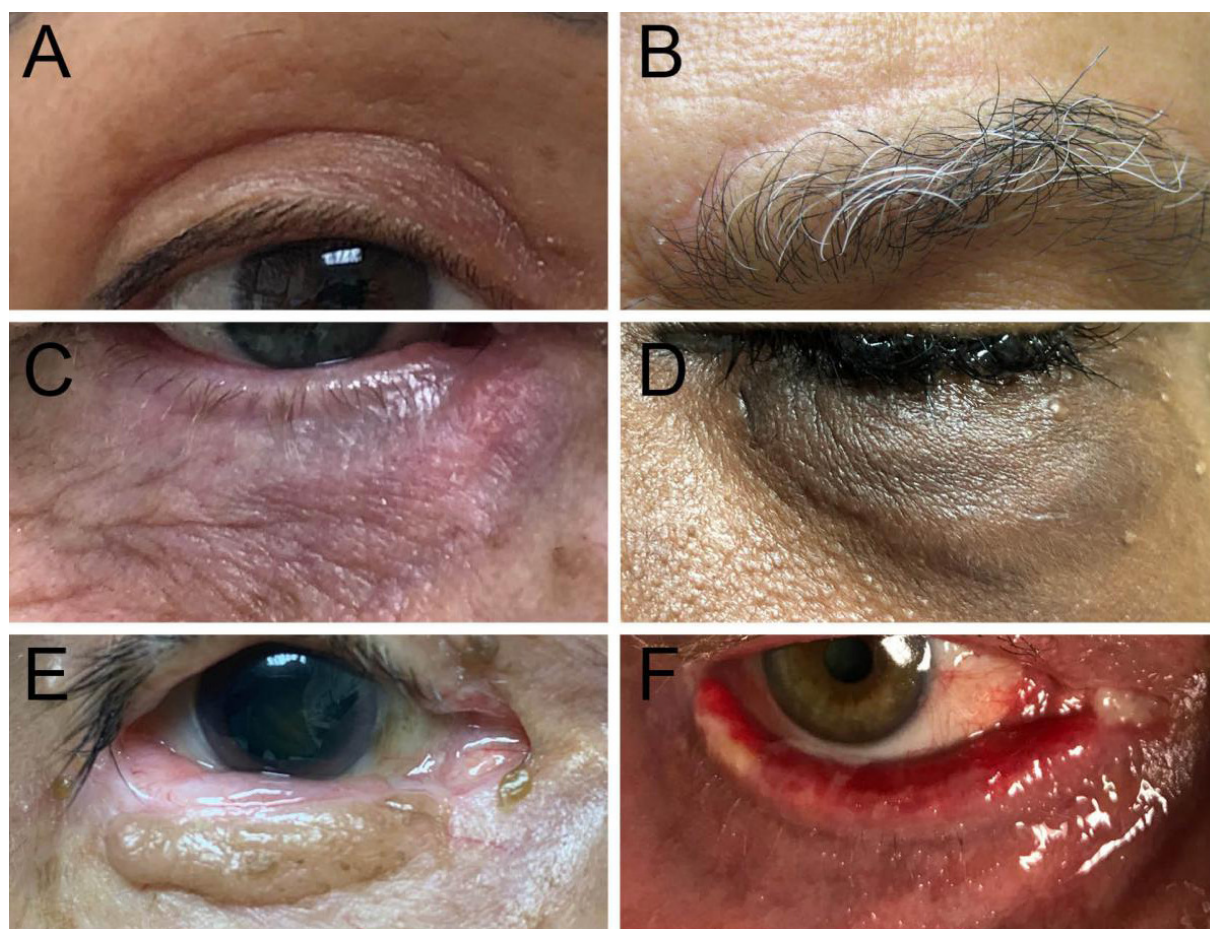
As with laser use in other areas of the face, there are few true absolute contraindications to the use of periocular laser, but these are important considerations when determining appropriate timing of treatment. These contraindications apply more directly to the use of ablative lasers due to the induced loss of epidermis. Examples include oral retinoid use within the last six months and active skin infections<sup>[13]</sup>. Other relative contraindications include history of poor wound healing, personal history of abnormal scarring or keloids, smoking, or diabetes. Patients with a history of herpetic lesions should be started on prophylactic doses of antivirals prior to laser treatment. Practices vary by practitioners as to timing and dose, and many practitioners advocate prophylactic treatment in all patients undergoing ablative laser resurfacing regardless of prior history.

## ASSESSMENT OF PERIOULAR SCARS

For the purposes of selecting the appropriate treatment, whether that involves a laser modality, pharmacologic therapy, a combination or expectant observation, the pertinent factors to consider are whether the scar is under tension, if and how the scar negatively impacts the eye, and what are the scar characteristics (form, depth, and age). Scars that cause skin contracture or are under significant tension may affect the opening and closure of the eyelid, and therefore require prompt treatment to avoid permanent damage to the eye. Scars that may be observed or treated less urgently include hypo- and hyperpigmented lesions, hypertrophic raised lesions, and erythema that do not impact eyelid function. Many of these scars can respond well to laser resurfacing provided that the appropriate chromophore and tissue depth can be safely targeted. For instance, most pigmented or vascular scars do best with PDL and IPL/BBL<sup>TM[14]</sup>.

In the periorbital region, the thickness of epidermis ranges from 130 to 202 μm and from 215 to 969 μm for dermis [Table 2]<sup>[15]</sup>. Eyelid skin is among the thinnest found on the body. The thickest skin in the periocular region is found on the forehead and cheek. Ablative CO<sub>2</sub> lasers can target tissue up to 2 mm in depth, deeper than is necessary for periorbital cosmetic skin resurfacing. With fractional CO<sub>2</sub> lasers, the treatment depth can be controlled by adjusting treatment power and spot size<sup>[16]</sup>. This is useful for safely treating large hypertrophic scars, periorbital rhytids, and laxity. Non-ablative lasers such as Nd:YAG are limited to depths less than 1 mm, which is sufficient to treat most superficial scars and limits the risk of adverse outcomes.

Post-surgical scars present a unique challenge as patients may associate the presence of scars with the overall surgical outcome<sup>[17,18]</sup>. Many procedures performed by oculoplastic surgeons serve both functional and cosmetic purposes (e.g., blepharoplasties, ptosis repair, and browlifts). Smaller surgical scars such as from blepharoplasties may hide well under skin folds or cilia [Figure 1A], but scarring at exposed incision



**Figure 1.** Post-surgical scars occurring in the periorbital region: (A) a well-concealed surgical incision within the lid crease following upper eyelid blepharoplasty; (B) a surgical scar above the brow following a direct browplasty procedure; (C) prolonged ecchymosis and dyspigmentation of the lower eyelid skin following a canalicular laceration repair; (D) hyperpigmentation of eyelid skin following complex repair of a lower eyelid avulsion; (E) dyspigmentation and hypertrophy of skin following a Hughes tarsconjunctival flap and bipedicle flap to reconstruct the lower eyelid years after excision of a basal cell carcinoma by an outside provider; and (F) lower eyelid ectropion in a patient with an underlying inflammatory dermatosis

sites can be aesthetically displeasing, as well as cause itchiness or pain. For instance, the direct browplasty, which is used to treat moderate to severe degrees of brow ptosis in select cases for whom other approaches are not possible, can leave a visible scar immediately above the brow that is displeasing to patients [Figure 1B].

Scars that arise from reconstructive eyelid surgeries are often unpredictable and range from prolonged ecchymosis [Figure 1C] and mild hyperpigmentation [Figure 1D] to hypertrophy and contractures leading to malposition of the eyelid [Figure 1E]. Earlier intervention is indicated in cases of eyelid malposition causing severe ectropion, where the ocular surface may quickly become compromised. Specific procedures more prone to causing eyelid malposition include skin flaps, full thickness skin grafts, and lower eyelid blepharoplasties where excess skin is excised. Scars that deform the eyelid require prompt attention as eversion of the eyelid margin exposes the conjunctiva and cornea and leads to chronic ocular irritation, redness, and corneal compromise that can ultimately result in corneal ulcers or even corneal melt. Similarly, severe scars are seen with trauma, radiation, chemical injury, thermal injuries, and chronic inflammation [Figure 1F], and they have been found to benefit from laser resurfacing<sup>[2,19]</sup>.

## CHOOSING THE APPROPRIATE TIMING FOR TREATMENT

Choosing the appropriate time for treatment depends on the underlying etiology, appearance of the scar, location and effect on eyelid closure, and patient preference in some cases. While it may be tempting to intervene early on all scars that appear in the postoperative period, it is important to remember that many may resolve or improve with time and massage. Normal wound healing proceeds through three phases: inflammatory (Days 1-3), proliferative, (Days 4-21), and remodeling (three weeks to two years)<sup>[20]</sup>. Advocates of early laser treatment reason that intervention during the inflammatory or proliferative phases can break this cycle and induce regenerative healing<sup>[21]</sup>. Several studies have demonstrated that PDL when applied as early as immediately following suture removal results in superior scar appearance when compared to no laser treatment<sup>[22-24]</sup>. Because PDL is preferentially absorbed by hemoglobin, its effect is likely greatest when erythema is still present early in the postoperative period. In contrast, scars already undergoing remodeling may respond better to other modalities. A randomized blinded study comparing non-ablative fractional laser (NAFL) to PDL performed at least two months following surgery demonstrated that, in this scenario, NAFL significantly outperformed PDL<sup>[25]</sup>. Interestingly, earlier intervention (within one month) with NAFL appears to offer no significant benefit over observation when re-evaluated beyond one year<sup>[23,26]</sup>. Moreover, in a prospective randomized control trial evaluating NAFL vs. observation of surgical scars related to direct browplasties performed at our institute, two of eight subjects attributed negative changes in the appearance of their scars to laser treatment, and both of these patients had early intervention (7 and 12 days postoperative vs. 31-767 days)<sup>[27]</sup>. A study comparing ablative laser therapy performed at Postoperative Week 1 to observation similarly found no difference in scar appearance by 12 weeks<sup>[28]</sup>.

## SELECTING THE APPROPRIATE LASER TREATMENT

Provided that the laser light reaches the appropriate depth, how well tissue responds to laser treatment depends on the relative abundance of water, hemoglobin, and melanin (the target chromophores) within the tissue and how selectively each of these molecules absorbs the emitted wavelength. For instance, ecchymoses and erythematous scars may be seen following minor surgery or periorbital trauma. Due to the hemoglobin content within these scars, they respond well to PDL and IPL/BBL<sup>TM</sup><sup>[29,30]</sup>. Hyperpigmented lesions, especially tattoo related scars and oculodermal melanocytosis (Nevus of Ota), respond well to nanosecond (Q-switched) and picosecond lasers at 694 and 755 nm wavelengths, which are preferentially absorbed by blue/green pigment<sup>[30,31]</sup>.

Scars with greater degrees of hypertrophy (> 3 mm) often require supplemental treatment with more powerful non-ablative lasers<sup>[30]</sup>. For very thick, mature hypertrophic scars and scars under tension, more powerful non-ablative lasers such as Nd:YAG, diode and Er:glass lasers and ablative lasers such as fractional CO<sub>2</sub> and Er:YAG lasers may be indicated<sup>[32-34]</sup>. Thick scars that cause a cicatricial ectropion may require prompt release of excessive tension due to the risk of ocular damage from prolonged exposure. Both ablative<sup>[2]</sup> and non-ablative fractional lasers<sup>[19]</sup> have been used successfully in these cases as an alternative to surgical correction. Although there is a greater risk of complication with ablative lasers, when the energy is fractionated (fractional ablative laser), the risks are lowered. Studies have demonstrated similar effectiveness of fractional CO<sub>2</sub> and Er:YAG lasers for improving texture, laxity, and dyschromia of the periorbital skin with improvement seen in approximately half of patients by six months<sup>[29]</sup>. One notable instance in which ablative lasers should be avoided, however, is in patients with Fitzpatrick skin types III-VI because of the greater risk of inducing postinflammatory dyspigmentation in this population<sup>[35,36]</sup>. Many providers may opt to avoid laser treatments in these patients altogether; however, certain fractional non-ablative lasers, in particular long-pulsed diode, Er:glass, and Nd:YAG lasers, have been demonstrated to be both safe and effective<sup>[37-39]</sup>.

Not all scars contain a predominant chromophore that can be used to achieve sufficient tissue selectivity by changing the laser wavelength. In these instances, tissue selectivity can be achieved by exploiting differences in thermal relaxation times. Ultimately, the goal of treatment is to break down scar tissue, induce neocollagenesis, and stimulate surrounding melanocytes. Both ablative and non-ablative lasers have been used to manage atrophic and hypopigmented scars in periorbital skin that occur in the settings of thermal injury, chemical burns, chronic inflammation, and topical steroid use<sup>[30,40,41]</sup>. Moreover, multiple modalities may be combined in a single session to efficiently address different scar characteristics, e.g., PDL combined with fractional CO<sub>2</sub> to target erythema and texture, respectively<sup>[42-45]</sup>.

## LASER ASSISTED DRUG DELIVERY

For the most severe scars, lasers may be insufficient to achieve the desired correction. In these cases, laser resurfacing can be combined with intralesional and topical application of antifibrotic agents such as triamcinolone and 5-fluorouracil (5-FU) for enhanced effect<sup>[2,30]</sup>. Laser treatment can be used to create channels within the stratum corneum where topically applied medications can then penetrate deep into the dermis. Fractional photothermolysis further helps to distribute medication across evenly spaced zones. Both erythematous and hypertrophic scars respond well to combination therapy<sup>[2,30]</sup>. For hypertrophic scars, botulinum toxins are increasingly being used in conjunction with steroids and 5-FU to decrease tension and decrease fibroblast activity, although they can only be used in areas where muscle paralysis would not affect eyelid closure (e.g., for medial or lateral canthal scars)<sup>[46]</sup>. In addition to antifibrotic agents, topical application of poly-L-lactic acid and prostaglandin analogs have been used with good effect to treat atrophic scars and improve contour<sup>[47]</sup> and to enhance re-pigmentation<sup>[48]</sup>, respectively.

## PARAMETERS FOR TREATING PERIOCCULAR TISSUE

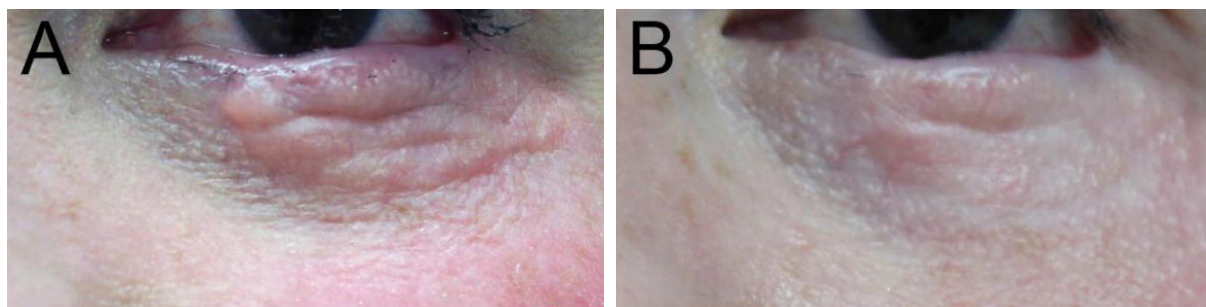
The key parameters for any laser are wavelength, pulse width, fluence (i.e., energy), spot size, and repetition rate. Wavelength is determined by the lasing medium and filter selection. Pulse width determines the interval of time over which energy is delivered. Fluence is the amount of energy delivered per unit area. In some machines, the total energy is selected. In devices capable of fractional photothermolysis, the energy is further divided into microthermal treatment zones (MTZs). MTZs refer to the number of fractionated spots within a treatment area. Spot size is the diameter of the beam at the surface. Repetition rate refers to the number of pulses per second. Frequently, treatments must be completed over multiple sessions to minimize excess thermal injury and allow for adequate collagen remodeling until the desired result is achieved.

Although periorbital skin is among the thinnest found on the body, higher fluences and higher treatment densities are sometimes used depending on the scar being addressed<sup>[49]</sup>. The rich blood supply to the ocular adnexa facilitates rapid healing from thermal injury<sup>[29]</sup>. Treatment depth can be controlled by adjusting both energy and treatment density. Specific treatment parameters vary by device. For the 2790-nm Er:YSGG laser used in our practice (Pearl Fractional<sup>TM</sup>, Cutera, Brisbane, CA), typical settings may range from 60 to 160 mJ at 4%-12% density<sup>[50]</sup>. For the Ultrapulse Encore fractional CO<sub>2</sub> laser (Lumenis, Israel), either the Active FX handpiece set at 60-90 mJ and 55%-82% density (Settings 1-3) is used for superficial scars or the Deep FX handpiece set at 8-10 mJ and 5%-15% density is used for deeper scars<sup>[51]</sup>. Higher energy and treatment density with this laser, however, can ablate completely through eyelid skin.

## COMPLICATIONS

Reported complications of laser periocular skin resurfacing include persistent erythema; undesired dyspigmentation; eyelid malposition, viral, bacterial, and fungal infections; burns; corneal injuries; and vision loss. It is imperative that providers adequately inform patients and set realistic expectations. Patients should be advised that they will experience some skin irritation for 24-48 h following treatment. Ablative





**Figure 2.** Appearance of a lower eyelid scar from a Hughes reconstruction before (A) and one year after laser resurfacing (B)

lasers entail even more downtime. If blistering occurs, generally due to excessive energy or insufficient cooling, the patient should not remove any scabs. Between sessions, energy can be increased by 10%-20% as tolerated or until the desired result is achieved. Patients should be instructed to avoid sunlight between sessions and wear broad-spectrum SPF 30 or higher sunscreen. Sessions should be scheduled approximately four weeks apart to allow adequate recovery. Most laser devices now have built in cooling, but, if this is absent, contact gel should be applied prior to treatment, and the skin should be cooled for 30 min post treatment.

Bulk heating resulting from excessive laser therapy can cause skin damage such as erythema to be as high as 8.8% with CO<sub>2</sub> laser<sup>[52]</sup>. Scarring and dyspigmentation may also be seen with non-ablative lasers such as Nd:YAG, although less frequently<sup>[29]</sup>. Laser to the periorbital skin presents with the added potential risk of causing harm to the eyes. Ocular structures are highly sensitive to both ablative and non-ablative lasers, but injuries can be entirely prevented with proper eye protection. Although fully occlusive goggles may be sufficient for some cases, more often corneal shields are indicated when eyelid skin is treated. Reported ocular complications include permanent loss of eyelashes and vitreous floaters due to PDL<sup>[29]</sup>; iritis, iris atrophy and posterior synechiae due to IPL; and vision loss in rare cases<sup>[11]</sup>. These complications all occurred when protective goggles were not appropriately placed or were removed to reach periocular skin.

## CASE PRESENTATION

A 48-year-old female with a history of left lower lid melanoma underwent Mohs micrographic surgery followed by lower eyelid reconstruction via a Hughes flap and a full thickness skin graft from post-auricular skin. Significant granulation and hypertrophy were noted along the lower lid five months after second stage Hughes without causing significant ectropion. The patient therefore underwent a series of three treatments with 2790-nm Er:YSGG fractional laser resurfacing (Pearl Fractional™, Cutera, Brisbane, CA) spaced 6-8 weeks apart. For the initial treatment, 120 mJ were applied at 12% treatment density followed by a second pass at 80 mJ applied at 8% treatment density. At subsequent sessions, 160 mJ at 12% density were applied followed by a second pass at 120 mJ at 8% density. The patient received prophylactic acyclovir before each session and a corneal shield was placed prior to each laser application. At one year follow up, touch up laser was performed. The patient's appearance before and one year after laser resurfacing are shown in [Figure 2](#).

## CONCLUSION

Laser skin resurfacing has become an integral tool for the management of periorbital scars. While several studies have demonstrated that early pre-planned treatment with multiple laser modalities can be used to minimize the appearance of postoperative scars, older hypertrophic surgical scars can also respond well to laser treatment, particularly in the periocular region. With the growing number of laser modalities and the capacity to combine laser with topical medications, physicians can tailor treatments to individual skin types



and scars. As with all interventions performed around the eyes, a cautious, conservative approach with adequate shielding of ocular structures is recommended to minimize potential complications.

## DECLARATIONS

### Acknowledgments

We would like to acknowledge Andrew J. Rong and Ann Q. Tran for their assistance in caring for the patients described in this manuscript.

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Pirakitikulr N, Martin JJ, Wester ST

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

The Bascom Palmer Eye Institute is supported by NIH Center Core Grant (P30EY014801), Research to Prevent Blindness Unrestricted Grant (New York, NY). This research was supported by the above grant and private donor funding.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Bui AD, Grob SR, Tao JP. 5-Fluorouracil management of oculo-facial scars: a systematic literature review. *Ophthalmic Plast Reconstr Surg* 2020;36:222-30.
2. Lee BW, Levitt AE, Erickson BP, et al. Ablative fractional laser resurfacing with laser-assisted delivery of 5-fluorouracil for the treatment of cicatricial ectropion and periocular scarring. *Ophthalmic Plast Reconstr Surg* 2018;34:274-9.
3. Orringer JS, Rittie L, Baker D, Voorhees JJ, Fisher G. Molecular mechanisms of nonablative fractionated laser resurfacing. *Br J Dermatol* 2010;163:757-68.
4. Orringer JS, Kang S, Johnson TM, et al. Connective tissue remodeling induced by carbon dioxide laser resurfacing of photodamaged human skin. *Arch Dermatol* 2004;140:1326-32.
5. Lowe NJ, Wieder JM, Shorr N, Boxrud C, Saucer D, Chalet M. Infraorbital pigmented skin. Preliminary observations of laser therapy. *Dermatol Surg* 1995;21:767-70.
6. Roberts TL, Lettieri JT, Ellis LB. CO<sub>2</sub> laser resurfacing: recognizing and minimizing complications. *Aesthet Surg J* 1996;16:142-8.
7. Kwon HH, Lee WY, Choi SC, Jung JY, Bae Y, Park GH. Combined treatment for skin laxity of the aging face with monopolar radiofrequency and intense focused ultrasound in Korean subjects. *J Cosmet Laser Ther* 2018;20:449-53.
8. Wenande E, Olesen UH, Nielsen MM, et al. Fractional laser-assisted topical delivery leads to enhanced, accelerated and deeper cutaneous 5-fluorouracil uptake. *Expert Opin Drug Deliv* 2017;14:307-17.
9. Neaman KC, Baca ME, Piazza RC, VanderWoude DL, Renucci JD. Outcomes of fractional CO<sub>2</sub> laser application in aesthetic surgery: a retrospective review. *Aesthet Surg J* 2010;30:845-52.
10. Alexiades-Armenakas MR, Dover JS, Arndt KA. Fractional laser skin resurfacing. *J Drugs Dermatol* 2012;11:1274-87.
11. Lee WW, Murdock J, Albini TA, O'Brien TP, Levine ML. Ocular damage secondary to intense pulse light therapy to the face. *Ophthalmic*

- Plast Reconstr Surg* 2011;27:263-5.
12. Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulsed radiation. *Science* 1983;220:524-7.
13. Waldman A, Bolotin D, Arndt KA, et al. ASDS guidelines task force: consensus recommendations regarding the safety of lasers, dermabrasion, chemical peels, energy devices, and skin surgery during and after isotretinoin use. *Dermatol Surg* 2017;43:1249-62.
14. Smit JM, Bauland CG, Wijnberg DS, Spauwen PH. Pulsed dye laser treatment, a review of indications and outcome based on published trials. *Br J Plast Surg* 2005;58:981-7.
15. Ichhpujani P, Spaeth LG, Yanoff M. Expert techniques in ophthalmic surgery. JP Medical Ltd; 2019.
16. Farkas JP, Hoopman JE, Kenkel JM. Five parameters you must understand to master control of your laser/light-based devices. *Aesthet Surg J* 2013;33:1059-64.
17. Vaidya TS, Mori S, Khoshab N, et al. Patient-reported aesthetic satisfaction following facial skin cancer surgery using the FACE-Q skin cancer module. *Plast Reconstr Surg Glob Open* 2019;7:e2423.
18. Arora A, Swords C, Garas G, et al. The perception of scar cosmesis following thyroid and parathyroid surgery: a prospective cohort study. *Int J Surg* 2016;25:38-43.
19. Nicoli F, Orfanotis G, Ciudad P, et al. Correction of cicatricial ectropion using non-ablative fractional laser resurfacing. *Lasers Med Sci* 2019;34:79-84.
20. Profyris C, Tziotziou C, Do Vale I. Cutaneous scarring: pathophysiology, molecular mechanisms, and scar reduction therapeutics Part I. The molecular basis of scar formation. *J Am Acad Dermatol* 2012;66:1-10; quiz 11-2.
21. Leclère FM, Mordon SR. Twenty-five years of active laser prevention of scars: what have we learned? *J Cosmet Laser Ther* 2010;12:227-34.
22. Nouri K, Jimenez GP, Harrison-Balestra C, Elgart GW. 585-nm pulsed dye laser in the treatment of surgical scars starting on the suture removal day. *Dermatol Surg* 2003;29:65-73; discussion 73.
23. Kent RA, Shupp J, Fernandez S, Prindeze N, DeKlotz CMC. Effectiveness of early laser treatment in surgical scar minimization: a systematic review and meta-analysis. *Dermatol Surg* 2020;46:402-10.
24. Artzi O, Friedman O, Al-Niaimi F, Wolf Y, Mehrabi JN. Mitigation of postsurgical scars using lasers: a review. *Plast Reconstr Surg Glob Open* 2020;8:e2746.
25. Tierney E, Mahmoud BH, Srivastava D, Ozog D, Kouba DJ. Treatment of surgical scars with nonablative fractional laser versus pulsed dye laser: a randomized controlled trial. *Dermatol Surg* 2009;35:1172-80.
26. Karmisholt KE, Banzhaf CA, Glud M, et al. Laser treatments in early wound healing improve scar appearance: a randomized split-wound trial with nonablative fractional laser exposures vs. untreated controls. *Br J Dermatol* 2018;179:1307-14.
27. Tenzel PA, Patel K, Erickson BP, et al. Split face evaluation of long-pulsed non-ablative 1,064 nm Nd:YAG laser for treatment of direct browplasty scars. *Lasers Surg Med* 2016;48:742-7.
28. Sobanko JF, Vachiramon V, Rattanaumpawan P, Miller CJ. Early postoperative single treatment ablative fractional lasing of Mohs micrographic surgery facial scars: a split-scar, evaluator-blinded study. *Lasers Surg Med* 2015;47:1-5.
29. Yates B, Que SK, D'Souza L, Suchecki J, Finch JJ. Laser treatment of periocular skin conditions. *Clin Dermatol* 2015;33:197-206.
30. Kauvar ANB, Kubicki SL, Suggs AK, Friedman PM. Laser therapy of traumatic and surgical scars and an algorithm for their treatment. *Lasers Surg Med* 2020;52:125-36.
31. Aurangabadkar S. QYAG5 Q-switched Nd:YAG laser treatment of nevus of Ota: an Indian study of 50 patients. *J Cutan Aesthet Surg* 2008;1:80-4.
32. Cho SB, Lee SJ, Chung WS, Kang JM, Kim YK. Treatment of burn scar using a carbon dioxide fractional laser. *J Drugs Dermatol* 2010;9:173-5.
33. Al-Mohamady Ael-S, Ibrahim SM, Muhammad MM. Pulsed dye laser versus long-pulsed Nd:YAG laser in the treatment of hypertrophic scars and keloid: a comparative randomized split-scar trial. *J Cosmet Laser Ther* 2016;18:208-12.
34. Koike S, Akaishi S, Nagashima Y, Dohi T, Hyakusoku H, Ogawa R. Nd:YAG laser treatment for keloids and hypertrophic scars: an analysis of 102 cases. *Plast Reconstr Surg Glob Open* 2015;2:e272.
35. Rudolph B, Harbott J, Lampert F. Fragile sites and neuroblastoma: fragile site at 1p13.1 and other points on lymphocyte chromosomes from patients and family members. *Cancer Genet Cytogenet* 1988;31:83-94.
36. Park SS, Khalid AN, Graber NJ, Fedok FG. Current trends in facial resurfacing: a survey of American academy of facial plastic and reconstructive surgery members. *Arch Facial Plast Surg* 2010;12:65-7.
37. Woolery-Lloyd H, Viera MH, Valins W. Laser therapy in black skin. *Facial Plast Surg Clin North Am* 2011;19:405-16.
38. de Angelis F, Kolesnikova L, Renato F, Liguori G. Fractional nonablative 1540-nm laser treatment of striae distensae in Fitzpatrick skin types II to IV: clinical and histological results. *Aesthet Surg J* 2011;31:411-9.
39. Martin JJ. The 1540-nm nonablative fractional photothermolysis for facial surgical scars. *Am J Cosmetic Surg* 2012;29:25-9.
40. Waibel JS, Rudnick A, Arheart KL, Nagrani N, Gonzalez A, Gianatasio C. Re-pigmentation of hypopigmentation: fractional lasers vs laser-assisted delivery of bimatoprost vs epidermal melanocyte harvesting system. *J Drugs Dermatol* 2019;18:1090-6.
41. Gan SD, Bae-Harboe YS, Graber EM. Nonablative fractional resurfacing for the treatment of iatrogenic hypopigmentation. *Dermatol Surg* 2014;40:87-9.
42. Goldman MP, Marchell N, Fitzpatrick RE. Laser skin resurfacing of the face with a combined CO<sub>2</sub>/Er:YAG laser. *Dermatol Surg* 2000;26:102-4.
43. Trelles M, Allones I, Vélez M, Mordon S. Nd:YAG laser combined with IPL treatment improves clinical results in non-ablative

- photorejuvenation. *J Cosmet Laser Ther* 2004;6:69-78.
44. Mei XL, Wang L. Ablative fractional carbon dioxide laser combined with intense pulsed light for the treatment of photoaging skin in Chinese population: a split-face study. *Medicine (Baltimore)* 2018;97:e9494.
  45. Cohen JL, Geronemus R. Safety and efficacy evaluation of pulsed dye laser treatment, CO<sub>2</sub> ablative fractional resurfacing, and combined treatment for surgical scar clearance. *J Drugs Dermatol* 2016;15:1315-9.
  46. Rahman SHA, Mohamed MS, Hamed AM. Efficacy and safety of Nd:YAG laser alone compared with combined Nd:YAG laser with intralesional steroid or botulinum toxin A in the treatment of hypertrophic scars. *Lasers Med Sci* 2020; doi: 10.1007/s10103-020-03120-0.
  47. Rkein A, Ozog D, Waibel JS. Treatment of atrophic scars with fractionated CO<sub>2</sub> laser facilitating delivery of topically applied poly-L-lactic acid. *Dermatol Surg* 2014;40:624-31.
  48. Massaki AB, Fabi SG, Fitzpatrick R. Repigmentation of hypopigmented scars using an erbium-doped 1,550-nm fractionated laser and topical bimatoprost. *Dermatol Surg* 2012;38:995-1001.
  49. Rahman Z, Alam M, Dover JS. Fractional laser treatment for pigmentation and texture improvement. *Skin Therapy Lett* 2006;11:7-11.
  50. Cutera. Pearl Fractional Treatment Guidelines. Brisbane, California; 2012.
  51. Ramsdell WM. Fractional carbon dioxide laser resurfacing. *Semin Plast Surg* 2012;26:125-30.
  52. Blanco G, Soparkar CN, Jordan DR, Patrinely JR. The ocular complications of periocular laser surgery. *Curr Opin Ophthalmol* 1999;10:264-9.

Systematic Review

Open Access



# Surgical management of jaw-winking synkinesis and ptosis in Marcus Gunn syndrome: a systematic outcomes analysis

Henry Bair<sup>1,2</sup>, Giancarlo A. Garcia<sup>1</sup>, Benjamin P. Erickson<sup>1</sup>

<sup>1</sup>Department of Ophthalmology, Byers Eye Institute at Stanford University, Palo Alto, CA 94303, USA.

<sup>2</sup>Stanford University School of Medicine, Stanford, CA 94305, USA.

**Correspondence to:** Dr. Giancarlo A. Garcia, Department of Ophthalmology, Byers Eye Institute at Stanford University, 2452 Watson Court, Palo Alto, CA 94303, USA. E-mail: [garcia.giancar@gmail.com](mailto:garcia.giancar@gmail.com)

**How to cite this article:** Bair H, Garcia GA, Erickson BP. Surgical management of jaw-winking synkinesis and ptosis in Marcus Gunn syndrome: a systematic outcomes analysis. *Plast Aesthet Res* 2020;7:68. <http://dx.doi.org/10.20517/2347-9264.2020.74>

**Received:** 12 Apr 2020 **First Decision:** 19 Aug 2020 **Revised:** 5 Sep 2020 **Accepted:** 5 Nov 2020 **Published:** 5 Dec 2020

**Academic Editor:** Raúl González-García **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

**Aim:** Marcus Gunn jaw-winking synkinesis (MGJWS) is characterized by congenital ptosis in conjunction with rapid and involuntary elevation of the affected upper eyelid upon contraction of the ipsilateral external pterygoid muscle. Selecting an approach to the surgical management of eyelid malposition in this syndrome is challenging and requires careful discussion with each patient's family. In this systematic review, we describe reported surgical approaches, assess outcomes data, and attempt to identify areas of consensus in the management of MGJWS.

**Methods:** Twenty-seven peer-reviewed studies were identified, describing a variety of interventions.

**Results:** The most commonly-used surgical techniques included: bilateral levator excision with bilateral frontalis sling, unilateral levator excision with bilateral or unilateral frontalis sling, the Neuhaus/Lemagne method, and levator plication surgery. However, no clear outcomes-based consensus regarding choice of surgical approach was identified, highlighting the ongoing role of surgeon and family preference in the selection of management strategy. Further, there was considerable variability in the literature for reporting outcome measures, including grading schemes for ptosis and jaw-wink.

**Conclusion:** The existing literature on management of MGJWS does not enable the development of an evidence-based consensus algorithm regarding the selection of an appropriate surgical technique. The disorder is



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



treated according to a case-by-case approach governed by surgeon and family preference. Standardization of nomenclature and outcome measures is crucial for obtaining higher-quality, generalizable data in futures studies.

**Keywords:** Marcus Gunn jaw-winking synkinesis, pterygoid-levator synkinesis, maxillopalpebral synkinesis

## INTRODUCTION

Marcus Gunn jaw-winking synkinesis (MGJWS), first described by Robert Marcus Gunn in 1883, is a disorder characterized by congenital ptosis accompanied by synkinetic elevation of the affected eyelid upon movement of the jaw<sup>[1]</sup>. Electromyographic studies demonstrate that contraction of the levator palpebrae superioris muscle of the ptotic eye occurs with stimulation of the ipsilateral external pterygoid muscle (by mouth opening, suction, or lateral excursion of the mandible). It is therefore also known as “maxillopalpebral synkinesis” or “pterygoid-levator synkinesis”. Among individuals with congenital ptosis, MGJWS is relatively common, with a reported incidence of 12.1%, and should be ruled out in all patients with this presenting complaint<sup>[2]</sup>.

Although the exact etiology is unknown, a combination of genetic, myogenic, and neurological factors has been implicated. Some evidence suggests underlying KIF21A mutation, while histological analyses demonstrate a degree of levator muscle atrophy on both the affected and unaffected side, thus suggesting a critical role for neural input<sup>[3-5]</sup>. But while the existence of congenital aberrant connections is widely accepted, their exact location within the brain or peripheral nervous system remains unclear. One possible location is in the midbrain between the trigeminal mesencephalic nucleus and oculomotor nucleus while the most likely alternative is between the mandibular division of the trigeminal nerve (CN V3) and the oculomotor nerve (CN III)<sup>[6,7]</sup>. Patients may present with other congenital aberrant innervation disorders or neurological disorders such as morning glory disc anomaly, inverse Marcus Gunn syndrome, or Duane's retraction syndrome type 1<sup>[8-10]</sup>.

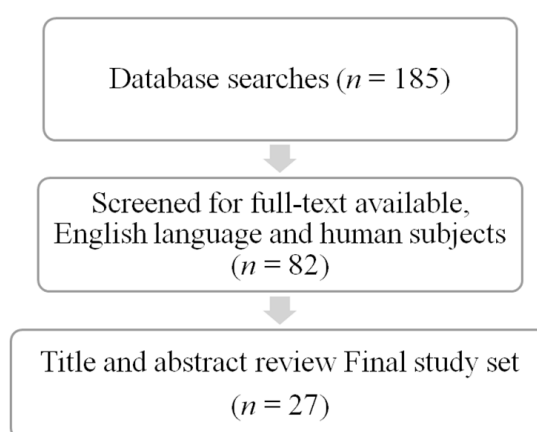
Treatment for MGJWS typically takes into account the extent of both functional and cosmetic impairments. Strabismus and amblyopia, when present, should be addressed. Repair of the baseline ptosis can be considered independent of jaw-winking correction, especially when the amplitude is low. However, in cases of moderate to severe jaw-winking, it is typically desirable to address the wink surgically. In general terms, surgery to eliminate the wink involves the disabling of levator function. Four types of surgeries are most commonly performed [Table 1]. These include: (1) bilateral levator excision with bilateral frontalis sling; (2) unilateral levator excision with bilateral or unilateral frontalis sling; (3) transection of the levator muscle followed by frontalis eyelid suspension using the distal segment of the muscle and aponeurosis, independently described by Neuhaus<sup>[11]</sup> and Lemagne *et al.*<sup>[12,17]</sup>; and (4) levator plication/resection without subsequent eyelid suspension (for patients with lower-amplitude jaw-winking). The primary difference between the Lemagne and Neuhaus procedure is that while the Lemagne procedure involves connecting the levator muscle to the frontalis muscle, with the intent of neurotizing the former with the latter, the Neuhaus procedure involves dividing the levator muscle into three strips as a mechanical sling. Apart from these four groups of procedures, several less commonly deployed options exist, including the Motaïs-Parinaud procedure (suspension of upper eyelid tarsus from the superior rectus muscle), the Friedenwald-Guyton technique (anchoring of the upper lid to the frontalis muscle with a single buried suture), and the Reese method (attachment of strips of orbicularis to the frontalis muscle)<sup>[18,19]</sup>.

The variety of surgical approaches for MGJWS, coupled with the absence of systematic and comprehensive outcomes analysis, present clinicians with significant challenges in terms of evidence-based technique selection. We herein evaluate the peer-reviewed outcomes literature pertaining to various surgical approaches for the management of MGJWS and attempt to identify common themes or areas of consensus.



**Table 1. Description of commonly performed surgical techniques for MGJWS**

Surgical technique	Description
Bilateral levator excision with bilateral frontalis sling	This procedure is well suited for severe blepharoptosis. Both levator muscles are dissected. The bilateral frontalis suspension is then most commonly performed using autogenous fascia lata. This procedure helps in achieving a symmetrical result in primary gaze and also during a downgaze <sup>[13]</sup>
Unilateral levator excision with bilateral (or unilateral) frontalis sling	In this approach, only the levator muscle to the ptotic eyelid is resected, while frontalis suspension is performed either only for the ptotic lid or in the normal lid as well <sup>[14]</sup> . Bilateral frontalis suspension after unilateral levator excision is designed to provide symmetrical lid height in downgaze while allowing the intact levator muscle to function in primary gaze <sup>[16]</sup>
Neuhauser/Lemagne method	In 1985, Russell Neuhaus and Jean-Michel Lemagne independently described a method to treat MGJWS by transection of the levator muscle followed by suspension of the eyelid to the frontalis using the distal segment of the muscle and aponeurosis, as opposed an autograft of fascia lata or a silicone rod <sup>[11,12]</sup> . While the Lemagne procedure involves connecting the levator muscle to the frontalis muscle with the intent of neurotizing the former with the latter, the Neuhaus procedure involves dividing the levator muscle into three strips as a mechanical sling
Levator plication or excision	In this technique, the levator muscle is resected or levator aponeurosis is plicated to effectively shorten the levator muscle and correct the ptosis. The amount of levator plication/resection to be performed depends on the desired eyelid height to be obtained. This a simpler technique but primarily addresses only the ptosis component and may not resolve jaw-winking <sup>[15]</sup>

**Figure 1.** Flowchart of the review process to identify the final study sample

## METHODS

A systematic review of the peer-reviewed literature as of March 31, 2020 was conducted using the search terms “Marcus Gunn jaw”, “Marcus Gunn jaw synkinesis”, “Marcus Gunn jaw ptosis”, “Marcus Gunn syndrome” and “maxillopalpebral synkinesis”. Terms related to acquired forms of eyelid ptosis and synkinesis, such as “Marin-Amat syndrome”, were omitted. The databases searched were PubMed, Medline, and Cochrane. Search term and database selection, as well as inclusion/exclusion criteria, were based upon guidelines from the Cochrane Handbook of Systematic Reviews of Interventions<sup>[20]</sup>.

The initial search retrieved 185 unique articles. This pool was further limited to human studies in the English language, available in full-text either online or through interlibrary loan. These parameters narrowed the sample size to 82 articles. Upon title and abstract review, we identified 27 articles related to surgical management of the disorder, which were included in the final study sample [Figure 1].

Full text of the articles was reviewed for each article and the following data extracted: (1) publication details (authors, year, references); (2) sample size; (3) ptosis and jaw-wink severity, (4) management approach (type of surgery performed, sling material, and follow-up); (5) documented efficacy outcomes; and (6) short- and long-term complications.

## RESULTS

### Study characteristics

The 27 identified articles included seven case reports, one case series, and 19 retrospective analyses [Table 2]. Studies that describe jaw-winking synkinesis in broader contexts of non-ophthalmic conditions were not included in our analysis. The remaining 23 studies reported a variety of surgical interventions, some of which were experimental in nature or modifications of established procedures. Larger case series often included the results of multiple types of surgical intervention. No randomized controlled surgical trials were identified.

Reported surgical interventions included bilateral levator excision with bilateral frontal sling<sup>[13,19,21]</sup> (3 articles), unilateral levator excision with bilateral frontal sling<sup>[16,19,23,33]</sup> (4 articles), unilateral levator excision with unilateral frontal sling<sup>[16,28,29,32,33]</sup> (5 articles), the Neuhaus/Lemagne method<sup>[11,13,17,29,35,37]</sup> (6 articles), levator plication/resection without eyelid suspension<sup>[13,15,30,33]</sup> (4 articles), the Motaïs-Parinaud procedure<sup>[26,33]</sup> (2 articles), and the use of an orbicularis oculi muscle flap<sup>[27,30]</sup> (2 articles). Although the Neuhaus and Lemagne methods differ slightly, the procedures are not significantly differentiated with regard to practice and analyses of outcomes in the majority of studies we found. For details of studies that discuss experimental procedures, see Table 2.

### Efficacy outcomes

There was considerable variability between studies in terms of how severity of ptosis and jaw-winking was documented and reported. Eyelid position was described using a variety of parameters, including quantitative measures such as eyelid height (margin to reflex distance, or MRD-1), palpebral aperture, and upper eyelid excursion; and qualitative measures such as eyelid contour and eyelid symmetry. Even in instances when studies employed similar outcome measures, definitions of these parameters were noted to vary. For instance, Doucet and Crawford<sup>[35]</sup> categorized jaw-wink amplitude as Grade I (< 2 mm of change in MRD-1), Grade II (2-6 mm), and Grade III (> 6 mm), while Dave *et al.*<sup>[22]</sup> considered < 2 mm mild, 2-4 moderate, and > 5 mm severe. Raw data for individual patients were not provided. Doucet and Crawford acknowledged that the jaw-winking severity scale they utilized was arbitrary, based on personal observation of the magnitude of eyelid movement deemed “noticeable”. The degree of eyelid symmetry was also reported using varying scales; Morris *et al.*<sup>[34]</sup> defined the outcome of “satisfactory” symmetry as a difference of ≤ 1 mm between the MRD-1 of the operated and unoperated eyes, while Cates and Tyers<sup>[21]</sup> and Ibrahim<sup>[35]</sup> reported symmetry as “good” or “acceptable” without quantitatively defining these terms. Eyelid contour was reported descriptively in all studies.

Given the variability of grading schemes used, we evaluated the studies that reported surgical outcomes of a given procedure for a discrete number of MGJWS patients. Three out of 3 studies that analyzed bilateral levator excision followed by bilateral frontalis suspension resulted in satisfactory improvement of both ptosis and jaw-winking<sup>[13,19,21]</sup>. Two out of 4 studies that reported on unilateral levator excision followed by bilateral frontalis suspension resulted in improvement of both ptosis and jaw-winking<sup>[16,33]</sup>; the other two studies<sup>[19,23]</sup> revealed unsatisfactory outcomes for ptosis but improvement in jaw-winking. Four out of 5 studies that described unilateral levator excision with unilateral frontalis suspension resulted in satisfactory improvement of both ptosis and jaw-winking<sup>[16,28,29,32]</sup>; however, one study<sup>[33]</sup> did not report any improvement in either ptosis or jaw-winking. Six out of 6 studies that assessed the Neuhaus/Lemagne method resulted in satisfactory improvement of both ptosis and jaw-winking (with significantly more improvement in jaw-winking). Of 4 studies that analyzed levator plication/resection without eyelid suspension, one resulted in satisfactory improvement of both ptosis and jaw-winking<sup>[15]</sup>; two of these four studies<sup>[13,30]</sup> revealed resolution of ptosis but undercorrection of jaw-winking; and one of these four studies<sup>[33]</sup> revealed only moderate correction of ptosis (in 63% of patients) and no improvement in jaw-winking.

**Table 2. Included peer-reviewed articles on surgical management of Marcus-Gunn jaw-winking synkinesis**

Author(s)	Year	Sample size	Ptosis severity	Management	Outcome
Beard <sup>[25]</sup>	1965	1	Severe	Levator excision of the unaffected eyelid followed by bilateral brow suspension	This approach is recommended for a failed prior surgery. Resulted in satisfactory outcomes
Nagpaul and Charan <sup>[26]</sup>	1968	1	5 mm	Motais-Parinaud procedure	Moderate improvement shown after the operation. Paretic superior rectus did not improve
Tsai <i>et al.</i> <sup>[27]</sup>	2002	1	Severe	Orbicularis oculi muscle flap	Used the orbicularis oculi muscle flap to elevate dynamically the ptotic eyelid and to eliminate the synkinetic reflex without levator excision. This approach had successful outcomes
Yoshikata and Yanai <sup>[28]</sup>	1999	1	Severe	Unilateral excision of levator muscle followed by unilateral frontalis suspension	33-yr-old patient had satisfactory surgical outcomes
Carbajal <sup>[18]</sup>	1959	5	N/A	A case-by-case approach: levator tucking, Blaskovics, tenectomy and Friedenwald-Guyton, tenectomy and Reese	Except for one case, all patients experienced recurrence between 6 and 23 months
Bajaj <i>et al.</i> <sup>[15]</sup>	2015	10	4.25 ± 0.79 mm	Levator plication	10 patients underwent modified levator plication surgery. 9 patients showed correction of ptosis and 3 had resolution of MGJWS. Resolution of MGJWP was defined as less than 1 mm of excursion of upper eyelid with synkinetic mouth movement. Ptosis correction (2.40 ± 0.50 mm) was statistically significant
Betharia and Kumar <sup>[14]</sup>	1987	15	Severe ( <i>n</i> = 9); mild-moderate ( <i>n</i> = 6)	Unilateral levator transection with levator aponeurosis for frontalis suspension (Neuhaus/Lemagne method)	Good correction in 10 cases. Under-correction in 5 cases
Bartkowski <i>et al.</i> <sup>[29]</sup>	1999	19	Marked ( <i>n</i> = 15)	Unilateral levator transection with levator aponeurosis for frontalis suspension (Neuhaus/Lemagne method; <i>n</i> = 16); unilateral levator transection followed by unilateral frontalis suspension ( <i>n</i> = 3)	84% patients showed no symptoms after the surgery. 1 patient had lagophthalmos
Park <i>et al.</i> <sup>[30]</sup>	2008	20	Mild-moderate ptosis	Unilateral levator resection only ( <i>n</i> = 10); frontalis muscle flap or orbicularis oculi muscle flap ( <i>n</i> = 10)	After ~30 months, blepharoptosis was corrected; however, there was only mild to moderate resolution of jaw-winking reflex
Shah <i>et al.</i> <sup>[31]</sup>	2019	23	Moderate to severe	Unilateral tarsofrontal silicone sling without levator excision	Unilateral tarsofrontal silicone sling without disinsertion or extirpation of the levator reduces the severity of symptoms in MGJWS. "good" = upper eyelid height was <1 mm, "fair" = 1-2 mm and "poor" ≥ 2 mm
Khwarezmi <i>et al.</i> <sup>[19]</sup>	1999	24	Minimal ( <i>n</i> = 5); moderate ( <i>n</i> = 11); severe ( <i>n</i> = 9)	Bilateral ( <i>n</i> = 19) or unilateral ( <i>n</i> = 5) levator excision, all followed by bilateral frontalis suspension	The procedure provides satisfactory correction (62% cases). But 5 patients reported recurrence
Bowyer and Sullivan <sup>[13]</sup>	2004	31	Severe ( <i>n</i> = 10); mild-moderate ( <i>n</i> = 21)	Unilateral levator advancement surgery ( <i>n</i> = 4, mild cases); bilateral levator weakening followed by bilateral frontalis suspension ( <i>n</i> = 13, moderate-severe cases)	The surgical approach will differ according to the condition. Patients with bilateral surgery had wink elimination while unilateral surgery had detectable wink
Ning <i>et al.</i> <sup>[32]</sup>	2019	42	Mild ( <i>n</i> = 7); moderate ( <i>n</i> = 24); severe ( <i>n</i> = 11)	Unilateral levator excision followed by unilateral frontalis suspension	34 patients with moderate to severe MGJWS underwent surgery and had satisfactory outcomes at 6-month follow-up
Demirci <i>et al.</i> <sup>[16]</sup>	2010	48	Mild ( <i>n</i> = 8); moderate ( <i>n</i> = 36); severe ( <i>n</i> = 4)	Unilateral levator excision followed by bilateral/unilateral frontalis suspension	The management was effective. Symptoms resolved in 97% patients and improved in 3%

Doucet and Crawford <sup>[33]</sup>	1981	55	Severe ( $n = 34$ ); mild-moderate ( $n = 21$ )	Unilateral levator disinsertion with bilateral ( $n = 2$ ) or unilateral ( $n = 12$ ) fascial suspension; Fasanella-Servat procedure ( $n = 1$ ); Motaïs-Parinaud procedure ( $n = 11$ ); levator excision only ( $n = 26$ ); no treatment ( $n = 17$ )	Bilateral fascial suspension had superior outcomes as compared to other treatments or no treatments
Ho <i>et al.</i> <sup>[24]</sup>	2017	8 out of 319 patients with ptosis	N/A	Levator muscle excision; frontalis suspension; frontalis muscle flap advancement (procedures not stratified by MGJWS status)	Presence of MGJWS had poorer outcomes after surgical correction for congenital ptosis
Cates and Tyers <sup>[21]</sup>	2008	7 out of 13 patients with ptosis	N/A	Bilateral frontalis brow suspension after bilateral levator excision	The researchers report satisfactory cosmetic results with good symmetry of lid movement and position
Morris <i>et al.</i> <sup>[34]</sup>	2008	7 out of 89 patients with ptosis	MRD1 > 2 mm	Silicone rod frontalis suspension	The surgery was found to be modestly effective (57% cases improved). Postoperative eyelid symmetry (< 1 mm = satisfactory)
Ibrahim <sup>[35]</sup>	2007	3 out of 8 patients with ptosis	N/A	Use of the distal portion of levator aponeurosis as a flap for frontalis suspension (similar to the Neuhaus/Lemagne method)	Synkinetic muscle movements disappeared, and hence, it is an effective treatment
Dave <i>et al.</i> <sup>[22]</sup>	2019	43 out of 95 patients	Severe ( $n = 91$ )	Frontalis sling with silicone; unilateral levator excision	Outcomes were not stratified by association with MGJWS. For all ptosis repairs, as compared to LR, FS gives a better eyelid elevation but also has greater regression requiring more surgeries
Kemp and MacAndie <sup>[23]</sup>	2001	3 out of 29	NA	Unilateral levator excision followed by bilateral Mersilene mesh brow suspension	MGJWS was associated with poorer outcomes
Neuhaus <sup>[11]</sup>	1985	1	Severe	Unilateral levator transection with distal levator muscle and aponeurosis for frontalis suspension (Neuhaus/Lemagne method)	No residual aberrant eyelid movement
Lemagne <sup>[17]</sup>	1988	1 out of 2	Severe	Unilateral levator transection with distal levator muscle and aponeurosis for frontalis suspension (Neuhaus/Lemagne method)	Synkinetic muscle movements disappeared. Moderate ptosis recurred 6 months postoperatively but was later corrected with an additional levator excision
Xiang <i>et al.</i> <sup>[36]</sup>	2010	13	Minimal ( $n = 1$ ); moderate ( $n = 7$ ); severe ( $n = 5$ )	Unilateral anastomosis of levator and frontal muscles	For moderate-to-severe MGJWS, this procedure provided generally satisfactory outcomes of both ptosis and jaw-winking
Manners <i>et al.</i> <sup>[37]</sup>	1996	28 out of 35	2-4 mm ( $n = 20$ ); 5-7 mm ( $n = 8$ )	Unilateral levator transection with distal levator muscle and aponeurosis for frontalis suspension (Neuhaus/Lemagne method)	This method was effective in eliminating jaw-winking. Ptosis often required additional levator excision to resolve

NA: not available

In studies with internal comparison of techniques, there was an apparent advantage of bilateral levator excision with bilateral frontalis suspension over other procedures. Khwarg *et al.*<sup>[19]</sup> reported that 100% of patients (19/19, including 3 who had bilateral MGJWS) who received bilateral levator excision with bilateral frontalis suspension saw significant improvement of ptosis, *vs.* only 40% of patients (2/5) who saw improvement after unilateral levator excision with bilateral frontalis suspension; across all operated eyelids, 37% (10/27) saw complete resolution of jaw-winking, while 48% (13/27) had mild residual jaw-winking. Bowyer and Sullivan<sup>[17]</sup> reported that all patients (13/13) who received bilateral levator excision with bilateral frontalis suspension had complete resolution of jaw-winking, *vs.* all 4 patients who received unilateral levator advancement who had persistent jaw-winking, despite the former group having more severe baseline MGJWS.

There was also an apparent slight advantage of unilateral levator excision with bilateral frontalis suspension over unilateral levator excision with unilateral frontalis suspension. Doucet and Crawford<sup>[33]</sup> reported

**Table 3. Types of sling materials**

Types of sling materials	Number of studies
Autogenic fascia lata	9
Muscle flaps	5
Silicone or other materials	5
None	7

**Table 4. Reports of post-surgical complications**

Post-surgical complications	Number of studies
Present	11
Absent	14

that all patients (2/2) who received bilateral frontalis suspension had complete resolution of both ptosis and jaw-winking, whereas patients who received unilateral frontalis suspension had residual moderate/severe ptosis (8%; 1/12), jaw-winking (33%; 4/12), and lid lag (100%; 12/12). They concluded that bilateral frontalis suspension is the more desirable option when possible. Demirici *et al.*<sup>[16]</sup> reported that patients who received bilateral (88%; 23/26) frontalis suspension had better upper eyelid symmetry than those who received unilateral (75%; 3/4) frontalis suspension, though this was not statistically significant.

For broader studies of ptosis that included a subset of MGJWS patients, ascertaining the efficacy of surgical intervention was hampered in some instances due to lack of reported postoperative outcomes for jaw-winking as well as for ptosis. For example, neither Dave *et al.*<sup>[22]</sup>, who assessed 95 ptosis patients, nor Ho *et al.*<sup>[24]</sup>, who assessed 319 ptosis patients, reported postoperative presence or absence of jaw-winking for MGJWS patients. Thus, for these studies, the efficacy of surgical interventions for MGJWS could not be definitively ascertained. Nevertheless, Ho *et al.*<sup>[24]</sup> found that patients with MGJWS achieved a less ideal lid height (postoperative MRD-1 < 3 mm) than in those without the condition (25% *vs.* 75.7%,  $P = 0.004$ ), while Dave *et al.*<sup>[22]</sup> did not find the presence of MGJWS to significantly affect outcome.

### Frontalis sling materials

Among articles describing the use of frontalis slings ( $n = 19$ ), the greatest number ( $n = 9$ ) used autologous materials such as tensor fascia lata, temporalis fascia, or frontalis fascia. Studies using autologous fascia lata did not report any post-surgical complications directly related to the choice of sling material. Synthetic materials such as silicone slings and tantalum wires were used in a smaller number of studies ( $n = 5$ ). Two studies reported post-surgical complications such as a sling-associated abscess or sling migration ( $n = 1$  for each). Five studies proposed the use of a muscle flap instead of a sling, using either the levator or orbicularis oculi muscle. These case studies did not report any subsequent complications [Table 3].

### Postoperative complications and recurrences

In total, there were 383 patients with MGJWS across all studies. Many of the series reported post-surgical complications [Table 4], including suture granuloma (3 patients), eyelash ptosis (12 patients), entropion (3 patients), undercorrection of ptosis (46 patients), overcorrection of ptosis (5 patients), lagophthalmos (26 patients), exposure keratopathy (22 patients), silicone sling complications (2 patients), and lid contour abnormalities (8 patients). Nonetheless, the noted number of patients with the above complications is complicated by the studies that did not specify whether the reported complications occurred in the subset of patients with MGJWS among all ptosis patients analyzed<sup>[22-24,34]</sup>.

Six studies reported recurrence of MGJWS symptoms. Carbajal<sup>[18]</sup> reported recurrence of both ptosis and jaw-winking in 2/2 patients who underwent levator plication and recurrence of ptosis in 1/1 patient who underwent the Reese procedure and 1/1 patient who underwent the Friedenwald-Guyton procedure.



**Table 5. Follow-up period**

Follow-up period	Number of studies
Less than 1 year or not reported	14
Between 1 and 5 years	9
More than 5 years	4

Khawarg *et al.*<sup>[19]</sup> reported recurrence of ptosis in 1 of 5 patients (20%) who underwent bilateral levator excision and bilateral frontalis suspension and in 3/19 patients (16%; of whom 3 had bilateral jaw-winking) who underwent bilateral levator excision and bilateral frontalis suspension. Cates and Tyers<sup>[21]</sup> reported recurrence of ptosis in 1/7 patients (14%) who received bilateral levator excision and bilateral frontalis suspension. Ho *et al.*<sup>[24]</sup> reported recurrence of ptosis in 2/8 patients (25%), although the procedures involved were not specified. Ning *et al.*<sup>[32]</sup> reported recurrence of jaw-winking in 1/34 patients (2.9%) who received unilateral levator excision and unilateral frontalis suspension. Demirci *et al.*<sup>[16]</sup> reported recurrences of ptosis in 3/30 of patients (10%) who received unilateral excision and bilateral frontalis suspension. These pooled results are similar to prior reports suggesting that the presence of MGJWS was associated with poorer surgical outcomes when compared to isolated congenital ptosis<sup>[23]</sup>. Recurrence of ptosis was more common than recurrence of jaw-winking. It is difficult to determine whether particular surgeries yielded fewer recurrences due to the limited sample size and the fact that the severity of preoperative ptosis and jaw-winking was often used as criteria for determining the type of surgery performed.

In these studies, the follow-up interval varied from two months to 16 years [Table 5]. The length of reported follow-up did not correlate with the type of surgery performed or postoperative complications recorded. In one study, although the patients were systematically followed for six months, late recurrences (e.g., 8 years) were also noted<sup>[21]</sup>.

## DISCUSSION

Surgical approaches for the management of MGJWS have historically been nuanced, with varying considerations employed for management of the ptosis, jaw-wink, and/or both. Procedures that correct only the ptosis component, such as levator plication, can potentially exaggerate the presentation of the jaw-winking. Therefore, management of MGJWS with clinically significant ptosis and jaw-winking typically involves disabling levator muscle function and suspension of the eyelid to the frontalis muscle. In this systematic review assessing reported outcomes of surgery for MGJWS, we found marked heterogeneity in management, even among cases with similar baseline clinical characteristics. Additionally, meta-analysis was challenging due to considerable differences in grading schemes for ptosis and jaw-wink as well as in reported outcome measures and follow-up intervals. Accordingly, even after thorough evaluation of the published literature, it was not possible to articulate a consensus algorithm regarding the selection of appropriate surgical technique.

Several articles<sup>[16,25,28,30,33]</sup> suggest that bilateral levator excision followed by bilateral frontalis suspension is the theoretically ideal surgical intervention for MGJWS from the perspective of achieving improvement of eyelid symmetry and jaw-wink. Nonetheless, they also acknowledge the difficulty this can present in practice, as excising a normally functioning levator on the unaffected side requires significant confidence on the part of the surgeon, and trust on the part of the patient and his or her family. Understandably, the potential ethical implications of operating on a normal eyelid and eyebrow must be carefully considered by the surgeon and weighed against potential functional and cosmetic benefit.

Along with the lack of consensus regarding choice of surgical approach, we also found considerable variability in the methodologies for reporting outcome measures. In particular, reports characterizing

ptosis and MGJWS typically describe surgical outcomes using qualitative terms such as “satisfactory”, “cosmetically acceptable”, “good symmetry”, or “improved”. In some studies<sup>[22-25]</sup>, the postoperative jaw-winking status was not mentioned. In cohort studies, we observed a greater preponderance of four objective measurements: magnitude of jaw-wink, MRD-1, levator excursion, and eyelid height. However, there remained no consistent manner by which these parameters were graded and reported [Table 6]. For future studies and case reports, we recommend including millimeter measurements of jaw-wink amplitude, MRD-1, levator excursion, and lagophthalmos in evaluations of MGJWS, with qualitative characterizations included as ancillary outcome descriptors. This will enable future post-hoc statistical analyses of outcome measures to better quantify the effectiveness of particular surgical approaches.

It is important to note that the efficacy of individual procedures may be affected by the severity of MGJWS, as this is in turn often a factor that determined the type of surgery that authors chose to perform. For example, Bowyer and Sullivan<sup>[17]</sup> performed unilateral levator advancement on patients with mild jaw-winking but bilateral levator weakening and brow suspension on patients with severe jaw-winking. In spite of this documented preference on the part of some surgeons, there was insufficient evidence on systematic review to support this as a consensus practice.

We observed that postoperative recurrence of both ptosis and jaw-wink was relatively common even in studies with limited follow-up duration, suggesting that a more structured approach to outcomes research would be beneficial for optimizing clinical results. Autologous fascia was the preferred sling material in most series, and the available evidence suggests that autogenous materials may be associated with fewer complications. The majority of case series reported only minor post-surgical complications with a limited impact on cosmetic or functional results, but heterogeneity of cohort size, reporting and analysis make it difficult to ascertain whether there are meaningful underlying lessons regarding surgical technique and sling material selection.

This review highlights findings that can be applied to clinical practice. First, we recommend that clinicians report preoperative and postoperative clinical findings, as described herein, in a quantitative, consistent manner, to enable more reliable systematic analyses. In addition, physicians should thoroughly counsel patients and families that the literature on management of MGJWS does not provide clear consensus guidelines, and that there is no clearly defined optimal approach to all cases. Furthermore, thorough discussions regarding potential complications - including the possibility for over-/undercorrection and recurrence - are critical to properly manage expectations preoperatively.

There were a number of limitations associated with this review. The primary limitation was the small sample size of 26 peer-reviewed articles (comprising 383 patients) that met inclusion criteria and the heterogenous reporting of pre-surgical metrics and outcome measures. Furthermore, in some studies with more statistical power<sup>[21,23,34]</sup> patients with MGJWS were a subset of a larger ptosis population. This analysis was a systematic review of the existing literature and therefore does not provide new prospective data. In addition, this review is limited to the peer-reviewed literature and does not describe surgical techniques that may be employed by surgeons anecdotally.

In conclusion, evidence-based lessons on the surgical management of MGJWS are limited, even when post-hoc analysis is applied to the existing literature in a systematic fashion. No clear consensus was noted, and at present, the disorder is treated according to a case-by-case approach governed by surgeon and family preference. Patients and physicians alike should be aware that recurrences in either ptosis or synkinetic jaw-winking movements are not uncommon after initial surgeries, but that in some cases, postoperative decreases in lid elevation can be corrected with subsequent interventions (such as additional levator excisions for patients who received the Neuhaus/Lemagne procedure). Future analyses may identify

**Table 6. Outcome measures reported in case series and retrospective reviews**

Author(s)	Postoperative jaw-winking	MRD-1	Upper eyelid excursion	Eyelid height
Bajaj <i>et al.</i> <sup>[15]</sup>	Resolution (< 1 mm of excursion of upper eyelid with synkinetic mouth movement) in three patients. Improvement in MGJWP (> 2 mm decrease but > 1 mm of excursion of upper eyelid with synkinetic mouth movement) in seven patients	N/A	Lid lag 1.20 ± 0.48	Postoperative lagophthalmos was 0.80 ± 0.88 mm. Amount of ptosis correction 2.40 ± 0.50
Betharia and Kumar <sup>[14]</sup>	Resolution in all patients	N/A	N/A	"Good correction" in 66.6% of patients; lagophthalmos average 2 mm ("minimal")
Bartkowski <i>et al.</i> <sup>[29]</sup>	Resolution in 84% of patients; improvement in 31.6% of patients	N/A	N/A	68% of patients had proper width and symmetry of palpebral fissures. Remaining had marked improvement
Park <i>et al.</i> <sup>[30]</sup>	Moderate degree of residual jaw-winking	N/A	N/A	"Ideal"
Shah <i>et al.</i> <sup>[31]</sup>	Resolution in 39.1% of patients, "improvement" in 47.8% of patients, and no improvement in 13.04% of patients	From -1.13 ± 0.916 to 3.17 ± 0.865 mm	N/A	Ptosis correction was "good" in 65.21% of patients and "fair" in 26.08% of patients
Khwarg <i>et al.</i> <sup>[19]</sup>	Resolution in 37.0% of patients, mild winking (1 mm or less) on the lateral jaw movement in 48.2% of patients	For bilateral frontalis suspension with unilateral levator excision: 40% of patients had "good" and "60%" had poor results. For bilateral frontalis suspension with bilateral levator excision: 68.4% of patients had good and 31.6% had fair results	Only initial excursion was recorded: poor (≤ 4 mm) in 22.2% of eyelids, fair (5-7 mm) in 19%, and good (8 mm or more) in 59%	N/A
Bowyer and Sullivan <sup>[13]</sup>	Unilateral levator advancement: persistent. Bilateral levator weakening with bilateral frontalis suspension: resolution	N/A	Only initial excursion was recorded: poor (≤ 4 mm) in 7% of eyelids, fair (5-7 mm) in 25%, and good (8 mm or more) in 68%	Improved
Ning <i>et al.</i> <sup>[32]</sup>	Resolution in all patients	From -0.57 mm to 2.96 ± 0.48 mm.	Preoperative mean excursion was 3.69 ± 1.09 mm	Postoperative palpebral fissure height of the operated eye in primary gaze was 7.93 ± 0.58 mm, with no significant difference to the unaffected side
Demirici <i>et al.</i> <sup>[16]</sup>	Resolved in 97% of patients, improved (from 6 mm to 2 mm) in 3%	N/A	From 7.5 ± 2.3 mm for the ptotic eyelid and 13 ± 2.2 mm for the normal eyelid, to 3.2 ± 0.9 mm for the ptotic eyelid and 10.3 ± 3.3 mm for the opposite normal eyelid	Upper eyelid margin distance from 0.1 ± 1.8 to 1.1 ± 0.9 mm
Doucet and Crawford <sup>[33]</sup>	For bilateral suspension: resolution. For unilateral suspension: no improvement	N/A	N/A	Moderate/severe ptosis in 37.5% of patients with levator muscle excision and 8.3% of patients with unilateral fascial suspension
Ho <i>et al.</i> <sup>[24]</sup>	N/A	Final MRD-1 > 3 mm in 25% of patients	N/A	Lid height determined by MRD-1

Cates and Tyers <sup>[21]</sup>	Resolution in 85.7% of patients	N/A	N/A	Successful lid height (within 1 mm of desired height) in 85.7% of patients
Morris <i>et al.</i> <sup>[34]</sup>	NA	Mean MRD from 0 to 2 mm; satisfactory MRD-1 elevation in 85.7% pts	N/A	57% of patients had satisfactory symmetry (< 1 mm difference between eyelid height)
Ibrahim <sup>[35]</sup>	Resolution in all patients	Mean MRD from 3.6 mm to 5.3 mm	N/A	N/A
Dave <i>et al.</i> <sup>[22]</sup>	N/A (results not stratified by MGJWS status)	N/A	NA	N/A
Xiang <i>et al.</i> <sup>[36]</sup>	Resolution in all patients	N/A	N/A	77% of patients had equal palpebral apertures, 23% had mild residual ptosis
Manners <i>et al.</i> <sup>[37]</sup>	Resolution in 93% of patients	N/A	N/A	62% of patients had improved ptosis, 21% had no change in ptosis, and 14% had worsened ptosis

whether particular surgical approaches are more appropriate, effective, and safe for particular clinical scenarios.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study, performed data analysis and interpretation, and wrote the manuscript: Bair H, Garcia GA, Erickson BP

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

Stanford University, Department of Ophthalmology is a recipient of an institutional Research to Prevent Blindness unrestricted grant and the National Eye Institute (P30-EY026877).

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Copyright

© The Author(s) 2020.

## REFERENCES

- Gunn RM. Congenital ptosis with peculiar associated movements of the affected lid. *Trans Ophthalmol Soc UK* 1883;3:283-7.
- Pearce FC, McNab AA, Hardy TG. Marcus Gunn jaw-winking syndrome: a comprehensive review and report of four novel cases. *Ophthalmic Plast Reconstr Surg* 2017;33:325-8.
- Yamada K, Hunter DG, Andrews C, Engle EC. A novel KIF21A mutation in a patient with congenital fibrosis of the extraocular muscles and Marcus Gunn jaw-winking phenomenon. *Arch Ophthalmol* 2005;123:1254-9.
- Kacar Bayram A, Per H, Quon J, et al. A rare case of congenital fibrosis of extraocular muscle type 1A due to KIF21A mutation with Marcus Gunn jaw-winking phenomenon. *Eur J Paediatr Neurol* 2015;19:743-6.

5. Lyness RW, Collin JR, Alexander RA, Garner A. Histological appearances of the levator palpebrae superioris muscle in the Marcus Gunn phenomenon. *Br J Ophthalmol* 1988;72:104-9.
6. Kannaditharayil D, Geyer H, Hasson H, Herskovitz S. Bilateral Marcus Gunn jaw-winking syndrome. *Neurology* 2015;84:1061.
7. Carman KB, Ozkan S, Yakut A, Ekici A. Marcus Gunn jaw winking synkinesis: report of two cases. *BMJ Case Rep* 2013;2013.
8. Alshamrani AA, Alghulaydhawi FA, Al Shamrani M. Marcus Gunn jaw-winking syndrome associated with morning glory disc anomaly. *Middle East Afr J Ophthalmol* 2019;26:37-9.
9. Kumar V, Goel N, Raina UK, Ghosh B. "See-saw" Marcus Gunn syndrome. *Ophthalmic Plast Reconstr Surg* 2011;27:e144-5.
10. Oltmanns M, Khuddus N. Duane retraction syndrome type I, Marcus Gunn jaw-winking and crocodile tears in the same eye. *J Pediatr Ophthalmol Strabismus* 2010;47 Online:e1-3.
11. Neuhaus RW. Eyelid suspension with a transposed levator palpebrae superioris muscle. *Am J Ophthalmol* 1985;100:308-11.
12. Lemagne JM, Brucher JM, Michiels J. Clinical, biochemical and histological results of a levator muscle transposition for ptosis in Cynomolgus monkeys. *Orbit* 1985;1:141-6.
13. Bowyer JD, Sullivan TJ. Management of Marcus Gunn jaw winking synkinesis. *Ophthalmic Plast Reconstr Surg* 2004;20:92-8.
14. Betharia SM, Kumar S. Levator sling for Marcus Gunn ptosis. *Br J Ophthalmol* 1987;71:685-9.
15. Bajaj MS, Angmo D, Pushker N, Hada M. Modified technique of levator plication for the correction of Marcus Gunn jaw-winking ptosis: a case series. *Int Ophthalmol* 2015;35:587-91.
16. Demirci H, Frueh BR, Nelson CC. Marcus Gunn jaw-winking synkinesis: clinical features and management. *Ophthalmology* 2010;117:1447-52.
17. Lemagne JM. Transposition of the levator muscle and its reinnervation. *Eye*. 1988;2:189-92.
18. Carbajal UM. Surgery of ptosis associated with jaw-winking. *Am J Ophthalmol* 1959;47:352-7.
19. Khwarg SI, Tarbet KJ, Dortzbach RK, Lucarelli MJ. Management of moderate-to-severe Marcus-Gunn jaw-winking ptosis. *Ophthalmology* 1999;106:1191-6.
20. Cumpston M, Li T, Page MJ, et al. Updated guidance for trusted systematic reviews: a new edition of the cochrane handbook for systematic reviews of interventions. *Cochrane Database Syst Rev* 2019;10:ED000142.
21. Cates CA, Tyers AG. Results of levator excision followed by fascia lata brow suspension in patients with congenital and jaw-winking ptosis. *Orbit* 2008;27:83-9.
22. Dave TV, Sharma P, Nayak A, Moharana R, Naik MN. Outcomes of frontalis sling versus levator resection in patients with monocular elevation deficiency associated ptosis. *Ophthalmic Plast Reconstr Surg* 2019;35:251-5.
23. Kemp EG, MacAndie K. Mersilene mesh as an alternative to autogenous fascia lata in brow suspension. *Ophthalmic Plast Reconstr Surg* 2001;17:419-22.
24. Ho YF, Wu SY, Tsai YJ. Factors associated with surgical outcomes in congenital ptosis: a 10-year study of 319 cases. *Am J Ophthalmol* 2017;175:173-82.
25. Beard C. A new treatment for severe unilateral congenital ptosis and for ptosis with jaw-winking. *Am J Ophthalmol* 1965;59:252-8.
26. Nagpaul PN, Charan H. Marcus Gunn phenomenon. *Br J Ophthalmol* 1968;52:484-5.
27. Tsai CC, Lin TM, Lai CS, Lin SD. Use of the orbicularis oculi muscle flap for severe Marcus Gunn ptosis. *Ann Plast Surg* 2002;48:431-4.
28. Yoshikata R, Yanai A. A clinical sign of the Marcus Gunn phenomenon. Case report. *Scand J Plast Reconstr Surg Hand Surg* 1999;33:237-41.
29. Bartkowski SB, Zapala J, Wyszynska-Pawelec G, Krzystkova KM. Marcus Gunn jaw-winking phenomenon: management and results of treatment in 19 patients. *J Craniomaxillofac Surg* 1999;27:25-9.
30. Park DH, Choi WS, Yoon SH. Treatment of the jaw-winking syndrome. *Ann Plast Surg* 2008;60:404-9.
31. Shah AD, Kumar AB, Kothari K. Bilateral Marcus Gunn jaw winking synkinesis with monocular elevation deficiency: a case report and literature review. *Int Ophthalmol* 2012;32:199-201.
32. Ning Q, Cao J, Xie J, Gao Q, Wang C, Ye J. Unilateral levator aponeurosis excision for Marcus Gunn syndrome and risk factors of residual jaw winking. *J Ophthalmol* 2019;2019:2058047.
33. Doucet TW, Crawford JS. The quantification, natural course, and surgical results in 57 eyes with Marcus Gunn (jaw-winking) syndrome. *Am J Ophthalmol* 1981;92:702-7.
34. Morris CL, Buckley EG, Enyedi LB, Stinnett S, Freedman SE. Safety and efficacy of silicone rod frontalis suspension surgery for childhood ptosis repair. *J Pediatr Ophthalmol Strabismus* 2008;45:280-8; quiz 9-90.
35. Ibrahim HA. Use of the levator muscle as a frontalis sling. *Ophthalmic Plast Reconstr Surg* 2007;23:376-80.
36. Xiang N, Hu WK, Li B, Liu R. Management of moderate-to-severe Marcus-Gunn syndrome by anastomosis of levator and frontal muscles. *Int J Ophthalmol* 2010;3:342-345.
37. Manners RM, Rosser P, Collin JRO. Levator transposition procedure: a review of 35 cases. *Eye* 1996;10:539-44.



Review

Open Access



# *Deschampsia antarctica* extract (Edafence®) as a powerful skin protection tool against the aging exposome

Manuel Mataix<sup>1,\*</sup>, Azahara Rodríguez-Luna<sup>2</sup>, María Gutiérrez-Pérez<sup>1</sup>, Massimo Milani<sup>3</sup>, Alberto Gandarillas<sup>4</sup>, Jesús Espada<sup>5,6</sup>, Azahara Pérez-Davó<sup>7,\*</sup>

<sup>1</sup>Department of Biology, Faculty of Sciences, Autónoma University of Madrid (UAM), Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid 28049, Spain.

<sup>2</sup>Innovation and Development, Cantabria Labs, Madrid 28043, Spain.

<sup>3</sup>Medical Department, Cantabria Labs Difa Cooper, Caronno Pertusella, VA 21042, Italy.

<sup>4</sup>Cell Cycle, Stem Cell Fate and Cancer Laboratory, Institute for Research Marqués de Valdecilla (IDIVAL), Santander 39011, Spain.

<sup>5</sup>Experimental Dermatology and Skin Biology Group, Ramón y Cajal Institute for Health Research, Ramón y Cajal University Hospital, Madrid 28034, Spain.

<sup>6</sup>Centro Integrativo de Biología y Química Aplicada (CIBQA), Universidad Bernardo O'Higgins, Santiago 8370993, Chile.

<sup>7</sup>Medical Affairs Department, Cantabria Labs, Madrid 28043, Spain.

\*These authors contributed equally to this work.

**Correspondence to:** Dr. Azahara Rodríguez-Luna, Innovation and Development, Cantabria Labs, Calle Arequipa 1, Madrid 28043, Spain. E-mail: azahara.rodriguez@cantabrialabs.es; Dr. Azahara Pérez-Davó, Medical Affairs Department, Cantabria Labs, Calle Arequipa 1, Madrid 28043, Spain. E-mail: azahara.perez@cantabrialabs.es

**How to cite this article:** Mataix M, Rodríguez-Luna A, Gutiérrez-Pérez M, Milani M, Gandarillas A, Espada J, Pérez-Davó A. *Deschampsia antarctica* extract (Edafence®) as a powerful skin protection tool against the aging exposome. *Plast Aesthet Res* 2020;7:69. <http://dx.doi.org/10.20517/2347-9264.2020.138>

**Received:** 25 Jun 2020 **First Decision:** 9 Oct 2020 **Revised:** 27 Oct 2020 **Accepted:** 10 Nov 2020 **Published:** 5 Dec 2020

**Academic Editor:** Salvador Gonzalez, Raúl González-García **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

The impact of the interaction of all combined environmental agents to which an individual is exposed during his/her lifetime, as well as how his/her organism responds to these influences, defines health, aging, and disease. The systematic, integrative characterization of the different elements making up the “exposome” is thus necessary to identify and exploit the potential of compounds capable of conferring protection with minimal side effects. Extracts from the natural world, containing synergistic combinations of compounds with antioxidant and protective properties, have long been used in traditional medicine. Modern science has the opportunity to leverage these substances honed by evolution and use them safely and reliably, with a profound mechanistic knowledge and guaranteeing standardization and absence of toxicity. Here, we discuss our current knowledge regarding the potential of a soluble extract of the hair grass *Deschampsia antarctica* (as its standardized commercial preparation Edafence®) to counteract the skin exposome and its impact on skin aging and disease.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Keywords:** Exposome, skin aging, skin homeostasis, detoxifying, *Deschampsia antarctica*, Edafence®, pollution

## INTRODUCTION

Environmental agents, both natural (e.g., sunlight, moisture, and endogenous active compounds such as sweat) and derived from human activity (e.g., air pollutants, plastics, cosmetics, textiles, and tobacco), compound a sustained challenge for our organism in general, and the skin in particular, to maintain homeostasis. Our modern lifestyle and increased life expectancy demand the identification and characterization of substances simultaneously safe (i.e., non-toxic) and effective to confer protection, either directly or through boosting our endogenous defense mechanisms, including stress and DNA damage repair pathways, antioxidant and proteostatic programs, and tissue architecture remodeling. Popular wisdom through the ages has identified, basically by tinkering and intuitive experimentation, products from nature that consistently exert protection from environmental wearing of our organism. However, we have now the opportunity to, beyond practical conveniences such as safety and consistency, understand with unprecedented detail how these compounds intersect with both environmental and endogenous stressor agents, to confer protection against tissue damage.

Here, we frame our current knowledge about the activity of a soluble aqueous extract from the hair grass *Deschampsia antarctica* (Edafence®), a tracheophyte adapted to extreme environmental conditions and endowed with remarkable protective and antioxidant properties, on this integrative perspective and discuss underlying candidate mechanisms and therapeutic potential.

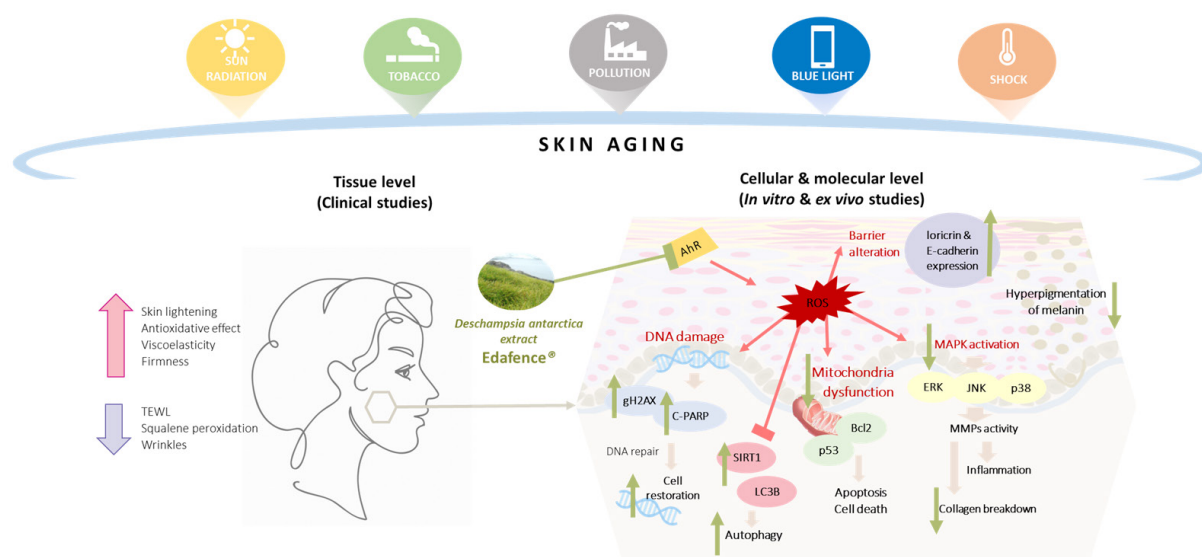
## THE SKIN EXPOSOME AND SKIN HEALTH AND AGING

The basic concept that phenotype arises from the interplay between genotype and environment is most clearly portrayed by the impact external agents have on aging and disease. While environmental agents may account on their own for up to ~16% of total deaths worldwide<sup>[1]</sup>, their impact on global health is far larger, as prime challenges to public health such as cardiovascular disease have a very limited (well below 50%) genetic basis<sup>[2]</sup>. At present, ~5000 toxic chemical species are identified as posing a significant threat to human population across the globe<sup>[1]</sup>. Thus, approaching the study of the impact of environment on the organism and their interplay, from a systematic and integrative perspective, is warranted. The term “exposome”, proposed in 2005 by cancer epidemiologist Christopher Wild<sup>[3]</sup>, aims at capturing this dynamic, reciprocal complexity and is currently defined as the totality of exposures an organism receives from conception to death and their interplay with the organism’s response<sup>[4]</sup>.

The skin is the first body barrier environmental cues encounter in our daily lives. As such, it is an organ whose physiopathology cannot be understood without considering this external influence, and the skin exposome is the central driver of skin aging and diseases such as cancer or chronic inflammatory conditions<sup>[5,6]</sup>. While classifying the wide variety of environmental agents our skin encounters is cumbersome and their interdependence or even synergy must additionally be taken into account, for simplicity, we briefly enumerate them as: (1) air pollution; (2) tobacco; (3) light radiations; and (4) other environmental agents including temperature and humidity, different chemicals from daily activities (nutrition, cosmetics, plastics), and endogenous factors such as stress and sleep deprivation [Figure 1].

### Air pollution

Human activity in the industrialized era releases into the atmosphere different pollutants that, apart from inducing damage to respiratory airways and associated conditions, have a direct impact on skin homeostasis<sup>[1,5,7,8]</sup>. The list of these agents is extensive and includes ionizing molecular gas species [ozone (O<sub>3</sub>), carbon monoxide/carbon dioxide (CO/CO<sub>2</sub>), nitrogen species, and sulphide dioxide (SO<sub>2</sub>)], volatile



**Figure 1.** Damaging activity of external aggressive factors (exposome). Current knowledge (primarily from *in vitro/ex vivo* studies) of cell/tissue damage mechanisms, their counteracting defense pathways, and how they are affected by Edafence® are summarized

compounds such as hydrocarbon molecules, and particulate matter, either coarse or fine (usually termed  $PM_{10}$  and  $PM_{2.5}$  according to their size in microns). All these compounds have been shown experimentally to induce damage and stress responses in skin cells and tissues and correlate with aging (in fact, they are precursory to a major share of differences in skin aging between urban and rural areas<sup>[9]</sup>), but their net effect must also take into account synergistic effects among each other, as well as with radiations (see below)<sup>[10,11]</sup>. Their molecular action mechanism is varied, but most of these agents induce oxidative stress and damage of cell structures [for example, nitric oxide (NO) and  $O_3$  can promote lipid peroxidation<sup>[5,12]</sup>] and activate adaptive responses primarily aimed at reducing cell damage, which in the long term contribute to the aging phenotype [most prominently, the aryl hydrocarbon receptor (AhR), see below].

### Tobacco

It may be considered “portable pollution”, as it constitutes an efficient means to deliver more than 2000 harmful substances to our organism and skin, including CO, formaldehyde, hydrocarbons, different toxic elements (cadmium and mercury), and tar, and, together with the characteristic effects of gestural wrinkling and skin pigmentation, its dismal impact on skin aging is well-established<sup>[5,13]</sup>. Tobacco smoke induces generic oxidative stress (partly due to its impact on mitochondrial function) and DNA damage, and, importantly, it has been shown to impair activated motility and alignment of fibroblasts and wound healing. It also induces stress hallmarks of connective tissue remodeling [matrix metalloproteinase 1 (MMP1)] and compromises skin barrier integrity<sup>[5,14-16]</sup>.

### Light radiation

Solar exposure is currently recognized as a prime environmental agent contributing to skin damage and aging, and the term photoaging has been coined to specifically describe this effect<sup>[5]</sup>. Because of their sustained, cumulative impact throughout an individual’s lifespan and their relevance as a prime oncogenic agent, as precursor of melanomas and other skin cancers, the impact of light radiations on skin homeostasis has been intensively studied for a long time<sup>[17,18]</sup>. Solar radiation, and particularly its high-energy ultraviolet radiation (UVR) spectrum, induces profuse alterations in the genomic material of skin cells, even before transformation phenotypes become apparent<sup>[19]</sup>. These alterations are direct precursors of tumorigenesis and senescence<sup>[18,20,21]</sup>. Light radiations are also powerful inducers of adaptive responses that can primarily counteract direct cell or tissue damage, but also intersect with pathways regulating immunity<sup>[5,18,20,22]</sup>;

these links attract interest because of their potential involvement in the onset and progression of immune dysregulation, underpinning conditions such as rosacea or lupus erythematosus<sup>[23]</sup>.

The solar spectrum comprises different wavelengths, ranging from short, high-energy wavelength radiation (UVR; < 380 nm) to low-energy infrared radiation (> 800 nm), through the visible spectrum (380-800 nm). UVR comprise ~5% of the total radiation spectrum reaching the skin<sup>[24]</sup> but is the most energetic and is likely one of the best-studied components of the skin exposome. A major impact of short-wave ionizing radiation on skin cells is either direct DNA damage by covalent alteration of nucleic acids (mostly exerted on pyrimidine bases) or indirect damage provoked by reactive oxygen species (ROS) and other highly reactive products, derived from both generic oxidative stress and the ionizing damage of other cell structures<sup>[5,18]</sup>. A relevant principle to mention is the fact that the contribution of ionizing radiations to skin damage and aging stems from a primary impact on the dermis (including the fibroblasts that serve the connective tissue and nurture other components of the dermis and the epidermal layer)<sup>[25]</sup>.

While lower energy wavelengths have long been regarded as irrelevant, several studies have demonstrated that radiation across the visible spectrum and even infrared radiations can induce significant responses in skin cells and tissues (such as pigmentation and expression of stromal remodeling enzymes for tissue repair such as MMP1), and therefore an impact on their physiology and molecular constituents<sup>[5,26-28]</sup>. As their net load is much higher than higher-energy radiations across time, increasing attention is being devoted to their effect. Wavelengths within the visible blue spectrum are capable of inducing oxidative stress *in vivo*, driving significant gene expression reprogramming in skin cells and reducing keratinocyte proliferation<sup>[29-31]</sup>. They may also promote a dysregulation of homeostatic molecular systems, such as those regulating osmotic balance<sup>[32]</sup>. This specific wavelength range is currently being intensively studied because of its higher relative energy and increasing widespread exposure due to electronic devices and artificial lighting, also called *digital pollution*<sup>[33]</sup>. Infrared light can exert a distinct impact on skin homeostasis and promotes specific gene expression signatures, including MMP1 upregulation; these effects may partially derive from its promotion of heat (intrinsically linked to skin aging, see below) apart from direct molecular mechanisms<sup>[5,27,34]</sup>.

### Miscellanea

Additional environmental factors, such as recurrent exposure to acute temperature changes, can promote aging, as evidenced by upregulation of different biomarkers indicating tissue damage (inflammatory infiltration, neovascularization, and oxidative DNA damage) upon exposure to heat<sup>[34,35]</sup>. Indeed, severe skin aging has been observed in exposed body parts in certain occupations such as glass blowers and bakers<sup>[5]</sup>. Dryness is also considered a hallmark of skin aging, and molecular changes such as aquaporin expression are altered with this process. It is thus not surprising that dry climates are associated with increased skin aging, commonly combining with high solar exposure and extreme temperatures<sup>[5,36]</sup>.

Modern lifestyle exposes our skin to a remarkable number of agents that can have an impact on skin health. Cosmetics can deliver different damaging compounds to our skin and are thus regularly screened not only for intrinsic toxicity but, most importantly, also for their sensitizing effect in the presence of other agents such as light radiation<sup>[37]</sup>. An additional class of external agents that can provoke skin damage are dietary components that exert metabolic stress, and byproducts of endogenous metabolism are associated with disturbed patterns of sleep and stress<sup>[5,38]</sup>. Apart from major imbalances such as insulin resistance and diabetes, which are linked to systemic inflammation<sup>[39]</sup>, high levels of certain nutrients such as carbohydrates or animal saturated fats and high-protein diets promote adverse metabolic states in otherwise “healthy” individuals and are linked to tissue aging, including skin aging<sup>[5,39-41]</sup>. An interesting additional direct adverse effect of high carbohydrate intake on skin and other tissues has been proposed through the formation of aberrant protein-glycan adducts, whose deposition may disrupt glycoprotein structures such

as those formed by fibrillary components of the connective tissue. Additionally, these compounds may, in a similar fashion to specific pharmacological agents, sensitize skin to UV radiation<sup>[5,42]</sup>.

## ENDOGENOUS MOLECULAR MECHANISMS DETERMINING THE IMPACT OF THE SKIN EXPOSOME

A majority of environmental stressors provoke skin damage and aging through either direct disruption of cell and tissue structures, such as DNA damage by light radiation, or by fostering the accumulation of toxic molecules, such as ROS, upon perturbation of cell metabolism - most importantly, mitochondrial function<sup>[18,43]</sup>. While these events can trigger proapoptotic signaling networks, such as the p53/BclX/Bcl-2 axis and the caspase activation cascade, adaptive mechanisms have evolved to counteract these aggressions to cell integrity and promote repair, as well as for efficiently and safely disposing of xenotoxins [Figure 1]. Reflecting the intimate relationship those molecular mechanisms have with the natural process of aging, these adaptive networks are integrated with general cell stress responses and repair mechanisms, including autophagy, proteostatic Unfolded Protein Responses (UPR), inflammation, and the DNA damage response (DDR)<sup>[44-48]</sup>. All of these mechanisms have been found essential to counteract skin damage and aging and leveraging on them is considered a priority strategy for therapeutic intervention<sup>[43,49,50]</sup>.

### Antioxidant and proteostatic responses

A major aspect of cell response to exposome aggression is the deployment of adaptive responses aiming at reducing the impact of oxidative damage to cell components. Reflecting the multiple sources of oxidant molecular species, both endogenous (e.g., physiological metabolism, inflammatory states) and exogenous, several stress responses also converge on the activation of these programs, as is the case for proteostatic responses such as UPR, DDR (polyADP rybosylation, H2AX phosphorylation, and downstream networks), the ERK/p38/JNK stress signaling network, and both bulk autophagy and mitophagy<sup>[47,48,51-55]</sup>. Importantly, inflammation signaling (such as the NF-kB transcriptional node) is integrated with these stress responses, feeding from and into ROS levels, and can drive tissue repair and protection as well as damage, depending on its amplitude<sup>[49,50,54-57]</sup>. Evidence supports all these responses exerting protective and antiaging roles in different organisms, and in human skin in particular<sup>[44-46]</sup>. In fact, natural aging is intimately associated with the decline of these mechanisms. Identifying compounds to specifically intervene in these mechanisms is therefore a priority for the prevention of skin aging exposome influence<sup>[58-60]</sup>.

### AhR axis

AhR is a conserved helix-loop-helix nuclear receptor that, upon binding with certain low molecular weight ligands, is released from quenching chaperones in the cytoplasm and orchestrates the expression of different gene subsets, primarily detoxifying and antioxidant enzymes. Both exogenous, “synthetic contaminants” and cyclic compounds generated endogenously upon exposure to UV radiation can activate AhR<sup>[61-64]</sup>. Importantly, the sustained activation of this pathway itself underlies the physiopathology of the impact of different xenotoxins, and its controlled modulation is currently studied intensively for therapeutic purposes.

### Stromal remodeling and repair enzymes

As stated above, a prime target of environmental damage and aging progression in the skin is the connective tissue servicing other structures. Indeed, a key hallmark of skin insult (which can be readily detected upon rather moderate cues such as visible light exposure) is the upregulation of certain extracellular matrix (ECM) remodeling enzymes such as matrix metalloproteases [e.g., MMP1, matrix metalloprotease 3 (MMP3)] and subsequent alterations in the architecture of ECM fibers<sup>[5,14,15,27,65-67]</sup>. Other skin structural components ensuring skin barrier integrity and protection, such as lorixin, cell-cell adhesion complex components, and E-cadherin, are accordingly highly sensitive to these responses and their changes are likely to play



a relevant role in the progression of both acute and cumulative skin damage<sup>[5]</sup>. Finally, melanization is a specific structural adaptation of the skin to protect from ionizing radiations<sup>[5,57]</sup>. As part of skin tissue repair programs, all these architectural remodeling activities are tightly engrained with tissue damage responses and the inflammatory signaling exposed above<sup>[5,15,57,65-67]</sup>.

## THE POTENTIAL OF *DESCHAMPSIA ANTARCTICA* SOLUBLE EXTRACT (EDAFENCE®) TO COUNTERACT THE IMPACT OF THE SKIN EXPOSOME

As previously indicated, modern lifestyle has increased the intensity and variety of damaging environmental agents on our health, including skin. Moreover, an exponential effect may result from the combination of these different agents, as is the case for pollutant-mediated sensitization to UV radiation. As such, identifying solutions to reduce the effects of this sustained aggression is warranted<sup>[68]</sup>. A rich source of substances and compounds is found in the natural world, because organisms have confronted environmental damaging agents such as ionizing radiations and toxins from the beginning of time, and the molecular damage mechanisms also apply to byproducts of endogenous metabolism. Thus, compounds with antioxidant and protective activities, also capable of boosting endogenous defense mechanisms, are found in nature and have been explored for their therapeutic potential since ancient times<sup>[69-74]</sup>.

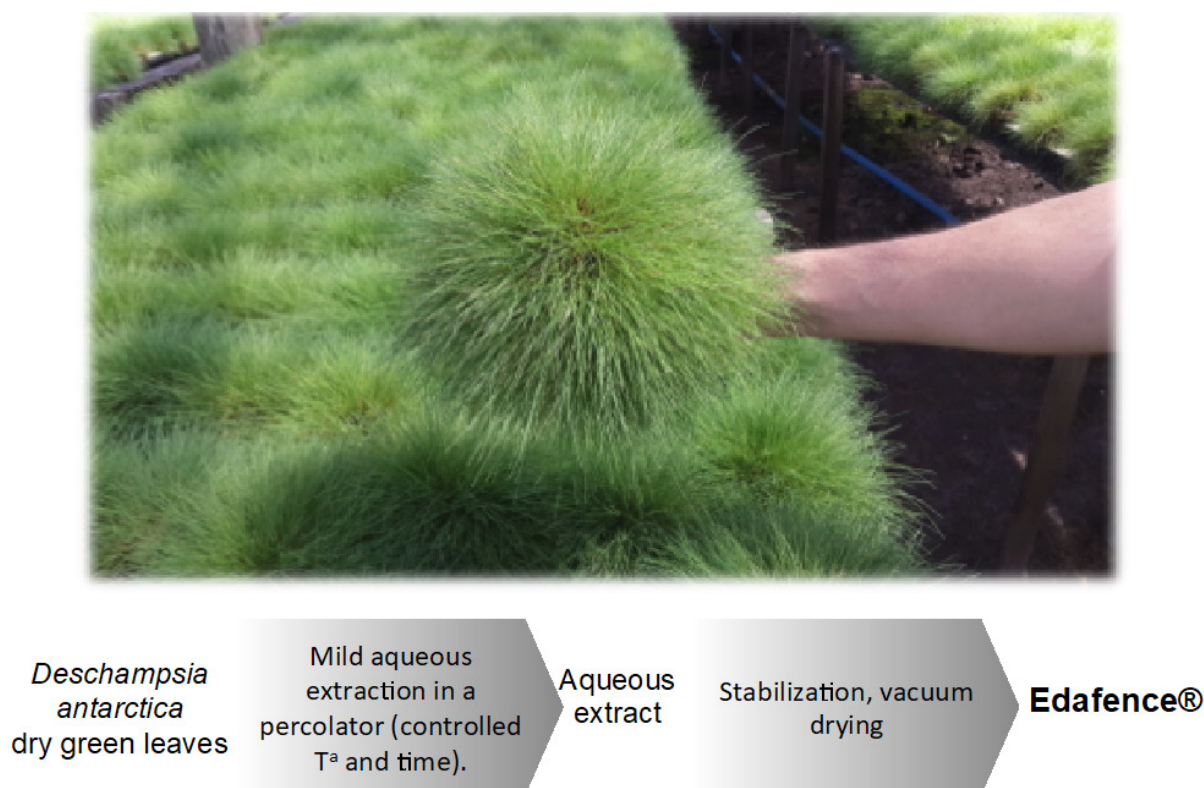
*Deschampsia antarctica* is a tracheophyte hair grass species, a polyextremophile Gramineae native to Antarctica, capable of thriving under extreme conditions of solar irradiation, temperature, dryness, salinity, and oxidative stress due to unique, evolutionary molecular mechanisms providing highly efficient protection against environmental aggression [Figure 2]. One of only two flowering plants in the Antarctic<sup>[75]</sup>, it partly owes its resilience to secondary metabolic routes which provide photoquenching compounds, “refolding” regulators, and dehydrins, as well as phenolic substances with strong antioxidant potential, including flavonoids such as apigenin and luteolin<sup>[76]</sup>. A standardized procedure for mild aqueous extraction of soluble fractions from *Deschampsia antarctica* has been established<sup>[77]</sup>, avoiding the use of organic solvents, whose associated contamination and residue carryout problems can be difficult to circumvent [Figure 2].

Briefly, dry green leaves obtained from the plant are introduced in a percolator through which water - or an aqueous solvent - is circulated under controlled temperature and time conditions. The obtained aqueous extract is then stabilized and vacuum dried. The resulting powder, Edafence®, presents activities against external aggressive factors<sup>[77]</sup>. Experimental and clinical evidence supports the potential of soluble extracts of this plant (Edafence®; see Figure 2) to counteract different detrimental effects of urban environment<sup>[78,79]</sup>.

### Experimental evidence showing Edafence® counteracting the effects of cutaneous environmental factors

#### *Damage from air pollutants*

This aqueous extract of *Deschampsia antarctica* counteracts damage induced by different xenotoxins and damaging agents. As a powerful oxidant commonly used as an experimental proxy of both endogenous ROS production and exogenous oxidative stress, exposure to H<sub>2</sub>O<sub>2</sub> induces in dermal fibroblasts senescence and DNA damage and reduces cell viability. Addition of Edafence® was shown to powerfully counteract these effects, as assessed by the reduction of molecular stress hallmarks [sirtuin 1 (Sirt1) and thioredoxin 2 (Trx2) expression upregulation and blunting of PCNA downregulation]<sup>[80]</sup>. Interestingly, this extract's protection against reduced cell viability was achieved under experimental conditions whereby the extract was added in advance to exposure to the stressor, suggesting that, in addition to intrinsic antioxidant properties, Edafence® is effectively capable of priming protective cell states, for example through inducing endogenous antioxidant responses<sup>[78]</sup>. This extract also exhibits efficient protection from dioxin toxicity, as modeled by 2,3,7,8-tetrachlorodibenzo-p-dioxin; blunts AhR expression; and rescues loricrin expression



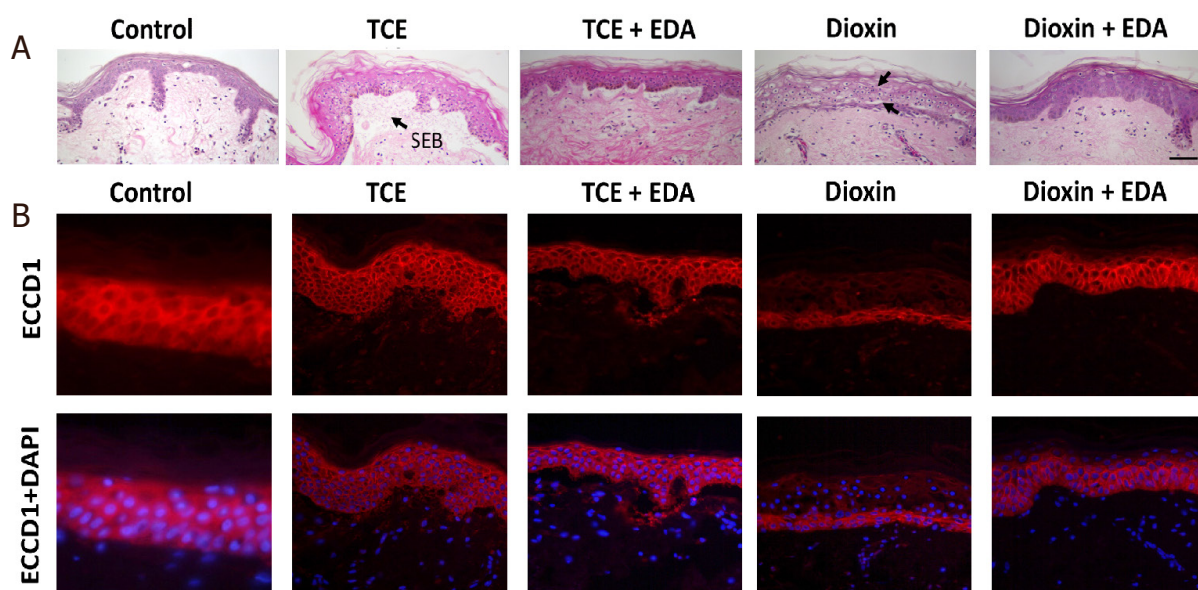
**Figure 2.** Outline of Edafence® extraction procedure

in keratinocytes<sup>[81]</sup>. The protective effect of this extract has also been demonstrated in an *in vitro* system to experimentally investigate the impact of specific toxic compounds (As, Cd, and Cr) on fibroblast homeostasis<sup>[82,83]</sup>.

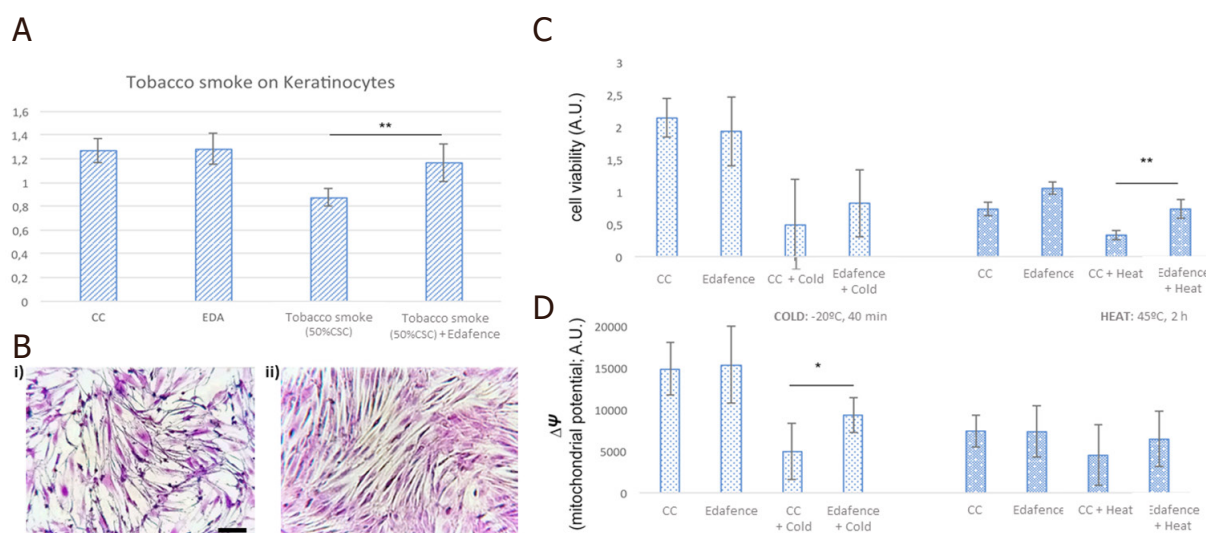
Recent studies provide evidence indicating that these protective mechanisms also apply to conditions closer to *in vivo* skin physiology. *Ex vivo* research on human skin organ cultures (hSOC; an experimental system that preserves physiological skin architecture) suggests that this aqueous extract of *Deschampsia antarctica* confers protection against both toxic compound models [combining arsenic and chromium I; toxic chemical elements (TCE)] and dioxins<sup>[82]</sup>. Indeed, addition of this extract prevented alterations to tissue architecture, skin barrier integrity (as assessed by E-cadherin expression and distribution), and dermal proliferation and significantly reduced oxidative DNA damage in hSOC exposed to TCE or dioxins<sup>[82]</sup> [Figure 3]. These results strongly support that the mechanisms by which Edafence® protects from different sources of cellular damage, as identified through the systematic *in vitro* experimentation described above, are relevant *in vivo*.

#### *Tobacco*

This extract has been tested on other components of the skin aging exposome to determine its activity [Figure 4]. Upon exposure to tobacco smoke (5% cigarette smoke condensate extract)<sup>[77]</sup> *in vitro*, this extract confers protection to human skin fibroblasts against loss of cell viability and collective organization and reverts aberrant morphological phenotypes. The effect is robust and reduces the impact of tobacco on cell viability by 66%; analogous positive results in increasing cell viability were also observed in human keratinocytes<sup>[77]</sup>. These observations support the potential for Edafence® in counteracting skin aging through maintaining and enhancing tissue repair mechanisms, a major target for tobacco-induced skin aging<sup>[13,14]</sup>.

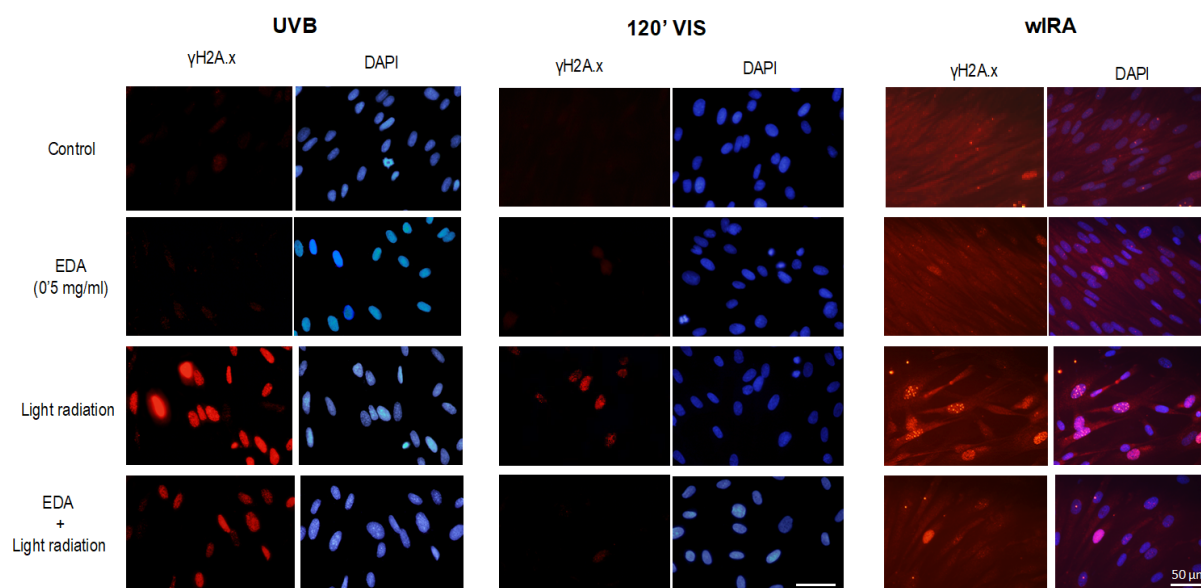


**Figure 3.** Edafence® protects against xenotoxic pollutants in ex vivo experimental models of skin integrity. Human Skin Organ Culture (hSOC) belongs to human skin biopsy samples from aesthetic surgeries. It was chosen as an experimental model to investigate the potential protective effects of this extract against exemplary chemical contaminants, including Toxic Chemical Elements (TCE: As and Cr) and dioxins. A: micrographs of H-E stained sections from hSOCs treated as indicated. Note severe morphological alterations induced by prolonged exposure (seven days) to common air pollutants, including Toxic Chemical Elements (TCE: 9 mmol/L As + 0.5 mmol/L Cr) and dioxins (10 nmol/L 2,3,7,8-tetrachlorodibenzen-p-dioxin). Note apparent subepidermal blister (SEB, arrows) in TCE-treated cells; exposure to dioxin also causes significant disorganization of epidermal layers (arrows). These alterations, indicative of a critical loss of skin function, are effectively prevented by Edafence® (2.5 mg/mL). Bars: 50  $\mu$ m; B: immunofluorescent staining of sections of hSOCs treated as indicated. Immunolocalization of the epithelial cell-cell adhesion molecule E-cadherin (ECCD1) confirms the extensive structural alterations of suprabasal epidermal layers induced by TCE/dioxin exposure and the protective effect against them conferred by the aqueous extract of *Deschampsia antarctica* treatment. Bars: 50  $\mu$ m. Adapted from [82]



**Figure 4.** Edafence's protective effects *in vitro* against other exposome agents. A, B: Edafence® incubation protects from damage induced by tobacco in human dermal fibroblasts (HDFs). It reduces loss of cell viability associated with exposure to tobacco smoke [(A) CSC: 5%, 3.5 h; Edafence® incubation: 10 mg/mL]. It also prevents alterations in collective organization (i.e., alignment) and morphological phenotype (B) compare (1) control CSC-exposed to (2) CSC-exposed supplemented with the extract. Scale bar: 100  $\mu$ m/L; C, D: Edafence® incubation protects human keratinocytes from both acute cold shock (data on the left, blue spotted pattern) and heat exposure (data on the right, blue background pattern). Primary human keratinocytes were subjected to the indicated treatments. Subsequently, cell viability was measured through crystal violet staining extension (C) and mitochondrial integrity/function was assessed by measuring mitochondrial potential with MitoTracker™ staining (D). \* $P < 0.05$ ; \*\* $P < 0.01$ . Adapted from [77,83]. CSC: cigarette smoke condensate





**Figure 5.** Edafence® attenuates oxidative DNA damage across different light radiation wavelengths in human fibroblasts. Human dermal fibroblasts were incubated with Edafence® for 24 h and then transiently exposed to different light radiations. Twenty-four hours later, cells were processed for immunofluorescence detection of  $\gamma$ H2A.x by confocal microscopy. Scale bar: 50  $\mu$ m. Adapted from [81,84,85]. UVB: ultraviolet B radiation; VIS: visible light spectrum; wIRA: infrared spectrum

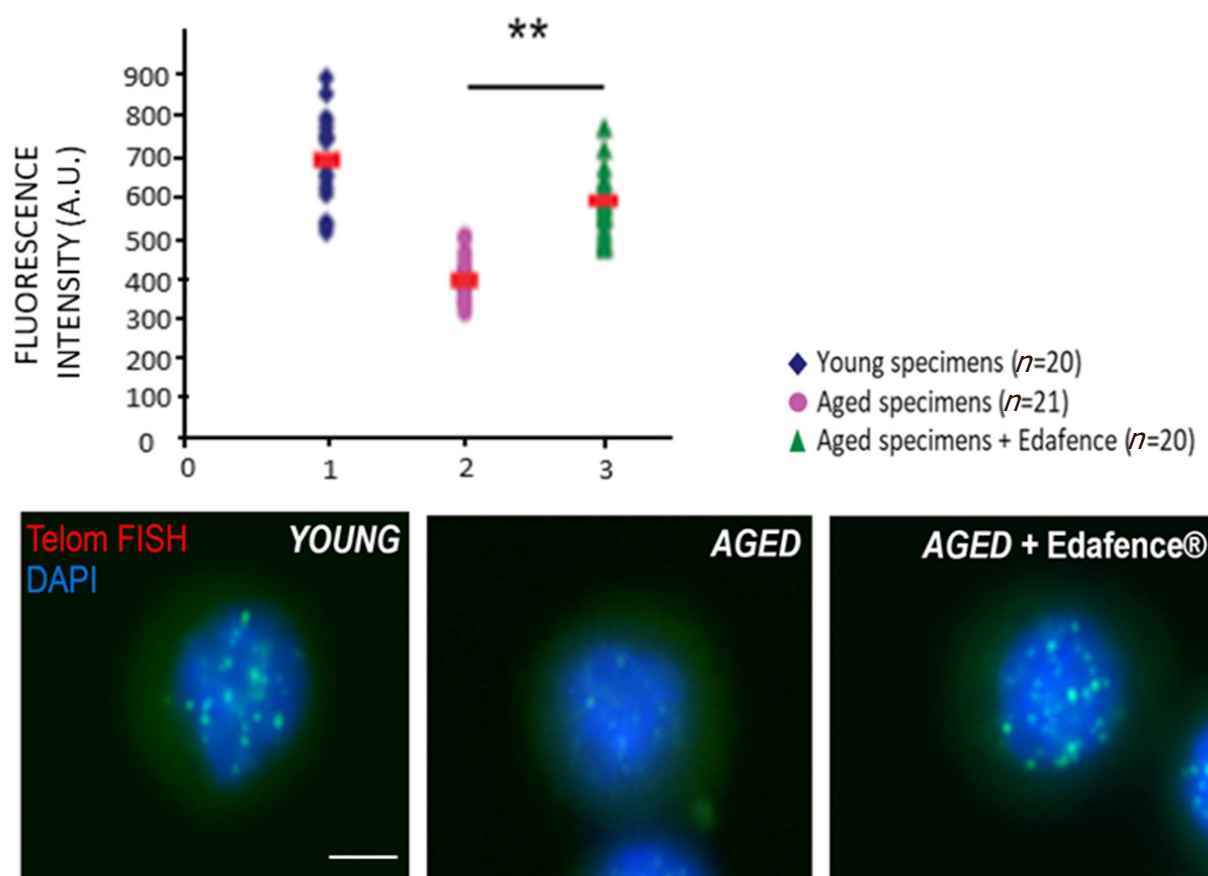
### Light radiations

Protection of skin cell types (mostly keratinocytes and skin fibroblasts) against the effects of solar radiations by this extract of *Deschampsia antarctica* has been explored in detail. Exposure to this extract protects human dermal fibroblasts from deleterious effects induced by high-energy UV radiation, including senescent visual phenotypes, oxidative DNA damage (as assessed by DDR hallmarks: poly-ADP ribose polymerase cleavage and H2AX induction), proapoptotic stress signaling (p38/JNK activation, caspase-3 cleavage, and increased autophagy flux), and expression of ECM remodeling enzymes (MMP1) [81].

Importantly, recent studies support that Edafence® also counteracts the damaging impact exerted by radiation within visible and infrared spectra on both human fibroblasts and keratinocytes [84]. Incubation with this extract reduces the accumulation of oxidative DNA damage-associated H2AX and the activation of autophagy and caspase signaling associated with visible light/infrared radiation (VL/IR) exposure [Figure 5]. Accordingly, exposure to Edafence® reverted reduced cell proliferation and the altered expression of ECM constituents (cathepsin K, MMP1, collagen I, and elastin) in a dose-dependent manner.

As aforementioned, an increasingly studied agent challenging skin homeostasis in modern life is high-energy blue light, a radiation wavelength emitted by digital devices and therefore reaching our body for extensive periods of time. Recent studies [85] mimicking artificial blue light exposure on *in vitro* cell cultures revealed that these wavelengths reduce cell viability (an effect more pronounced in melanocytes than in dermal fibroblasts), promote hyperpigmentation and morphological alterations, and induce mitochondrial dysfunction and oxidative stress. Of note, addition of Edafence® counteracted these effects and reduced oxidative stress and ROS accumulation, mitochondrial homeostasis, and stress markers in fibroblasts, as well as melanization of keratinocytes [85].

The molecular mechanisms at play remain to be fully understood, but both the intrinsic antioxidant activities of this extract bearing species capable of directly quenching oxidizing radicals as well as its ability to boost homeostatic programs in the cell, including endogenous antioxidant responses [78], could underpin the protective action of Edafence® against different sources of damaging light radiation.



**Figure 6.** Edafence® attenuates natural aging in human primary keratinocytes as assessed by telomeric length. Primary human keratinocytes were subject to *in vitro* aging (24 days) under indicated conditions with and without Edafence® treatment, 0.9 mg/mL, and processed for *in situ* hybridization [fluorescence *in situ* hybridization (FISH)] with a fluorescent probe targeting telomeric repeats (*Telom. FISH*, red signal; 4,6-diamidino-2-phenylindole counterstain, blue signal). Telomeric signal, as a proxy of telomere integrity/length, was acquired on a fluorescence microscope and computed as indicated. Scale bar: 10  $\mu$ m. \*\* $P < 0.01$ . Adapted from<sup>[86]</sup>

### Miscellanea

The studies described above suggest that the protective properties of this extract apply to a wide range of environmental agents driving cell and tissue aging. In fact, unpublished research<sup>[86]</sup> supports that this compound may attenuate “natural” aging and replicative exhaustion, as its supplementation reduces time-dependent telomere shortening in an *in vitro* human keratinocyte system [Figure 6] and positively regulates stem cell proliferation and DNA damage repair. Cellular DNA damage drives keratinocytes into terminal differentiation<sup>[87,88]</sup>. The aqueous extract of *Deschampsia antarctica* enhanced the potential of cellular repair and as a result protected the capacity of epidermal stem cells for self-renewal, supporting its positive effect on replication and differentiation potential<sup>[86]</sup>. These observations are particularly interesting as they might suggest that a protective mechanism for this extract relies on a positive impact on tissue repair and regeneration.

Temperature is another challenge against which Edafence® has been shown to confer significant protection under controlled experimental conditions [Figure 4]. Exposure to this extract protected human keratinocytes from both severe cold shock and heat, as assessed by measurements of cell viability and mitochondrial function (i.e., mitochondrial potential)<sup>[77]</sup>. The relative extent of this protective effect is higher in harsher conditions (i.e., during extended periods of heat shock; 60 min *vs.* 45 min at 42 °C), suggesting that protection conferred by this extract is sustained through time and is independent from



the specific nature of the external challenge applied. Similarly, exposure to Edafence® confers protection in keratinocytes and fibroblasts to moderate dehydration and hyperosmotic shock, improving viability in a dose-dependent manner<sup>[77]</sup>. These results suggest a genuine capability of Edafence® to counteract damaging stimuli, which likely extends to most elements in the skin aging exposome. Again, these *in vitro* studies further support the notion that Edafence-induced protection is durable (i.e., its effects continue after the exposure to the aqueous extract of *Deschampsia antarctica* and are comparatively higher upon longer exposure to damaging agents) and likely operates through integrated mechanisms, effective in the face of aggressions of different nature.

### Clinical studies on Edafence®

Bearing in mind the importance of correlating the *in vitro* and *ex vivo* findings with the potential *in vivo* relevance of these compounds, studies were conducted on the effect of topical preparations containing this extract on different parameters indicative of skin health and aging.

A first set of studies<sup>[89,90]</sup> explored the potential impact of this extract on skin aging under conditions of relatively high air pollution (metropolitan Rome at different times of the year). Milani *et al.*<sup>[89,90]</sup> reported improvement of skin barrier function (as inferred by transepidermal water loss measurements), reduction of squalene peroxidation ratios, and enhanced visual appearance as assessed by high-resolution digital imaging. Of note, these studies covered conditions of both high particulate air pollution (winter season<sup>[89]</sup>) and elevated O<sub>3</sub> levels (summer season<sup>[90]</sup>).

An additional recent study examined the impact of topic administration of Edafence-containing preparations on features indicative of general skin aging, among a homogeneous population (female, Caucasian with Fitzpatrick's Skin Types III and IV, aged 45-65 years)<sup>[91]</sup>. Quantitative measurement of features such as wrinkling (transient reduction ranging 20%-30% after four-week treatment, as evaluated quantitatively using digital imaging), firmness, and elasticity (up to 41.7% and 12.8% improvement, respectively, after 12-week treatment) indicated a positive effect of this extract on skin health and even moderate repair of aged skin, together with remarkable subjective improvement reported by tested subjects (100% of subjects stated significant improvement in skin texture and brightness) and tolerance of the relatively high-dose preparations<sup>[91]</sup>. This preliminary research encourages larger studies investigating the potential synergy with concomitant interventions such as nutraceuticals, moisturizing creams, and sunscreen preparations. Taken together, these studies support that the aqueous extract of *Deschampsia antarctica*, in combination with antioxidants and retinoids (products formulated by Cantabria Labs), bears potent anti-aging activity through the improvement of skin barrier integrity and function, normalizing skin tone and counteracting oxidative stress in polluted urban zones. These observations are in agreement with the aforementioned body of *in vitro* and *ex vivo* research outlining the biological basis of the protective potential of Edafence® against external aggressive factors.

### CONCLUSION

The critical impact exerted by environmental factors on skin and organism health is best understood within the integrated framework provided by the exposome concept. Accordingly, our search for preventive and/or therapeutic antiaging and antixenotic solutions should ideally aim for products conferring protection against a wide array of damaging agents. Edafence® may fit this objective: it confers protection against environmental stressors in urban areas and prevents different clinical signs of skin aging (e.g., dehydration, wrinkles, hyperpigmentation). Different experimental models, including advanced systems approximating *in vivo* skin architecture and complexity, support its activity on counteracting cell and tissue damage from different stressors such as ionizing radiation, toxic compounds, tobacco, or natural aging. On the basis of the observations outlined here [Table 1], future studies will shed light on the mechanistic basis of its

**Table 1. Summary of studies supporting a protective role for Edafence® against exposome agents**

	Exposome agent	Experimental model	EDA Concentrations	Phenotypes improved by EDA	Ref.
<i>In vitro</i>	H <sub>2</sub> O <sub>2</sub> /oxidative stress	<i>in vitro</i> (HF)	0.3-1 mg/mL	*Viability *Senescence (β-galactosidase, Sirt1, LmnC) *Proliferation [cytometry, proliferating cell nuclear antigen (PCNA)] *Antioxidant response (Trx2)	[78]
	Osmotic stress	<i>in vitro</i> (HF)	0.3-1 mg/mL	*Viability	[75]
	Thermal stress (heat and cold)	<i>in vitro</i> (HaCaT)	2.5 mg/mL	*Viability *Mitochondrial potential and corrected mitochondrial potential	[75]
	Natural aging	<i>in vitro</i> (Keratinocytes)	0.9 mg/mL	*Telomere length	[84]
	Tobacco (5%) Urban pollution: Cr (III y VI) 6 mcg; Cd 3 mcg y As 9 mmol/L	<i>in vitro</i> (HF)	5-10 mg/mL	*Viability *Cell morphology	[81]
	Urban pollution: Dioxin (TCDD, 10 nmol/L)/ UVA (3000 mJ/cm <sup>2</sup> ) & UVB (300 y 700 mJ/cm <sup>2</sup> )	<i>in vitro</i> (HF, HaCaT)	0.1-0.3 mg/mL	*Viability & cell morphology *DDR (γH2AX, PARP) *Stress/apoptotic signaling (caspase 3, survivin, autophagy (LC3B), AhR) *Tissue remodeling (MMP1, loricrin)	[79]
	Dioxins 10 nmol/L Urban pollution (As 9 mmol/L, Cr VI 0.5 mmol/L)	Human Skin organ Culture	2.5 mg/mL	*Tissue architecture and integrity *Viability *Apoptosis	[80]
	Visible light (400-730 nm) Infrared (550-1400 nm)	<i>in vitro</i> (HF, HaCaT)	0.1-0.5 mg/mL	*Viability & cell morphology *DDR (γH2AX, PARP) *Stress/apoptotic signaling [caspase-3, survivin, autophagy (LC3B), AhR] *Tissue remodeling (MMP1, loricrin, collagen I, elastin)	[82]
	Blue light	<i>in vitro</i> (HF, Melanocytes)	0.1 mg/mL	*Viability *ROS levels *Mitochondrial integrity (architecture and membrane potential) *Melanization	[83]
	Facial Photoaging (age 45-65 year)		Commercial compound (EDAFENCE®, RetinSphere, Niacinamide)	*Viscoelasticity & firmness (Cutometer®) *Wrinkles (Visia® & Visioline®)	[89]
<i>In vivo</i>	Urban pollution (In winter; age 35-45 year)		Commercial compound (EDAFENCE®, SCA®, vitamin C, ferulic acid)	*Barrier function (TEWL) *Antioxidant effect (SQOOH/SQ) *Remove dark spot (Colorimeter®)	[87]
	Urban pollution (In summer; age 35-45 year)		Commercial compound (EDAFENCE®, SCA®, vitamin C, ferulic acid)	*Barrier function (TEWL & Corneometry) *Antioxidant effect (SQOOH/SQ)	[88]

DDR: DNA damage response; γH2AX: phosphorylated H2A histone family member X; PARP: poly-ADP ribose polymerase; TEWL: transepidermal water loss

activity and, most importantly, on the translation of those promising *in vitro* and *ex vivo* findings to the effects attainable *in vivo*.

## DECLARATIONS

### Acknowledgments

Miguel Sánchez-Álvarez provided support for manuscript preparation and editing.

### Authors' contributions

Substantially contributed to the conception and design of the review: Pérez-Davó A

Organized and performed meta-analysis across *in vivo* and *in vitro* Edafence® studies: Mataix M,

Rodríguez-Luna A, Gutiérrez-Pérez M, Pérez-Davó A

Designed graphic art: Rodríguez-Luna A, Pérez-Davó A

Contributed to data analyses, critically revising it for relevant content: Mataix M, Rodríguez-Luna A, Gutiérrez-Pérez M, Milani M, Gandarillas A, Espada J, Pérez-Davó A

### Availability of data and materials

All unpublished data sources have been listed here or registered under European Patent Office number (EP 3471 835 B1). Further details and materials will be fully provided upon request to corresponding authors. Any print permits from copyrighted material as been confirmed.

### Financial support and sponsorship

Studies cited on the biological and clinical activity of Edafence® have been funded by Cantabria Labs.

### Conflicts of interest

Rodríguez-Luna A, Milani M and Pérez-Davó A are members of the Innovation and Development Department, the Medical Department of Cantabria Labs Difa Cooper, and the Medical Affairs Department, respectively, at Cantabria Labs. The remaining authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

All authors provided approval for publication of all content, contributed to manuscript revision, and read and approved the submitted version.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Landrigan PJ, Fuller R, Acosta NJR, et al. The lancet commission on pollution and health. *The Lancet* 2018;391:462-512.
2. Gakidou E, Afshin A, Abajobir AA, et al. Global, regional, and national comparative risk assessment of 84 behavioral, environmental and occupational, and metabolic risks or clusters of risks, 1990-2016: a systematic analysis for the global burden of disease study 2016. *Lancet* 2017;390:1345-422.
3. Wild CP. Complementing the genome with an “exposome”: the outstanding challenge of environmental exposure measurement in molecular epidemiology. *Cancer Epidemiol Biomarkers Prev* 2005;14:1847-50.
4. Vermeulen R, Schymanski EL, Barabási AL, Miller GW. The exposome and health: Where chemistry meets biology. *Science* 2020;367:392-6.
5. Krutmann J, Bouloc A, Sore G, Bernard BA, Passeron T. The skin aging exposome. *J Dermatol Sci* 2017;85:152-61.
6. Gracia-Cazaña T, González S, Parrado C, Juarraz Á, Gilaberte Y. Influence of the exposome on skin cancer. *Actas Dermosifiliogr* 2020;111:460-70.
7. Magnani ND, Muresan XM, Belmonte G, et al. Skin damage mechanisms related to airborne particulate matter exposure. *Toxicol Sci* 2016;149:227-36.
8. Puri P, Nandar SK, Kathuria S, Ramesh V. Effects of air pollution on the skin: a review. *Indian J Dermatol Venereol Leprol* 2017;83:415-23.
9. Li M, Vierkötter A, Schikowski T, et al. Epidemiological evidence that indoor air pollution from cooking with solid fuels accelerates skin aging in Chinese women. *J Dermatol Sci* 2015;79:148-54.
10. Marrot L. Pollution and sun exposure: a deleterious synergy. Mechanisms and opportunities for skin protection. *Curr Med Chem* 2018;25:5469-86.
11. Schikowski T, Hüls A. Air pollution and skin aging. *Curr Environ Health Rep* 2020;7:58-64.
12. Chen C, Arjomandi M, Balmes J, Tager I, Holland N. Effects of chronic and acute ozone exposure on lipid peroxidation and antioxidant capacity in healthy young adults. *Environ Health Perspect* 2007;115:1732-7.
13. Prieux R, Eeman M, Rothen-Rutishauser B, Valacchi G. Mimicking cigarette smoke exposure to assess cutaneous toxicity. *Toxicol In Vitro* 2020;62:104664.

14. Morita A, Torii K, Maeda A, Yamaguchi Y. Molecular basis of tobacco smoke-induced premature skin aging. *J Invest Dermatol Symp Proc* 2009;14:53-5.
15. Lahmann C, Bergemann J, Harrison G, Young AR. Matrix metalloproteinase-1 and skin ageing in smokers. *The Lancet* 2001;357:935-6.
16. Prins JM, Wang Y. Quantitative proteomic analysis revealed N<sup>7</sup>-nitrosornicotine-induced down-regulation of nonmuscle myosin II and reduced cell migration in cultured human skin fibroblast cells. *J Proteome Res* 2013;12:1282-8.
17. Kligman AM. Early destructive effect of sunlight on human skin. *JAMA* 1969;210:2377.
18. Rittié L, Fisher GJ. Natural and sun-induced aging of human skin. *Cold Spring Harb Perspect Med* 2015;5:a015370.
19. Martincorena I, Roshan A, Gerstung M, et al. Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. *Science* 2015;348:880-6.
20. Bayerl C, Taake S, Moll I, Jung EG. Characterization of sunburn cells after exposure to ultraviolet light. *Photodermatol Photoimmunol Photomed* 1995;11:149-54.
21. Rigel DS. Cutaneous ultraviolet exposure and its relationship to the development of skin cancer. *J Am Acad Dermatol* 2008;58:S129-32.
22. Schäfer M, Farwanah H, Willrodt AH, et al. Nrf2 links epidermal barrier function with antioxidant defense. *EMBO Mol Med* 2012;4:364-79.
23. Barbhaiya M, Costenbader KH. Environmental exposures and the development of systemic lupus erythematosus. *Curr Opin Rheumatol* 2016;28:497-505.
24. Fu Q. Radiation (SOLAR). In: Holton JR, editor. Encyclopedia of atmospheric sciences. Academic Press: Oxford; 2003. pp. 1859-63.
25. Dorr MM, Guignard R, Auger FA, Rochette PJ. The use of tissue-engineered skin to demonstrate the negative effect of CXCL5 on epidermal ultraviolet radiation-induced cyclobutane pyrimidine dimer repair efficiency. *Br J Dermatol* 2020; doi: 10.1111/bjd.19117.
26. Liebel F, Kaur S, Ruvolo E, Kollias N, Southall MD. Irradiation of skin with visible light induces reactive oxygen species and matrix-degrading enzymes. *J Invest Dermatol* 2012;132:1901-7.
27. Schroeder P, Lademann J, Darvin ME, et al. Infrared radiation-induced matrix metalloproteinase in human skin: implications for protection. *J Invest Dermatol* 2008;128:2491-7.
28. Dupont E, Gomez J, Bilodeau D. Beyond UV radiation: a skin under challenge. *Int J Cosmet Sci* 2013;35:224-32.
29. Nakashima Y, Ohta S, Wolf AM. Blue light-induced oxidative stress in live skin. *Free Radic Biol Med* 2017;108:300-10.
30. Arthaut LD, Jourdan N, Mteyrek A, et al. Blue-light induced accumulation of reactive oxygen species is a consequence of the Drosophila cryptochrome photocycle. *PLoS One* 2017;12:e0171836.
31. Dong K, Goyarts EC, Pelle E, Trivero J, Pernodet N. Blue light disrupts the circadian rhythm and create damage in skin cells. *Int J Cosmet Sci* 2019;41:558-62.
32. Hudson L, Rashdan E, Bonn CA, Chavan B, Rawlings D, Birch-Machin MA. Individual and combined effects of the infrared, visible, and ultraviolet light components of solar radiation on damage biomarkers in human skin cells. *FASEB J* 2020;34:3874-83.
33. Ayaki M, Hattori A, Maruyama Y, et al. Protective effect of blue-light shield eyewear for adults against light pollution from self-luminous devices used at night. *Chronobiol Int* 2016;33:134-9.
34. Cho S, Lee MJ, Kim MS, et al. Infrared plus visible light and heat from natural sunlight participate in the expression of MMPs and type I procollagen as well as infiltration of inflammatory cell in human skin in vivo. *J Dermatol Sci* 2008;50:123-33.
35. Kim MS, Kim YK, Cho KH, Chung JH. Infrared exposure induces an angiogenic switch in human skin that is partially mediated by heat. *Br J Dermatol* 2006;155:1131-8.
36. Kim EJ, Han JY, Lee HK, et al. Effect of the regional environment on the skin properties and the early wrinkles in young Chinese women. *Skin Res Technol* 2014;20:498-502.
37. Fung ES, Towle KM, Monnot AD. Devising a tier-based skin sensitisation screening strategy for personal care and cosmetic products. *Altern Lab Anim* 2020;48:70-7.
38. Cao C, Xiao Z, Wu Y, Ge C. Diet and skin aging-from the perspective of food nutrition. *Nutrients* 2020;12:870.
39. Hotamisligil GS. Inflammation, metaflammation and immunometabolic disorders. *Nature* 2017;542:177-85.
40. Smith CJ, Perfetti TA, Hayes AW, Berry SC. Obesity as a source of endogenous compounds associated with chronic disease: a review. *Toxicol Sci* 2020;175:149-55.
41. López-Otin C, Galluzzi L, Freije JMP, Madeo F, Kroemer G. Metabolic control of longevity. *Cell* 2016;166:802-21.
42. Gill V, Kumar V, Singh K, Kumar A, Kim JJ. Advanced glycation end products (AGEs) may be a striking link between modern diet and health. *Biomolecules* 2019;9:888.
43. Gu Y, Han J, Jiang C, Zhang Y. Biomarkers, oxidative stress and autophagy in skin aging. *Ageing Res Rev* 2020;59:101036.
44. Santra M, Dill KA, de Graff AMR. Proteostasis collapse is a driver of cell aging and death. *Proc Natl Acad Sci U S A* 2019;116:22173-8.
45. Stead ER, Castillo-Quan JI, Miguel VEM, et al. Agephagy - adapting autophagy for health during aging. *Front Cell Dev Biol* 2019;7:308.
46. Morimoto RI. Cell-nonautonomous regulation of proteostasis in aging and disease. *Cold Spring Harb Perspect Biol* 2020;12:a034074.
47. Hetz C, Zhang K, Kaufman RJ. Mechanisms, regulation and functions of the unfolded protein response. *Nat Rev Mol Cell Biol* 2020;21:421-38.
48. Anderson NS, Haynes CM. Folding the mitochondrial UPR into the integrated stress response. *Trends Cell Biol* 2020;30:428-39.
49. Rinnerthaler M, Bischof J, Streubel MK, Trost A, Richter K. Oxidative stress in aging human skin. *Biomolecules* 2015;5:545-89.
50. Pasparakis M, Haase I, Nestle FO. Mechanisms regulating skin immunity and inflammation. *Nat Rev Immunol* 2014;14:289-301.
51. Lee S, Hur EG, Ryoo IG, Jung KA, Kwak J, Kwak MK. Involvement of the Nrf2-proteasome pathway in the endoplasmic reticulum stress response in pancreatic  $\beta$ -cells. *Toxicol Appl Pharmacol* 2012;264:431-8.
52. Riz I, Hawley TS, Marsal JW, Hawley RG. Noncanonical SQSTM1/p62-Nrf2 pathway activation mediates proteasome inhibitor

- resistance in multiple myeloma cells via redox, metabolic and translational reprogramming. *Oncotarget* 2016;7:66360-85.
53. Abrahams A, Mouchet N, Gouault N, et al. Integrating targeted gene expression and a skin model system to identify functional inhibitors of the UV activated p38 MAP kinase. *Photochem Photobiol Sci* 2016;15:1468-75.
54. Clementi E, Inglin L, Beebe E, Gsell C, Garajova Z, Markkanen E. Persistent DNA damage triggers activation of the integrated stress response to promote cell survival under nutrient restriction. *BMC Biol* 2020;18:36.
55. Dufey E, Bravo-San Pedro JM, Eggers C, et al. Genotoxic stress triggers the activation of IRE1 $\alpha$ -dependent RNA decay to modulate the DNA damage response. *Nat Commun* 2020;11:2401.
56. Wang Y, Wang L, Wen X, et al. NF- $\kappa$ B signaling in skin aging. *Mech Ageing Dev* 2019;184:111160.
57. Tian X, Cui Z, Liu S, Zhou J, Cui R. Melanosome transport and regulation in development and disease. *Pharmacol Ther* 2020;107707.
58. Rojo de la Vega M, Krajisnik A, Zhang DD, Wondrak GT. Targeting NRF2 for improved skin barrier function and photoprotection: focus on the achiote-derived apocarotenoid bixin. *Nutrients* 2017;9:1371.
59. Del Vecchio CA, Feng Y, Sokol ES, et al. De-differentiation confers multidrug resistance via noncanonical PERK-Nrf2 signaling. *PLoS Biol* 2014;12:e1001945.
60. Lu MC, Ji JA, Jiang ZY, You QD. The Keap1-Nrf2-ARE pathway as a potential preventive and therapeutic target: an update. *Med Res Rev* 2016;36:924-63.
61. Kudo I, Hosaka M, Haga A, et al. The regulation mechanisms of AhR by molecular chaperone complex. *J Biochem* 2018;163:223-32.
62. Hidaka T, Ogawa E, Kobayashi EH, et al. The aryl hydrocarbon receptor AhR links atopic dermatitis and air pollution via induction of the neurotrophic factor artemin. *Nat Immunol* 2017;18:64-73.
63. Rothhammer V, Quintana FJ. The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. *Nat Rev Immunol* 2019;19:184-97.
64. Lindén J, Lensu S, Tuomisto J, Pohjanvirta R. Dioxins, the aryl hydrocarbon receptor and the central regulation of energy balance. *Front Neuroendocrinol* 2010;31:452-78.
65. Quan T, Qin Z, Xia W, Shao Y, Voorhees JJ, Fisher GJ. Matrix-degrading metalloproteinases in photoaging. *J Invest Dermatol Symp Proc* 2009;14:20-4.
66. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol* 2014;15:786-801.
67. Szentléleky E, Szegezski V, Karanyicz E, et al. Pituitary adenylate cyclase activating polypeptide (PACAP) reduces oxidative and mechanical stress-evoked matrix degradation in chondrifying cell cultures. *Int J Mol Sci* 2019;20:168.
68. Shin JW, Kwon SH, Choi JY, et al. Molecular mechanisms of dermal aging and antiaging approaches. *Int J Mol Sci* 2019;20:2126.
69. Martel J, Ojcius DM, Ko YF, Chang CJ, Young JD. Antiaging effects of bioactive molecules isolated from plants and fungi. *Med Res Rev* 2019;39:1515-52.
70. Panagiotidou E, Chondrogianni N. We are what we eat: ubiquitin-proteasome system (UPS) modulation through dietary products. In: Barrio R, Sutherland JD, Rodriguez MS, editors. *Proteostasis and disease*. Cham: Springer International Publishing; 2020. pp. 329-48.
71. Gombau L, García F, Lahoz A, et al. Polypodium leucotomos extract: antioxidant activity and disposition. *Toxicol In Vitro* 2006;20:464-71.
72. Gonzalez S, Gilaberte Y, Philips N, Juarranz A. Fernblock, a nutraceutical with photoprotective properties and potential preventive agent for skin photoaging and photoinduced skin cancers. *Int J Mol Sci* 2011;12:8466-75.
73. Parrado C, Mascaraque M, Gilaberte Y, Juarranz A, Gonzalez S. Fernblock (Polypodium leucotomos extract): molecular mechanisms and pleiotropic effects in light-related skin conditions, photoaging and skin cancers, a Review. *Int J Mol Sci* 2016;17:1026.
74. Zamarrón A, Lorrio S, González S, Juarranz Á. Fernblock prevents dermal cell damage induced by visible and infrared A radiation. *Int J Mol Sci* 2018;19:2250.
75. Young M. Guinness book of world records 1997. London: Guinness Publishing Ltd; 1997. pp. 42-3.
76. Day T, Ruhland C, Xiong F. Influence of solar ultraviolet-B radiation on Antarctic terrestrial plants: results from a 4-year field study. *J Photochem Photobiol B* 2001;62:78-87.
77. Matji-Tuduri JA, Brieve-Delgado A, Domínguez M, et al. Use of extracts of *Deschampsia antarctica* for counteracting human skin barrier damage caused by environmental aggressions (Patent EP 3471 835 B1). ES: European Patent Office; 2019.
78. Köhler H, Contreras RA, Pizarro M, Cortés-Antiquera R, Zúñiga GE. Antioxidant responses induced by UVB radiation in *Deschampsia antarctica* desv. *Front Plant Sci* 2017;8:921.
79. Pérez-Torres E, García A, Dinamarca J, et al. The role of photochemical quenching and antioxidants in photoprotection of *Deschampsia antarctica*. *Funct Plant Biol* 2004;31:731-41.
80. Ortiz-Espín A, Morel E, Juarranz Á, et al. An extract from the plant *Deschampsia antarctica* protects fibroblasts from senescence induced by hydrogen peroxide. *Oxid Med Cell Longev* 2017;2017:2694945.
81. Zamarrón A, Morel E, Lucena SR, et al. Extract of *Deschampsia antarctica* (EDA) prevents dermal cell damage induced by UV radiation and 2,3,7,8-Tetrachlorodibenzo-p-dioxin. *Int J Mol Sci* 2019;20:1356.
82. Fernández-Maros S, Calvo-Sánchez M, Pérez-Davó A, Vitale M, Espada J. Protective effects of aqueous extract of *Deschampsia antarctica* against urban air pollutants in human skin model. In 28th EADV Congress. 2019. Available from: <https://eadvmadrid2019.org/wp-content/uploads/2019/09/e-Poster-list.pdf>. [Last accessed on 16 Nov 2020]
83. Ortiz-Espín AM, Delgado Rubín de Célix A, Brieve A, Guerrero A, González S, Sevilla F. The extract from *Deschampsia antarctica* (Edafence®) protects fibroblasts viability from the effects of environmental oxidants and pollutants. In 76th Society of Investigative Dermatology Annual Meeting. 2017. Available from: [https://cdn.ymaws.com/www.sidnet.org/resource/resmgr/docs/SID\\_Portland\\_Final\\_5\\_web.pdf](https://cdn.ymaws.com/www.sidnet.org/resource/resmgr/docs/SID_Portland_Final_5_web.pdf). [Last accessed on 16 Nov 2020]
84. Juarranz Á. IFC- P1403C: Efecto de EDAFENCE® sobre el daño al DNA, muerte celular, sufrimiento mitocondrial, MMP-1 y expresión



- de proteínas de diferenciación y adhesión en fibroblastos y queratinocitos humanos expuestos a UVB, UVA, IR y VIS. Industrial report. Spain: Universidad Autónoma de Madrid; 2017.
85. Lorrio S, Rodríguez-Luna A, Delgado-Wicke P, et al. Protective effect of the aqueous extract of *Deschampsia antarctica* (EDAFENCE®) on skin cells against blue light emitted from digital devices. *Int J Mol Sci* 2020;21:988.
  86. Gandarillas A. IF-CAF: Estudio de los mecanismos de protección de compuestos de tecnología IFC (EDA e IFC-CAF - Endocare retinage) en modelos celulares de epidermis humana *in vitro*. Industrial report. Spain: IDIVAL; 2014.
  87. Freije A, Molinuevo R, Ceballos L, et al. Inactivation of p53 in human keratinocytes leads to squamous differentiation and shedding via replication stress and mitotic slippage. *Cell Rep* 2014;9:1349-60.
  88. Gandarillas A. The mysterious human epidermal cell cycle, or an oncogene-induced differentiation checkpoint. *Cell Cycle* 2012;11:4507-16.
  89. Milani M, Hashtroody B, Piacentini M, Celleno L. Skin protective effects of an antipollution, antioxidant serum containing *Deschampsia antarctica* extract, ferulic acid and vitamin C: a controlled single-blind, prospective trial in women living in urbanized, high air pollution area. *Clin Cosmet Investig Dermatol* 2019;12:393-9.
  90. Milani M, Piacentini M, Celleno L. A serum containing *deschampsia antarctica* extract, ferulic acid and vitamin c has anti-pollutant effects on skin exposed to high tropospheric ozone levels: a controlled single-blind, prospective clinical trial in women living in urbanized, high air pollution area during the summer season. *J Clin Exp Dermatol Res* 2019;10:510.
  91. Pérez-Davó A, Truchuelo MT, Vitale M, González-Castro J. Efficacy of an antiaging treatment against environmental factors. *J Clin Aesthet Dermatol* 2019;12:65-70.

Original Article

Open Access



# Wound repair and scarring of genital skin

Ursula Mirastschijski<sup>1,2</sup>, Dongsheng Jiang<sup>3</sup>, Yuval Rinkevich<sup>3</sup>, Refaat Karim<sup>4</sup>, Heiko Sorg<sup>5,6</sup>

<sup>1</sup>Center for Biomolecular Interactions Bremen, University of Bremen, Bremen 28359, Germany.

<sup>2</sup>Mira-Beau gender esthetics, Berlin 10777, Germany.

<sup>3</sup>Comprehensive Pneumology Center, Institute of Lung Biology and Disease, Helmholtz Zentrum München, Munich 85764, Germany.

<sup>4</sup>A kliniken, Amstelveen 1182, The Netherlands.

<sup>5</sup>Department of Plastic, Reconstructive and Aesthetic Surgery, Klinikum Westfalen, Dortmund 44309, Germany.

<sup>6</sup>Department of Health, Faculty of Medicine, University Witten/Herdecke, Witten 58455, Germany.

**Correspondence to:** Prof. Ursula Mirastschijski, Center for Biomolecular Interactions Bremen, University of Bremen, Leobener Str/NW2, Bremen 28359, Germany. E-mail: mirastsc@uni-bremen.de

**How to cite this article:** Mirastschijski U, Jiang D, Rinkevich Y, Karim R, Sorg H. Wound repair and scarring of genital skin. *Plast Aesthet Res* 2020;7:70. <http://dx.doi.org/10.20517/2347-9264.2020.147>

**Received:** 7 Jul 2020 **First Decision:** 17 Sep 2020 **Revised:** 12 Oct 2020 **Accepted:** 10 Nov 2020 **Published:** 5 Dec 2020

**Academic Editor:** Alexis Desmoulière, Raúl González-García **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

**Aim:** Scarring is a physiological process in adult wound repair. Although keratinocytes and fibroblasts are the main cell types of the skin, they differ in migration behaviour and inflammatory responses depending on their location in the body. The aim of this article is to describe wound repair in genital skin and to depict differences with regard to skin anatomy and cellular responses to inflammatory stimuli in acute and chronic wound healing.

**Methods:** This report reviews data from patients undergoing reconstructive and aesthetic plastic surgery as well as published studies on genital wound repair. Genital surgery comprised plastic reconstructive surgery after urological interventions of biological men and women, tissue from trans-males and trans-females undergoing gender reassignment surgery and tissue from patients undergoing aesthetic genital surgery. The cohort comprised a total of 68 patients ( $32.9 \pm 11.3$  years), of which 31 were male (mean  $30.4 \pm 9.3$  years) and 37 were female ( $34.9 \pm 12.5$  years; mean  $\pm$  SD).

**Results:** Wound healing in genital skin markedly differs from other areas of the body due to its anatomical features, microbiome, and elevated hormonal responsiveness. Human genital skin is highly extensible and unusually rich in elastic fibres, and it lacks the mechanical anchorage and tensile properties typical of non-genital regions. Acute injury resolves rapidly due, in part, to rapid resolution of the inflammatory response. In contrast to scarring responses on other body surfaces, genital skin wounding is resolved by shrinkage or fistula formation.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



**Conclusion:** The embryological origins of genital skin fibroblasts, together with the gender-specific hormonal environment, contribute to the unique phenotype and healing properties of genital skin. When performing genital surgery, it is of utmost importance to be aware of the differing responsiveness of genital tissue to trauma, surgery, and repair.

**Keywords:** Genital skin, hormone sensitivity, shrinkage, scarless, oestrogen, testosterone

## INTRODUCTION

The skin is our largest organ, consisting of a variety of cells, layers, adnexa such as hair follicles, sebaceous and sweat glands, and with nerve endings sensing pain, pressure, vibrations and temperature. All these entities act in concert to sense and to protect us against the outside world, e.g., physical, chemical and biological influences (temperature, radiation, desiccation, trauma, chemicals, microbes, *etc.*). Furthermore, the skin is of utmost importance for us to perceive our environment and for communication. Our skeleton and the musculature provide the inner framework, which the skin covers as outer surface. The elasticity and robustness of the skin are optimized for growth, joint motion and shear and tear.

As stated above, the integrity of our outer envelope is the prerequisite for survival. A whole body of literature and knowledge is available on the physiology of skin wound healing and scarring as summarized in this PAR issue. Interestingly, little is known on the anatomical features and pathophysiology of genital wound repair. This may be due to the fact that trauma to the genitalia is rare with only 5.3% of combat<sup>[1]</sup> or 1.5% of burn injuries<sup>[2]</sup> afflicting the genital area. In contrast, infections are quite common. With the commensal microbial colonization of the vagina, urinary orifices or anus, the genital skin is constantly exposed to a high variety of putative intruders. Minor lesions can cause bacterial penetration into deeper tissue layers and manifest as abscess, gangrene or fasciitis, also known as Fournier's gangrene with a high mortality<sup>[3]</sup>. Due to the life-threatening character of genital infections, immediate and thorough debridement is the only cure followed by defect reconstruction after recovery in an interdisciplinary setting comprising urologists, gynaecologists, and general as well as plastic surgeons.

Despite of the fact that 30% of males are circumcised world-wide, almost no data exists on genital wound healing and scarring<sup>[4]</sup>. In recent times, the number of surgical interventions for gender reassignment surgery and genital aesthetic surgery, especially labioplasty, has increased enormously with the need for more information on genital skin repair processes. It is also striking that female genital mutilation/cutting (FGM/C) patients have little to no hypertrophic scarring or keloid formation. This is in contrast to clinical observations of normal scar tissue formation in pigmented skin with a higher tendency for excessive scarring. The aim of this article is to inform on the anatomy and microstructure of genital skin, to delineate healing differences compared to skin of other body parts, and to encourage further research in this hitherto neglected area of genital skin wound repair.

## METHODS

Data on genital postoperative scarring were derived from our own patient cohort with an observational period of 5 years. Pre- and postoperative examinations were performed as routine diagnostics and follow-ups for patient care. Genital surgery comprised plastic reconstructive surgery after urological interventions of biological men and women, tissue from trans-males and trans-females undergoing gender reassignment surgery and tissue from patients undergoing aesthetic genital surgery. The cohort comprised a total of 68 patients ( $32.9 \pm 11.3$  years), of which 31 were male (mean  $30.4 \pm 9.3$  years) and 37 were female ( $34.9 \pm 12.5$  years; mean  $\pm$  SD).

Tissue specimens were obtained after information and written patient's consent. The study was approved by the local ethical committees (Ethics Committee of the Medical Chamber of Bremen, no. 336/12 and no. RA/RE 336; and of Bavaria, 2018-157). Tissues were harvested directly after surgery and fixed with 2% paraformaldehyde and processed for cryosection.

### Histological assessment

For comparison of skin derived from the genitalia or from other body parts, 6 µm cryosections were cut and processed as previously described<sup>[5,6]</sup>. An overview of the cutaneous microstructure is provided by hematoxylin-eosin staining as described previously<sup>[5]</sup>. For visualization of collagen fibres, Masson's trichrome staining was performed by using commercially available kit from Sigma-Aldrich (#HT15-1KT)<sup>[6,7]</sup>. In brief, cryo-sections were fixed in cold acetone at minus 20 °C for 5 min and then incubated in preheated Bouin's solution (Sigma-Aldrich HT10132) at 56 °C for 5 min. After that the sections were sequentially incubated at room temperature in Biebrich scarlet-acid fuchsin solution for 5 min, working concentration of Phosphotungstic/ Phosphomolybdic acid for 5 min, aniline blue solution for 10 min, and 1% acetic acid for 2 min. After dehydration, the sections were cleared with Roti-Histol (Roth 6640) and mounted with a Roti-Histokitt (Roth 6638). Collagen was stained in blue, cells in red and nuclei in black. For elastic fibres, Elastica van Gieson staining was performed by using commercially available kit from Sigma-Aldrich (#115974), according to the manufacturer's instruction.

## RESULTS

### Anatomy and histology of genital skin

#### *Development of the outer genitalia and similarities between male and female tissues*

To understand the microscopic features of the genital skin in both sexes, one has to keep in mind the common origin of the genitalia with intrauterine differentiation due to hormonal influences. The outer genital organs derive from genital buds, which develop into the penis or small labia and into the scrotum or the big labia [Table 1, Figure 1]. Consequently, the microstructure of the genitalia is like the corresponding part of the other sex, e.g., penis and small labia (labia minora) or scrotum and big labia (labia majora).

#### *Skin architecture and biomechanics*

Human skin is structured into the epidermis, the dermis and the subcutaneous fat layer. On many sites of the human body, the subcutaneous fat is divided in two compartments by a thin fascia, the fascia cutanea superficialis, also named "Scarpa fascia". The Scarpa fascia is a remnant of the carnosus muscle found in fur bearing animals. In the neck, the ancient muscle is still present as platysma and, in the genitals, as Dartos muscle in the scrotal sac or Dartos fascia in the penis or Colles' fascia in the labia. The absence of anchoring structures provided by the subcutaneous fat layer is an explanation for the highly mobile genital skin. Besides, the penis and the small labia are devoid of fat, whereas fat tissue is commonly found in the big labia and in the scrotal sac in obese men. Another unique feature of the genitalia is the fact that they are devoid of any skeletal fixation, neither to bone or cartilage structures. The biomechanics of genital organs differ from tissues with underlying anchorage to stiff structures resulting in constant biophysical strain and tension to the covering connective tissue and skin layers. The biomechanical environment and the extracellular matrix architecture of the external genitalia create a low-tension state that may contribute to reduced mechanotransduction.

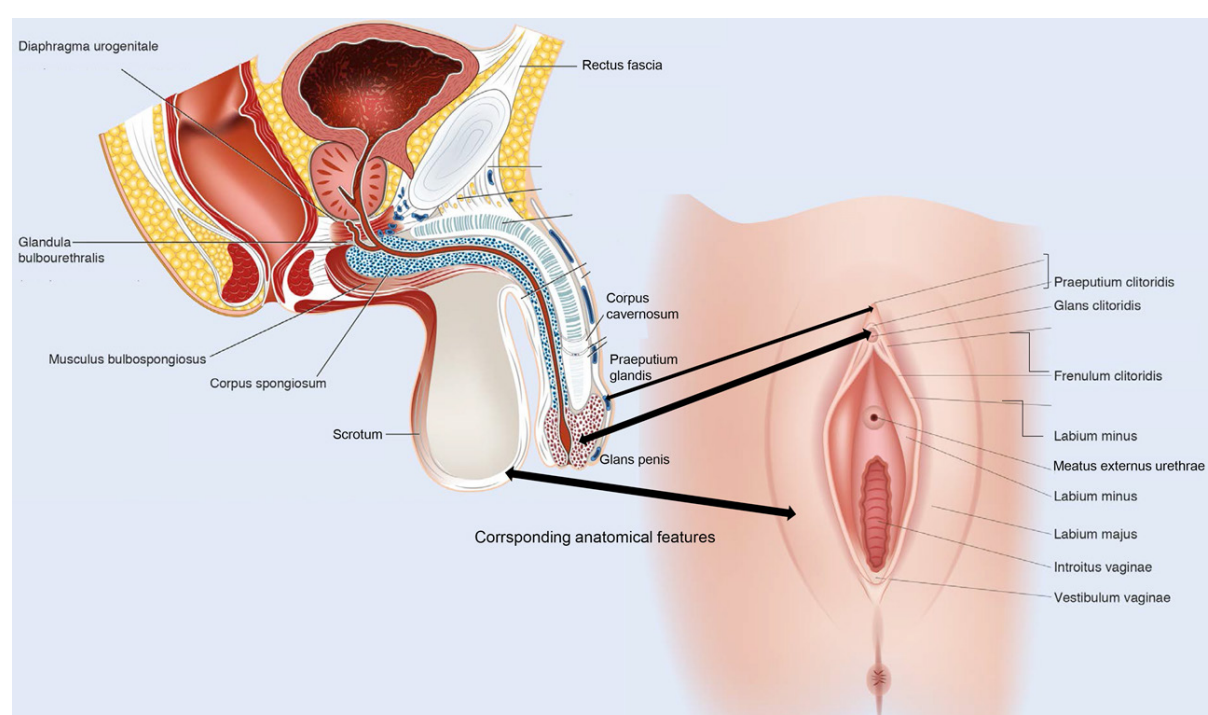
#### *Microscopic structure of genital skin*

The genital epidermis differs from skin of other body parts in two main aspects, namely by being devoid of hair (penis and small labia as well as the inner aspect of the big labia), and by being, in part, covered by mucous epithelium, which does not keratinize and has no cornified layer (clitoris, glans penis, inner part of the foreskin of glans and clitoris). The dermal structure of the genitalia differs from other skin sites as well. The separation in upper, papillary dermis and lower, reticular dermis is less prominent as in non-genital

**Table 1. Skin structure of homologous male and female outer genitalia**

	Male	Female	Histological microstructure
Glans	Of penis*	Of clitoris	Multilayered, non-keratinizing epidermis, dermal tissue with abundant nerve endings
Foreskin	Of penis	Of clitoris	Outer part: epidermis with cornified layer Inner part: non-keratinizing epidermis; mucous epithelium; no fat tissue
Frenulum	Frenulum penis	Frenula clitoridis (paired)	Non-keratinizing, mucous epithelium, no subcutaneous fat tissue
	Penile shaft skin	Small labia	Penis: epidermis with cornified layer, highly flexible attachment to underlying tissue via Dartos fascia (Fascia penis superficialis) Labia: outer surface with thin cornified layer; inner surface: no cornified layer Both: no hair; no fat tissue; many elastic fibers
	Scrotum	Big labia	Hair bearing epidermis (labia: only outer surface), epidermal cornified layer Labia: subcutaneous fat layer and smooth muscle cells Scrotum: no (or very little) fat, but contractile Tunica Dartos with smooth muscle cells and myofibroblasts; in obese patients: fat tissue

\*After circumcision, the epithelium changes into a keratinizing epidermis of the glans penis



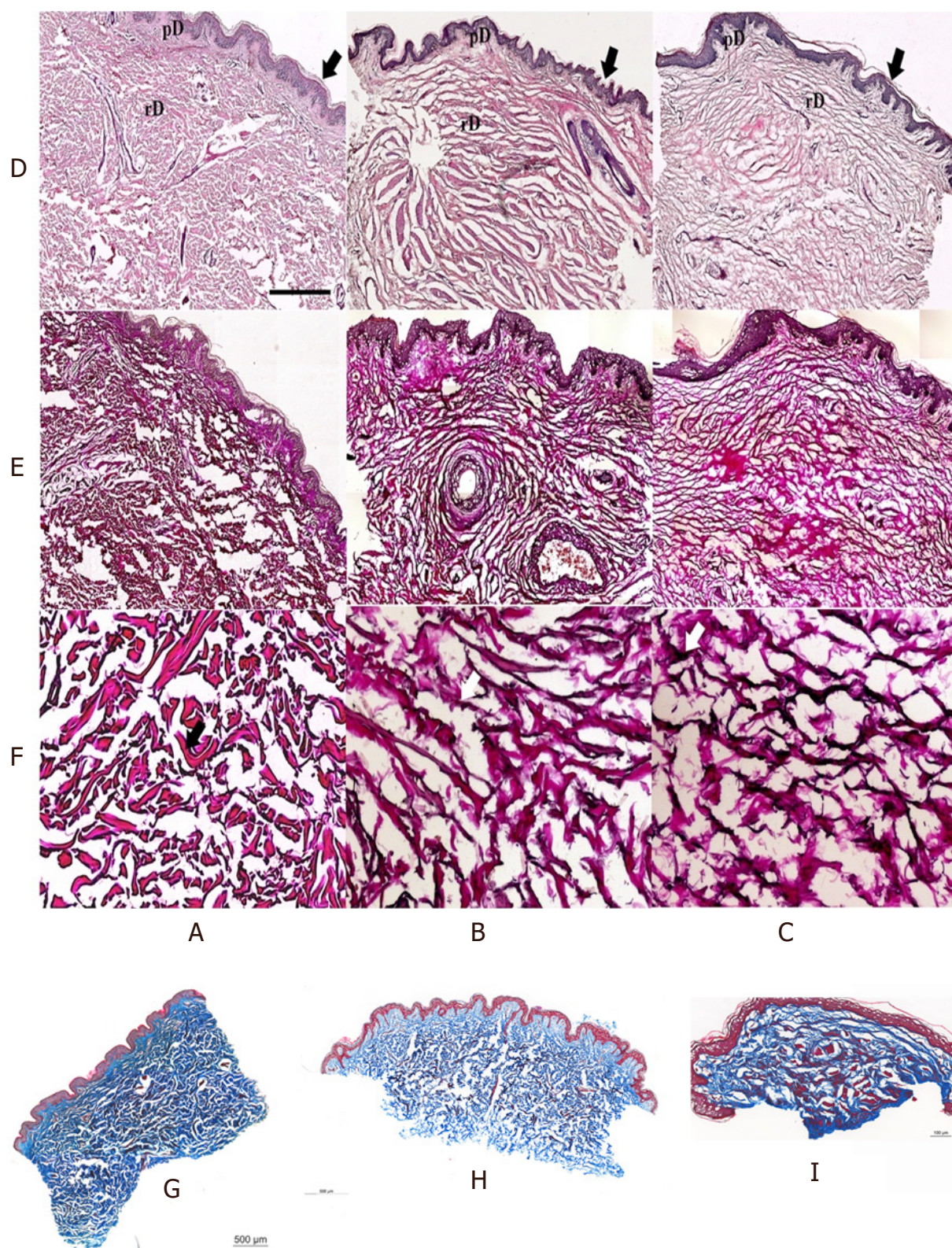
**Figure 1.** Comparison of corresponding anatomical features in male and female genitalia. Genital buds differentiate into penis or scrotum in males and into labia minora and majora in females respectively. Common features are the glans and the foreskin that covers the glans in both genders. In females, genital buds separate and become labia, in males genital buds fuse and become scrotum and penis. Remnants of the fusion process are seen in two parts of the septum scroti. Both septi contain their own vasculature<sup>[8]</sup>. (modified after Mirastschijski and Rimmel<sup>[9]</sup>)

skin. The genital dermal structure is more loosely woven and contains abundant elastic fibres and less collagen in comparison to other body parts [Figure 2]. High elasticity is a prerequisite for the frequent and fast changes in volume and expansion of the genital organs, e.g., during penile erection or excitement with higher perfusion and tumescence of the cavernous bodies, or for temperature regulation in the scrotal sac.

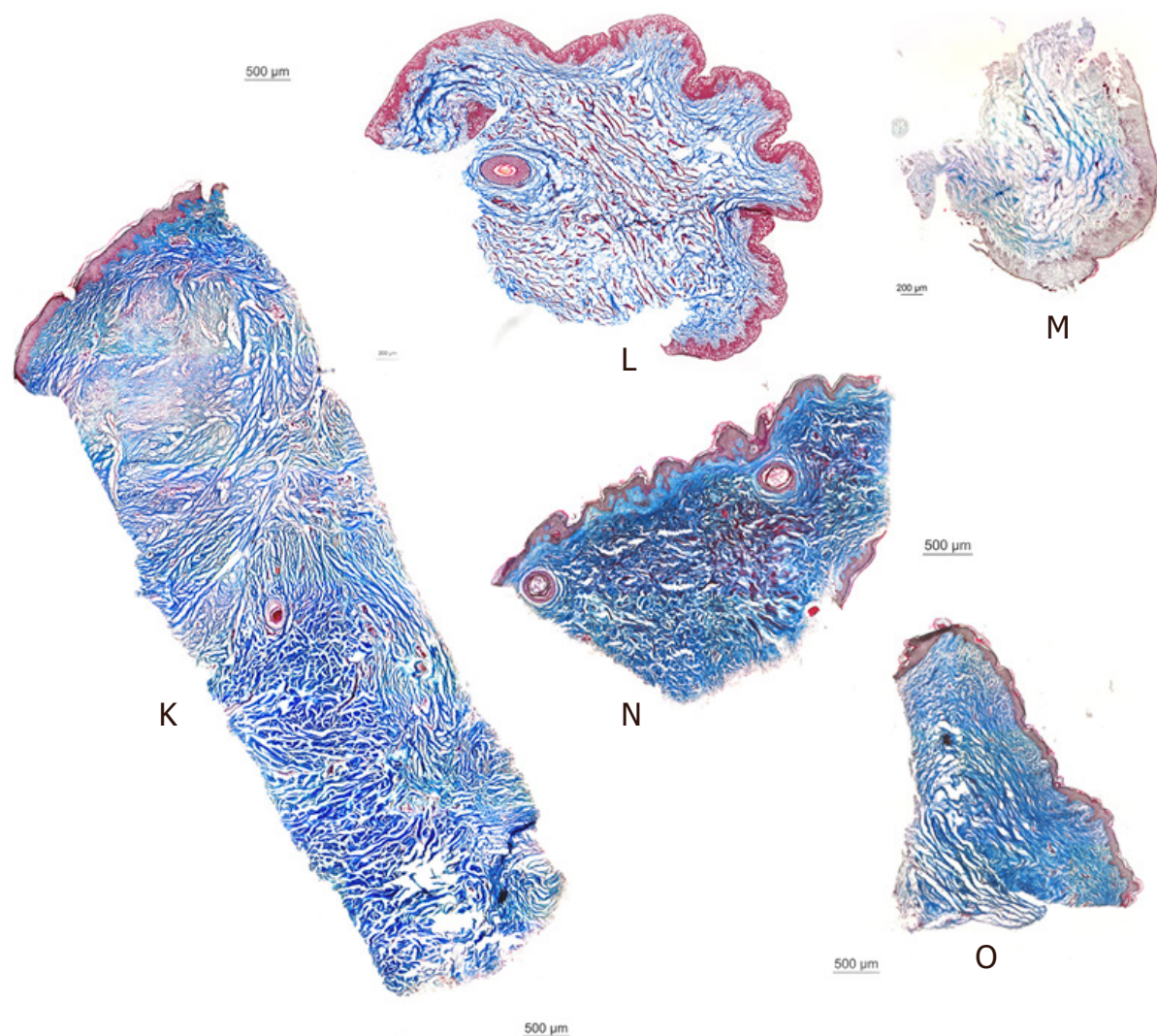
#### *Hormone responsiveness of genital skin*

Tissues and cells of genital and non-genital skin exhibit diverging expression of hormone receptors and processing of sex hormones [Table 2]. Hormone responsivity of tissues has important impact on skin wound repair<sup>[10]</sup>. Female and male hormones influence genital wound healing differently [Figure 3]. For









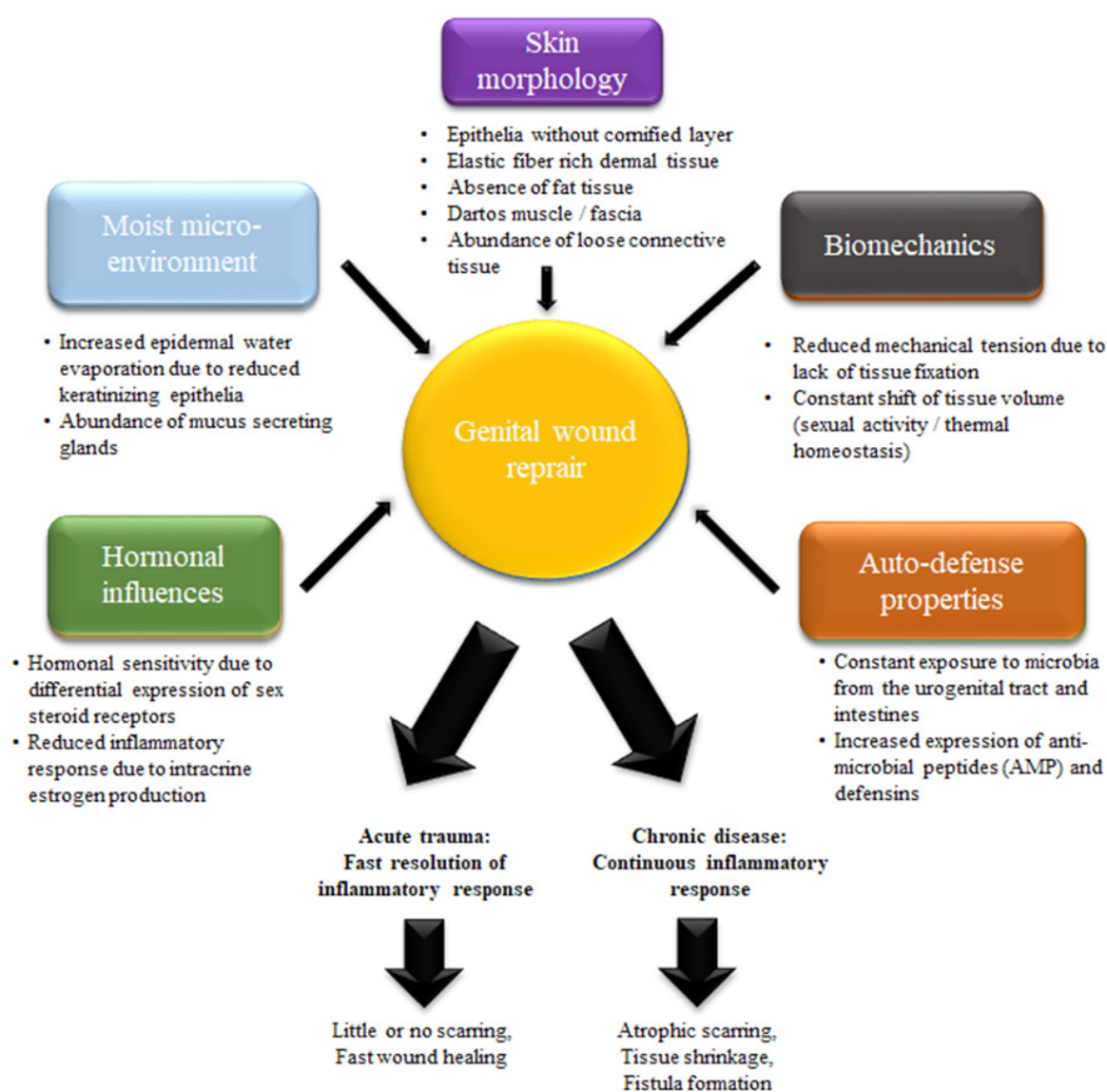
**Figure 2.** Microstructure of the skin from different body areas. A: lower left arm, B: penis, C: small labia. Note the dense dermal structure with multiple vessels in (A) and loose collagen bundles in (B) and (C) with high similarity of penile and labial skin. D: panel HE staining, E and F: Elastica van Gieson staining. Arrow upper panel depicting epidermis, pD papillary dermis, rD reticular dermis. Note abundance of collagen fibres in arm skin (red staining, black arrow; panel E and F) and abundant elastic fibres in penile and labial skin (black staining, white arrows). Scale bar in A for upper two panels 500  $\times$ m, lower panel 40 $\times$  magnification. G-O: Masson Trichrome staining for visualization of collagen fibres (blue staining) in normal and scar tissues of different origin. G, H, I, N normal skin, K, L, M, O scar tissue; G and K breast, H and L scrotum, I and M penis, N and O abdomen. Note abundant collagen bundles in normal breast and abdominal skin (G and N) and densely packed collagen bundles in scar tissue of breast and abdomen (K and O, respectively). Thickened dermal tissue in breast scar (K). Loose connective tissue with scrotal (H, L) and penile (I, M) normal and scar tissue without densely packed collagen bundles as seen in non-genital scar tissue. Scale bars next to each specimen indicating magnification

example, oestrogens accelerate wound closure, whereas testosterone delays healing<sup>[11]</sup>. Skin is a major source of extra-glandular sex steroid hormones. The intracellular enzyme aromatase converts the sex hormone precursor dehydroepiandrosterone downstream into estrone or via testosterone into the more potent 17 $\beta$ -oestradiol. Both oestrogens act via the oestrogen receptors and stimulate keratinocyte and fibroblast migration<sup>[10]</sup>. In genital fibroblasts, aromatase expression is androgen dependent and oestrogens stimulate fibroblast contractility without increasing alpha-smooth muscle actin expression or myofibroblast differentiation<sup>[12]</sup>. Upon wounding, aromatase activity increases 400-fold in keratinocytes with increased intracellular oestrogens. Oestrogens reduce the cellular inflammatory response via downregulation of the pro-inflammatory cytokine macrophage migration inhibitory factor<sup>[13]</sup>, by reduced toll-like-receptor-4 mediated mitogen activated protein kinase activation, by reduced macrophage infiltration into wounds and

**Table 2. Hormonal differences between genital and non-genital skin of both sexes**

	Genital skin	Non-genital skin
Androgen receptor	Higher expression in labia majora and minora; Upregulated in fibroblasts and basal keratinocytes; Co-localization with ER	Only present in hair follicles and pilo-sebaceous duct keratinocytes; Low expression in extra-genital skin
Oestrogen receptors	Highly expressed in penis and labia minora; Restricted to basal keratinocytes and stromal fibroblasts; Expression decreases with age	Lower expression compared to vulva or vagina; Expressed by keratinocytes and fibroblasts; Absence in skin appendages or blood vessels
Testosterone	AR binding capacity of Testosterone higher; 30 times faster degradation; Reduced effect on aromatase activity in low oxygen conditions	Higher rate of conversion testosterone into DHT; Higher 5- $\alpha$ -reductase activity with irreversible formation of DHT
Oestrogens	No conversion of 17 $\beta$ -estradiol into the weaker estrone; Stimulate fibroblast contractility without ASMA expression	3-fold increased metabolism of 17 $\beta$ -estradiol into the weaker estrone
Aromatase	Higher activity in fibroblasts with conversion of testosterone into 17 $\beta$ -estradiol; Dose-dependent reduced activity by testosterone; Aromatase expression is androgen dependent	Expression in skin fibroblasts, keratinocytes of the outer root sheath and in terminal hair follicles and in cells of sebaceous glands and ducts

AR: androgen receptor; ER: oestrogen receptors; DHT: dihydrotestosterone; ASMA: alpha smooth muscle actin



**Figure 3.** Genital wound repair is influenced by moisture, hormones, biomechanics, microbial environment and specific skin morphology that differs from skin of other body parts

reduced pro-inflammatory signalling of interleukin-6 and tumour necrosis factor (TNF)- $\alpha$ <sup>[14]</sup>. Furthermore, oestrogens are also important anti-oxidants that reduce cellular oxidative stress, apoptosis and increase keratinocyte migration and collagen synthesis by dermal fibroblasts<sup>[15]</sup>. In menopausal women, cutaneous oestrogen insufficiency manifests by atrophic skin changes, vulvar and vaginal exsiccation, and diminished defence against reactive oxygen species.

#### *Hormonal influences on genital skin during menopause*

As stated above, genital tissue is highly responsive to hormonal cues and changes. The menopause is a major incident in a woman's life with effects on her social, physical and psychological health. In 2014, a variety of menopausal symptoms were classified as Genitourinary Syndrome of Menopause<sup>[16]</sup>, which includes vulvovaginal atrophy in 84% of menopausal women<sup>[17]</sup>. Oestrogen insufficiency is the major cause for menopausal skin symptoms such as dryness, decreased elasticity and hydration. 17- $\beta$ -oestradiol protects skin cells against oxidative stress, induces collagen production, controls cutaneous water content and the dermal thickness. Oestrogen deprivation is followed by a decrease in tissue thickness and elasticity, and a decrease in wound healing and scar formation<sup>[18]</sup>. Regarding genital changes, mucous membranes of small labia and vaginal tissue react with dryness and atrophy on low oestrogen levels<sup>[19]</sup>. Topical oestrogen application was successful in reversing atrophic changes of genital tissues<sup>[18]</sup>.

### **Physiology of genital wound healing and scarring**

#### *Acute wound healing*

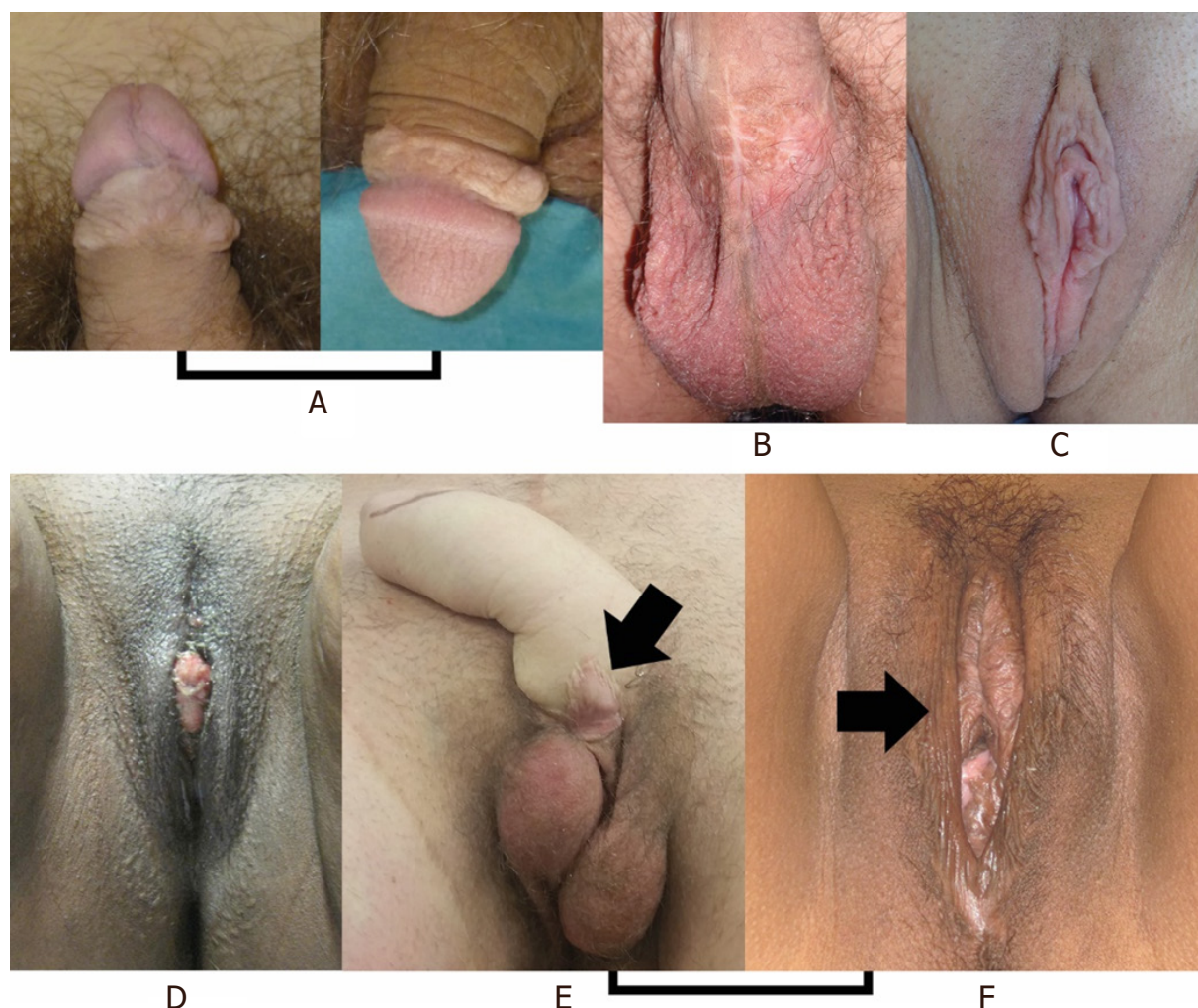
Despite constant commensal colonization of genital skin and an absent cornified layer as a potent barrier against microbial penetration, most genital wounds heal quickly and uneventfully. Communication with colleagues from gynecology or urology mirrors our observations from genital reassignment or aesthetic genital surgery. In contrast to skin of other body parts, genital wound healing is characterized by initial swelling with fast resolution and by almost invisible scarring [Figure 4].

It is a well-known fact that wound healing is promoted by a moist environment - present on mucous surfaces. Furthermore, inapparent scarring might be due to the fact that abundant elastic fibers are present in genital skin compared to normal skin and to the absence of tissue tension due to lack of fixation to underlying bone or cartilage. The disadvantage of the absent attachment to skeletal structures of genital skin is its tendency to shrink when a chronic inflammatory stimulus is present [Figure 3].

#### *Influence of microbial colonization*

Surfaces of mucous epithelia are inhabited by a microflora that differs from normal skin as well. Despite the missing cornified barrier and abundant commensal habitation, genital infections are rare (as in the oral cavity) but in the event of bacterial penetration, infections can be disastrous with high mortality. One example of a life-threatening genital infection is Fournier's gangrene that can only be cured by extensive and deep debridement of infected skin and underlying tissues as well as antibiotic therapy<sup>[3]</sup>. Constant exposure to commensal microbia is reflected by differential cellular immune responses with higher expression of antimicrobial peptides (AMPs) and defensins. The immune response and resolution are fast with conversion of M1 to M2 macrophages and reduced expression of pro-inflammatory cytokines<sup>[15]</sup>. Upon injury, skin cells increase IL-1 $\alpha$  production 15-fold in comparison to vaginal epithelial cells which show only a 3-fold increase. IL-1 $\beta$  and TNF- $\alpha$  are secreted by cutaneous epithelia in contrast to mucous epithelial cells<sup>[20]</sup>. With regard to pro-fibrotic mediators, transforming growth factor (TGF)- $\beta$  is significantly elevated in normal skin keratinocytes but not in mucosal epithelia and without induction of fibrotic processes in the underlying connective tissue. In summary, the reduced inflammatory response of mucosal epithelia to injury ensures fast wound closure.





**Figure 4.** Clinical examples for genital scarring in male and female genitalia. A: almost invisible scar after circumcision. Note color differences between the inner and the outer part of the foreskin; B: scrotal scarring after massive trauma and scrotal reconstruction; C: invisible scarring after esthetic reduction of the small labia; D: scarring seen in FGM/C after reconstructive surgery; E, F: scarring after gender reassignment surgery: E: female-to-male gender reassignment with scarring between the clitoral insertion sutures into the penoid (arrow) which was performed by a thigh flap; F: male-to-female gender reassignment situs with almost invisible scarring (arrow) seen on the big labia (constructed out of scrotal and penile skin)

### Pathophysiology of chronic genital repair processes

#### *Foreign body granuloma*

Many different substances are in use for penile enlargement, e.g., vaseline, paraffin, liquid mercury, silicone, or cod liver oil. A chronic inflammatory reaction due to foreign bodies was followed by granuloma formation, infections, swelling, and local tissue necrosis<sup>[21]</sup>. Polymethylmethacrylat microspheres, autologous fat or silicone implants are approved in certain countries for penile enlargement surgery<sup>[22,23]</sup>. The placement of permanent, alloplastic foreign body material in an environment populated by a variety of commensal microbes is risky due to the inherent danger of infection. In case of granuloma, tissue necrosis or implant infection, the foreign material must be removed with subsequent tissue loss. Foreign body materials can initiate a chronic inflammatory process with subcutaneous tissue fibrosis leading ultimately to a shrinkage of the entire penile shaft or to massive epitheloid cyst formation as seen in women after FGM/C. Of note, tissue shrinkage occurs in the subcutaneous compartment rather than in the penile shaft skin.



### *Autoimmune diseases*

Lichen sclerosus et atrophicus (LSC) is the most common chronic dermatitis of the genital skin which leads to fibrosis and tissue shrinkage and atrophy. Autoimmune responses govern the histological appearance with epidermal atrophy, hyalinization of the upper dermis and immune cell infiltrate. In patients with chronic disease, sclerotization of the tissue is found<sup>[24]</sup>. LSC is found in females and males with a ratio of up to 10:1 with increased occurrence in pre-pubertal and post-menopausal women. In men, LSC is the most common cause of acquired phimosis<sup>[25]</sup> and affects the glans and the prepuce. In chronic disease, these atrophic lesions can lead to a complete destruction of the vulva with shrinkage of the small labia and narrowing of the vaginal entrance. The patient's quality of life is severely reduced due to chronic itching and pain. LSC is associated with squamous cell carcinoma in 5% of women<sup>[26]</sup> and up to 30% of men<sup>[25]</sup>. Another related dermatosis, the Lichen planus, presents with similar symptoms and aetiology which makes the initial differentiation between Lichen sclerosus and Lichen planus difficult<sup>[27]</sup>. Finally, patients suffering from Behçet's or Crohn's disease may have genital manifestations of their primary autoimmune disease as well.

## **DISCUSSION**

Abundant knowledge is available regarding normal and pathological wound healing and scarring of skin tissue from the whole body except for the genital skin. Because the genital area is generally considered as embarrassing, patients rarely contact gynaecologists, urologists or plastic surgeons for reconstructive measures. If so, little expertise is present as reflected by the sparse literature available on genital wound healing and scarring. In the past decades, gender reassignment surgery, labioplasty, and plastic reconstructive surgery in FGM/C has been established in plastic surgery with rising awareness that genital skin pathophysiology differs from skin from other body areas.

Interestingly, hypertrophic scarring to the genitalia is uncommon even when dark skin types are considered. After circumcision or aesthetic labia reduction, almost invisible scars are the result. This phenomenon is explained by genital skin biomechanics and morphology with three key characteristics that are eminent to genital skin, namely: (1) lack of skeletal support and reduced tissue tension; (2) abundance of elastic fibers; and (3) presence of superficial cutaneous fasciae, e.g., the Dartos fascia. Tissue tension and TGF- $\beta$  are of pivotal importance for scarring and tissue fibrosis<sup>[28]</sup>. Bone and cartilage are part of skeletal structures that provide anchorage for muscles, tendons and other connective tissue structures with the skin spanning over all tissues as outer barrier. Hence, intact skin has an intrinsic, physiological tension which is released after full-thickness incisions or trauma and is visible as gaping wound edges. The human genitalia are not supported by a skeletal framework, and thus genital biomechanics differ from other body parts. The skin is loose and highly flexible - important characteristics for fast volume changes during sexual intercourse or child birth. Abundance of elastic fibres in genitalia is the prerequisite for tissue elasticity that is required for volume changes during erection. Elastic fibres are located to the Dartos fascia that is found beneath the dermis, reminiscent of the carnosus muscle found in fur bearing animals. In humans, the platysma muscle of the neck, palmaris brevis in the hand and the Dartos fascia belong to the panniculus carnosus. In pathological conditions such as buried penis or hypospadias, a significant reduction of elastic fibres and tissue elasticity is found in the Dartos fascia<sup>[29]</sup>. Furthermore, chronic genital inflammatory conditions such as LSC are characterized by decreased elastic fibres, tissue fibrosis and atrophy<sup>[25,30]</sup>. Our data show that the morphology of genital skin differs to skin from other body sites by having a thin epidermis and no fat tissue, but instead displaying a superficial cutaneous fascia (Dartos fascia in men or Colles fascia in women) with abundant elastic fibres.

In plastic surgery, tension-free wound margins are mandatory for unimpaired wound healing with almost invisible scarring. Lack of skeletal anchorage, highly elastic skin and abundance of tissue are advantageous for acute wound closure of genital skin. Interestingly, almost no scarring is found after routine circumcision

in men<sup>[31]</sup>. After traumatic skin loss of about half of the scrotal sac, the defect can be closed primarily with the remaining scrotal tissue<sup>[8,32]</sup>. Furthermore, abundant genital tissue provides the means for various local flaps which are commonly used for scrotal and penile reconstruction<sup>[33,34]</sup> or gender reassignment surgery<sup>[35]</sup>.

The importance of hormones on cutaneous repair is well established<sup>[10]</sup>. Unfortunately, most studies used skin tissue from non-genital body areas with no information on performance of genital skin in wound repair. Genital skin possesses the whole armamentarium to synthesize its own sex hormones<sup>[36]</sup> and an abundance of corresponding receptors to stimulate repair processes<sup>[15,37]</sup>. During aging, hormone levels decrease and the cytoprotective effect of oestrogens ceases<sup>[38]</sup>. Increased inflammation due to inflammatory cell recruitment, matrix metalloproteinase secretion and tissue degradation with subsequent loss of extracellular matrix are the cause for generalized tissue atrophy including genitalia with loss of elasticity<sup>[15]</sup>. In post-menopausal women, oestrogen deficiency is followed by vulvar and vaginal dryness and atrophy that can be - in part - reversed by local or systemic hormone replacement therapy<sup>[39]</sup>.

Chronic inflammatory diseases such as LSC lead to tissue fibrosis with epidermal thickening and to a shrinkage and atrophy with complete tissue destruction of the outer genitalia in the long-run. Interestingly, excessive scarring is rarely found in genitalia but atrophy and shrinkage is. In contrast to the genital skin, hypertrophic scarring and scar contractures are frequently seen after trauma or burns in body areas adjacent to the genitalia, e.g., the groin or the perineal crease<sup>[31]</sup>. A novel and seemingly successful approach to tackle LSC in women was published by Italian gynaecologists who used autologous lipofilling for vulvar atrophy<sup>[40]</sup>. Fat grafts are known for their pain-reducing and anti-inflammatory properties<sup>[41,42]</sup>. Aside from immunological effects, the fat graft restores the volume of vulvar structures and changes biomechanics as well<sup>[43,44]</sup>.

The presence of mucous epithelia characterizes not only genital skin but also the oral cavity. As stated above, little data is available on genital mucosal wound repair but abundant knowledge on oral mucosal cell behaviour is, which might be comparable for both body parts. Like oral wound repair<sup>[45]</sup>, genital wounds heal faster, with less scarring and faster resolution of the inflammatory response compared to normal skin<sup>[46]</sup>. An important observation was the diverging angiogenesis between oral and normal skin. Oral wounds develop less but functional vessels for wound tissue revascularization in contrast to abundant immature capillaries in granulation tissue of normal skin<sup>[47]</sup>. Unfortunately, no data is available on angiogenesis in genital wound repair.

Faster wound repair of oral keratinocytes was attributed to higher proliferation rates, faster migration and independence from paracrine stimuli by underlying connective tissue cells<sup>[48]</sup>. Seemingly, the epithelial response to injury governs the local inflammatory reaction and subsequently scar formation by the underlying dermal tissue. In previous studies it was shown that vaginal epithelium responds to injury with less IL-1 $\beta$  and absence of TNF- $\alpha$  secretion in comparison to skin keratinocytes with reduced scar formation<sup>[20]</sup>. Similar effects were found upon IL-1 $\beta$  stimulation with higher levels of IL-6 and TNF- $\alpha$  in epidermal compared to oral keratinocytes with faster wound closure implying a robust responsiveness of mucosal cells to inflammatory stimuli<sup>[49]</sup>. Further research on genital tissue is needed to verify if findings from the oral cavity correspond to genitalia as well.

Barrier epithelia are constantly exposed to the commensal microbial flora and elicit differential immune responses to continuously present bacteria in contrast to localized infections. Moreover, genital epithelia face exposure to foreign microbes during sexual intercourse. AMP such as defensins belong to the epithelial repertoire of antimicrobial defence mechanisms<sup>[50]</sup> and are physiologically secreted at low levels for protection against the commensal flora<sup>[51]</sup>. Bacterial infection with depletion of the indigenous microbial

population initiates the secretion of proinflammatory mediators with increased and differential AMP expression in the female reproductive tract<sup>[51,52]</sup>. While  $\beta$ -defensin-1 is constitutively expressed by mucosal epithelia,  $\beta$ -defensin-2 and -3 are found during inflammation and infection<sup>[53]</sup>. Less  $\beta$ -defensin-2 was found in migrating cells from a bioengineered skin construct composed of human foreskin cells in comparison to full-thickness skin graft sampled from the thigh<sup>[54]</sup>. Because foreskin derived cells are frequently used as human skin cells in experimental studies, further research is needed with comparison of cellular performance between genital and non-genital cutaneous cells.

In summary, genitalia comprise epithelial and connective tissues with varying morphology and inflammatory responses to trauma. Lack of skeletal anchorage, abundance of connective tissue with high content of elastic fibres and presence of superficial fasciae provide different biomechanics and scarring behaviour of genital skin in comparison to other body parts. Reconstructive procedures should take the characteristics of genital tissues into consideration when planning defect closure with functional restoration of micturition and sexuality. As a consequence, complex surgical interventions of the genitalia and adjacent body areas should exclusively be performed by experienced specialists, preferably in an interdisciplinary setting.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception and design of the study and performed data analysis and interpretation: Mirastschijski U, Jiang D, Rinkevich Y, Karim R, Sorg H

Performed data acquisition, as well as provided administrative, technical, and material support: Mirastschijski U, Jiang D

Writing and editing of the manuscript, advice and intellectual support: Mirastschijski U, Jiang D, Rinkevich Y, Karim R, Sorg H

Editing of figures, tables and photographs: Mirastschijski U, Jiang D, Karim R

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

Rinkevich Y was supported by the Human Frontier Science Program Career Development Award (CDA00017/2016), the German Research Foundation (RI 2787/1-1 AOBJ: 628819), the Fritz-Thyssen-Stiftung (2016-01277), and the European Research Council Consolidator Grant (ERC-CoG 819933).

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Tissue specimens were obtained after information and written patient's consent. The study was approved by the local ethical committees (Ethics Committee of the Medical Chamber of Bremen, No. 336/12 and No. RA/RE 336; and of Bavaria, 2018-157).

### Consent for publication

Written informed consent for anonymous publication of photographs are routinely obtained from every patient undergoing plastic surgery and are stored in the patients' files.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Balzano FL, Hudak SJ. Military genitourinary injuries: past, present, and future. *Transl Androl Urol* 2018;7:646-52.
2. Ismail Aly ME, Huang T. Management of burn injuries of the perineum. In: Herndon D, editor. London: Elsevier; 2018. pp. 609-17.
3. Lehnhardt M, Wallner C, Daigeler A. Reconstruction of the male genitals after Fournier gangrene. In: Mirastschijski U, Rimmel E, editors. Berlin: Springer; 2019. pp. 253-63.
4. World Health Organization. Male circumcision: global trends and determinants of prevalence, safety and acceptability. Geneva: IWHO Library Cataloguing-in-Publication Data; 2007. Available from: [https://apps.who.int/iris/bitstream/handle/10665/43749/9789241596169\\_eng.pdf?sequence=1](https://apps.who.int/iris/bitstream/handle/10665/43749/9789241596169_eng.pdf?sequence=1). [Last accessed on 17 Nov 2020]
5. Mirastschijski U, Schwab I, Coger V, et al. Lung surfactant accelerates skin wound healing: a translational study with a randomized clinical phase I study. *Sci Rep* 2020;10:2581.
6. Correa-Gallegos D, Jiang D, Christ S, et al. Patch repair of deep wounds by mobilized fascia. *Nature* 2019;576:287-92.
7. Jiang D, Correa-Gallegos D, Christ S, et al. Two succeeding fibroblastic lineages drive dermal development and the transition from regeneration to scarring. *Nat Cell Biol* 2018;20:422-31.
8. Mirastschijski U, Schwenke C, Schwab I, Buchhorn A, Schmiedl A. Midline raphe scroti artery flap for penile shaft reconstruction. *Plast Aesthet Res* 2020;7:1-13.
9. Mirastschijski U, Rimmel E. Intimchirurgie. Berlin: Springer Verlag; 2019.
10. Gilliver SC, Ashworth JJ, Ashcroft GS. The hormonal regulation of cutaneous wound healing. *Clin Dermatol* 2007;25:56-62.
11. Gilliver SC, Ashcroft GS. Sex steroids and cutaneous wound healing: the contrasting influences of estrogens and androgens. *Climacteric* 2007;10:276-88.
12. Pomari E, Dalla Valle L, Pertile P, Colombo L, Thornton MJ. Intracrine sex steroid synthesis and signaling in human epidermal keratinocytes and dermal fibroblasts. *FASEB J* 2015;29:508-24.
13. Emmerson E, Campbell L, Ashcroft GS, Hardman MJ. Unique and synergistic roles for 17beta-estradiol and macrophage migration inhibitory factor during cutaneous wound closure are cell type specific. *Endocrinology* 2009;150:2749-57.
14. Crompton R, Williams H, Ansell D, et al. Oestrogen promotes healing in a bacterial LPS model of delayed cutaneous wound repair. *Lab Invest* 2016;96:439-49.
15. Wilkinson HN, Hardman MJ. The role of estrogen in cutaneous ageing and repair. *Maturitas* 2017;103:60-4.
16. Portman DJ, Gass ML. Vulvovaginal atrophy terminology consensus conference p. genitourinary syndrome of menopause: new terminology for vulvovaginal atrophy from the international society for the study of women's sexual health and the north american menopause society. *Maturitas* 2014;79:349-54.
17. Santoro N, Komi J. Prevalence and impact of vaginal symptoms among postmenopausal women. *J Sex Med* 2009;6:2133-42.
18. Rzepecki AK, Murase JE, Juran R, Fabi SG, McLellan BN. Estrogen-deficient skin: the role of topical therapy. *Int J Womens Dermatol* 2019;5:85-90.
19. Nappi RE, Martini E, Cucinella L, et al. Addressing vulvovaginal atrophy (VVA)/genitourinary syndrome of menopause (GSM) for healthy aging in women. *Front Endocrinol (Lausanne)* 2019;10:561.
20. Gallant-Behm CL, Du P, Lin SM, Marucha PT, DiPietro LA, Mustoe TA. Epithelial regulation of mesenchymal tissue behavior. *J Invest Dermatol* 2011;131:892-9.
21. Schill S, Panfilov DE, Mirastschijski U. Intimchirurgie beim mann. In: Mirastschijski U, Rimmel E, editors. Berlin: Springer; 2019. pp. 49-68.
22. Lemperle G, Casavantes L. Penisvergrößerung durch Injektion von PMMA-Mikrosphären. In: Mirastschijski U, Rimmel E, editors. Berlin: Springer; 2019. pp. 79-89.
23. Lemperle G, Elist JJ, Jethon C. Penisvergrößerung mit dem penuma-silikon-implantat. In: Mirastschijski U, Rimmel E, editors. Berlin: Springer; 2019. pp. 69-78.
24. Lee A, Fischer G. Diagnosis and treatment of vulvar lichen sclerosis: an update for dermatologists. *Am J Clin Dermatol* 2018;19:695-706.
25. Clouston D, Hall A, Lawrentschuk N. Penile lichen sclerosis (balanitis xerotica obliterans). *BJU Int* 2011;108 Suppl 2:14-9.
26. Kirtschig G. Lichen sclerosis-presentation, diagnosis and management. *Dtsch Arztebl Int* 2016;113:337-43.
27. Terlou A, Santeoets LA, van der Meijden WI, et al. An autoimmune phenotype in vulvar lichen sclerosis and lichen planus: a Th1 response and high levels of microRNA-155. *J Invest Dermatol* 2012;132:658-66.
28. Hinz B, McCulloch CA, Coelho NM. Mechanical regulation of myofibroblast phenocconversion and collagen contraction. *Exp Cell Res* 2019;379:119-28.
29. Atmoko W, Shalmon G, Situmorang GR, Wahyudi I, Tanurahardja B, Rodjani A. Abnormal dartos fascia in buried penis and hypospadias: evidence from histopathology. *J Pediatr Urol* 2018;14:536.e1-7.
30. Canady J, Karrer S, Fleck M, Bosserhoff AK. Fibrosing connective tissue disorders of the skin: molecular similarities and distinctions. *J Dermatol Sci* 2013;70:151-8.
31. Mirastschijski U. Genital scars. In: Téot L, Mustoe TA, Middelkoop E, Gauglitz G, editors. London: Springer International Publishing; 2020. pp. 1-640.
32. Mirastschijski U, Schwenke C, Schmiedl A. Plastisch-chirurgische rekonstruktion des männlichen genitalen. In: Mirastschijski U, Rimmel E, editors. Berlin: Springer; 2019. pp. 189-206.
33. Mirastschijski U. Buried penis. In: Mirastschijski U, Rimmel E, editors. Berlin: Springer; 2019. pp. 107-14.
34. Mirastschijski U. Classification and treatment of the adult buried penis. *Ann Plast Surg* 2018;80:653-9.

35. Schaff J, Morath S, Mirastschijski U. Operative techniken bei mann-zu-frau-transsexualität. In: Mirastschijski U, Rimmel E, editors. Berlin: Springer; 2019. pp. 294-303.
36. Zouboulis CC, Chen WC, Thornton MJ, Qin K, Rosenfield R. Sexual hormones in human skin. *Horm Metab Res* 2007;39:85-95.
37. Choudhry R, Hodgins MB, Van der Kwast TH, Brinkmann AO, Boersma WJ. Localization of androgen receptors in human skin by immunohistochemistry: implications for the hormonal regulation of hair growth, sebaceous glands and sweat glands. *J Endocrinol* 1992;133:467-75.
38. Thornton MJ. Estrogens and aging skin. *Dermatoendocrinol* 2013;5:264-70.
39. Matthews N, Wong V, Brooks J, Kroumpouzou G. Genital diseases in the mature woman. *Clin Dermatol* 2018;36:208-21.
40. Boero V, Brambilla M, Sipio E, et al. Vulvar lichen sclerosus: a new regenerative approach through fat grafting. *Gynecol Oncol* 2015;139:471-5.
41. Fredman R, Edkins RE, Hultman CS. Fat grafting for neuropathic pain after severe burns. *Ann Plast Surg* 2016;76 Suppl 4:S298-303.
42. Prantl L, Rennekampff HO, Giunta RE, et al. Current perceptions of lipofilling on the basis of the new guideline on “autologous fat grafting”. *Handchirurgie Mikrochirurgie Plastische Chirurgie* 2016;48:330-6.
43. Clauser LC, Tieghi R, Galie M, Carinci F. Structural fat grafting: facial volumetric restoration in complex reconstructive surgery. *J Craniofac Surg* 2011;22:1695-701.
44. Jaspers ME, Brouwer KM, van Trier AJ, Groot ML, Middelkoop E, van Zuijlen PP. Effectiveness of autologous fat grafting in adherent scars: results obtained by a comprehensive scar evaluation protocol. *Plast Reconstr Surg* 2017;139:212-9.
45. Larjava H, Wiebe C, Gallant-Behm C, Hart DA, Heino J, Hakkinen L. Exploring scarless healing of oral soft tissues. *J Can Dent Assoc* 2011;77:b18.
46. Mak K, Manji A, Gallant-Behm C, et al. Scarless healing of oral mucosa is characterized by faster resolution of inflammation and control of myofibroblast action compared to skin wounds in the red Duroc pig model. *J Dermatol Sci* 2009;56:168-80.
47. DiPietro LA. Angiogenesis and wound repair: when enough is enough. *J Leukoc Biol* 2016;100:979-84.
48. Turabelidze A, Guo S, Chung AY, et al. Intrinsic differences between oral and skin keratinocytes. *PLoS One* 2014;9:e101480.
49. Glim JE, van Egmond M, Niessen FB, Everts V, Beelen RH. Detrimental dermal wound healing: what can we learn from the oral mucosa? *Wound Repair Regen* 2013;21:648-60.
50. Zasloff M. Innate immunity, antimicrobial peptides, and protection of the oral cavity. *Lancet* 2002;360:1116-7.
51. Yarbrough VL, Winkle S, Herbst-Kralovetz MM. Antimicrobial peptides in the female reproductive tract: a critical component of the mucosal immune barrier with physiological and clinical implications. *Hum Reprod Update* 2015;21:353-77.
52. Lai Y, Gallo RL. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. *Trends Immunol* 2009;30:131-41.
53. Chung WO, Dale BA. Innate immune response of oral and foreskin keratinocytes: utilization of different signaling pathways by various bacterial species. *Infect Immun* 2004;72:352-8.
54. Mirastschijski U, Bugdahl R, Rollman O, Johansson BR, Agren MS. Epithelial regeneration from bioengineered skin explants in culture. *Br J Dermatol* 2006;154:42-9.



Review

Open Access



# Botulinum toxin in facial plastic surgery

Nicole Favre, David Sherris

Department of Otolaryngology, Jacobs School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY 14209, USA.

**Correspondence to:** Dr. David Sherris, Department of Otolaryngology, Jacobs School of Medicine and Biomedical Sciences, State University of New York at Buffalo, 1237 Delaware Avenue, Buffalo, NY 14209, USA. E-mail: dsherris@buffalo.edu

**How to cite this article:** Favre N, Sherris D. Botulinum toxin in facial plastic surgery. *Plast Aesthet Res* 2020;7:71. <http://dx.doi.org/10.20517/2347-9264.2020.149>

**Received:** 8 Jul 2020 **First Decision:** 24 Aug 2020 **Revised:** 7 Sep 2020 **Accepted:** 2 Nov 2020 **Published:** 5 Dec 2020

**Academic Editor:** James E. Zins **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Since the U.S. Food and Drug Administration approved botulinum toxin (BoNT) type A in 2002 for glabellar rhytids, BoNT has been used successfully for many clinical indications in facial plastic surgery. The current usage of BoNT as a non-invasive procedure for rhytids of the aging face include but are not limited to rhytids of the forehead, glabella, lateral orbit, nasal sidewall, upper lip, vertical perioral rhytids, melomental fold, and chin. In addition to facial rhytids, BoNT has been shown to be effective for a variety of other clinical indications in facial plastic surgery, including masseter hypertrophy, facial paralysis, brow ptosis, and wound healing. This article will review the pharmacology and mechanism of action of BoNT. In addition, the suggested dosage and instruction for injection for facial rhytids will be discussed along with BoNT usage for clinical indications other than rhytids.

**Keywords:** Botulinum toxin, botox, neurotoxin

## INTRODUCTION

The first published report of botulinum toxin (BoNT) for aesthetic use in 1989 was just the beginning of the expansion of BoNT as one of the most common non-invasive techniques in the field of facial plastic surgery<sup>[1]</sup>. Shortly after this report, the safety and efficacy of BoNT-A to treat glabellar frown lines was demonstrated in study with injection into 18 patients by Carruthers and Carruthers<sup>[2]</sup>. Together with other trials, the U.S. Food and Drug Administration (FDA) approved the use of BoNT-A for glabellar rhytids in 2002. Although BoNT-A is currently approved by the FDA for the aesthetic treatment of various facial rhytids, there continues to be an increasing number of off-label aesthetic usages of BoNT<sup>[3]</sup>. According to



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



the American Society of Plastic Surgeons, in the United States specifically, the estimated BoNT-A injectable procedures performed in 2019 were approximately 7,697,798. This BoNT-A number is of anatomic sites injected and based on data from the 682 physicians included<sup>[4]</sup>. Because the face is the central aspect of a person's identity and a visible marker of one's age<sup>[5]</sup>, it is important to review the current non-invasive procedures used in aging face treatment. This article reviews the use of BoNT in the treatment of facial rhytids as well as other uses of BoNT in non-invasive procedures related to facial plastic surgery.

## MECHANISM OF ACTION

BoNT is produced by *Clostridium botulinum*, a spore-forming obligate anaerobe that resides in the soil. BoNT is a simple dichain polypeptide that consists of a 100-kd heavy chain joined by a single disulfide bond to a 50-kd light chain. BoNT acts by blocking the fusion of soluble N-ethylmaleimide sensitive factor attachment protein receptor (SNARE) proteins at the neuromuscular junction, resulting in the blocking of acetylcholine release and temporary flaccid paralysis<sup>[6]</sup>. The SNARE proteins targeted by different BoNTs vary. Although there are seven distinct types of BoNT, there are currently only two available for commercial usage, type A and type B. BoNT type A (BoNT-A) cleaves synaptosomal - associated protein (SNAP-25), whereas BoNT type B cleaves synaptobrevin or vesicle-associated membrane protein<sup>[7]</sup>. Although BoNT causes irreversible neuromuscular blockade as shown in both disease and therapy, muscle function does recover through the formation of axonal sprouts and new motor end plates, as well as the full recovery of the neuromuscular junction<sup>[3]</sup>.

There are currently three leading BoNT-A products available in the United States: OnabotulinumtoxinA (ONA; Botox), AbobotulinumtoxinA (ABO; Dysport), and IncobotulinumtoxinA (INCO; Xeomin). Although the conversion factors between the two formulations are not yet clearly stated, it is suggested in multiple studies that an ONA:ABO conversion ratio of exactly 1:2 is appropriate<sup>[8]</sup>. In a study by Hexsel *et al.*<sup>[9]</sup>, a conversion ratio of 1:2.5 for ONA:ABO showed significantly different fields of effect, which points to the fact that the difference in diffusion effect is specifically dose dependent. INCO was shown to be as effective as ONA with a comparable frequency of adverse effects when a clinical conversion ratio of 1:1 was used.

## INSTRUCTIONS FOR INJECTION

When performing injections of BoNT-A, it is important to understand the anatomy of the muscle as well as the toxin, dilution, and needle characteristic used for injection in order to achieve the desired clinical outcome and avoid any complications or adverse effects. Based on the senior author's experience, the effect of BoNT-A starts at 24-48 h after injection, peaking at 7-14 days, and lasting 3-6 months, with an average of 3-4 months. There are some possible adverse effects that should be discussed with the patient prior to injection, including but not limited to unexpected loss of strength or muscle weakness, injection site reactions or bleeding, and bruising. Patients are instructed to avoid anticoagulants or other blood thinning medications for two weeks in order to minimize the bleeding and bruising<sup>[10]</sup>. It is also important to consider the diffusion of the neurotoxin when choosing which neurotoxin to use in each anatomic location. Some anatomic locations require a larger field of effect for optimal treatment, while this same large field of effect may produce an undesirable outcome for other anatomic locations<sup>[9]</sup>. Based on the prescribing information for each of these products, the 1:1 conversion ratio of ONA:INCO and the 1:2.5 conversion ratio of ONA:ABO are used in the tables below which summarize the injection dosage of facial rhytids [Tables 1-3].

## BoNT TREATMENT FOR RHYTIDS OF THE FACE

### Upper face

#### Forehead

When treating the forehead to minimize rhytids, the main muscle of target is the frontalis muscle which attaches proximally to the galea aponeurotica and distally to the skin around the eyebrows and the nose.

**Table 1. Upper face rhytid dosage and injection instructions**

Muscle	Onabotulinum	Incobotulinum	Abobotulinum
Frontalis	2-4 units over 4-6 injection sites	2-4 units over 4-6 injection sites	5-10 units over 4-6 injection sites
Glabellar Complex	15-20 units over 5 injection sites	15-20 units over 5 injection sites	37.5-50 units over 5 injection sites
Lateral Orbit	2-4 units over 3 injection sites bilaterally	2-4 units over 3 injection sites bilaterally	5-10 units over 3 injection sites bilaterally

**Table 2. Midface rhytid dosage and injection instructions**

Muscle	Onabotulinum	Incobotulinum	Abobotulinum
Nasalis	2-4 units bilaterally or one central injection	2-4 units bilaterally or one central injection	5-10 units bilaterally or one central injection
Upper Lip (i.e., levator labii superioris alaeque nasi, levator labii superioris, zygomaticus minor)	4-6 units over 2-4 injection sites	4-6 units over 2-4 injection sites	10-15 units over 2-4 injection sites
Orbicularis Oris	3-7 units over 3 injection sites	3-7 units over 3 injection sites	7.5-17.5 units over 3 injection sites

**Table 3. Lower face rhytid dosage and injection instructions**

Muscle	Onabotulinum	Incobotulinum	Abobotulinum
Depressor Anguli Oris	2-3 units into each muscle belly	2-3 units into each muscle belly	5-7.5 units into each muscle belly
Mentalis	2 units into each muscle belly or 5 units into one midline injection	2 units into each muscle belly or 5 units into one midline injection	5 units into each muscle belly or 15 units into one midline injection

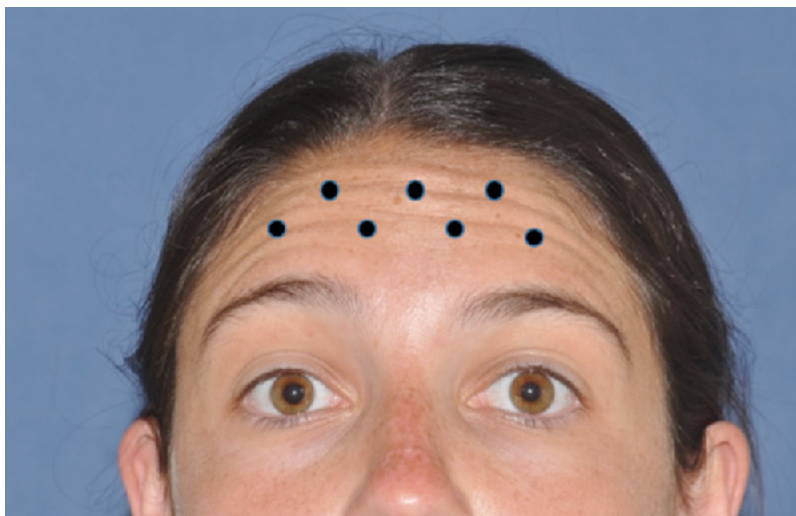
The main function of the frontalis muscle is to raise the eyebrows, and in doing so, it causes wrinkling of the forehead. In treating these rhytids caused by contraction of the frontalis muscle, it is important to differentiate between static and dynamic lines. Dynamic lines are a result of hyperfunctional muscle contraction, like that seen by the frontalis muscle when lifting the eyebrows, while static lines are unchanged with muscle movement and are more commonly treated with dermal fillers<sup>[11]</sup>.

Because the frontalis muscle is the only active muscle in this region, it is suggested that weakening of the muscle, instead of complete denervation, is preferred in order to avoid brow ptosis. The forehead furrows caused by the frontalis muscle respond favorably to either subcutaneous or intramuscular injection<sup>[10]</sup> [Figure 1].

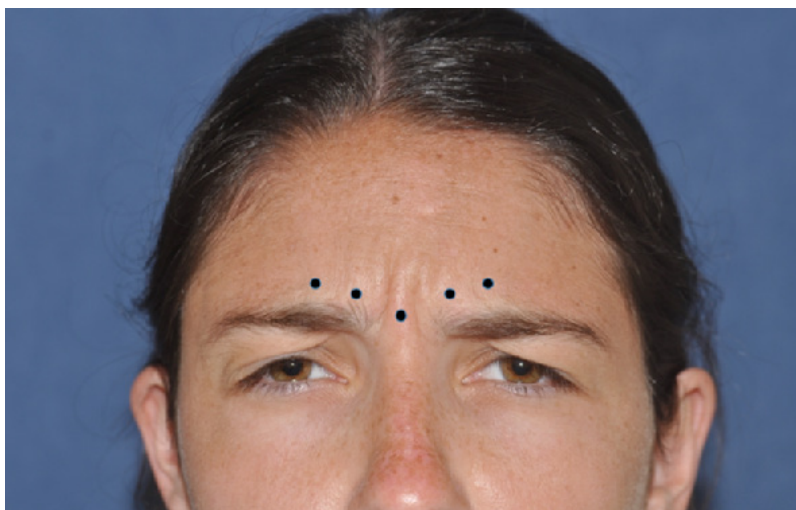
### Glabella

The glabellar complex, a medial brow depressor, consists of muscles including procerus, corrugator supercilii, and depressor supercilii. The two main depressors are the corrugator supercilii which originates from the superciliary arch of the frontal bone and inserts into the skin of the eyebrow, and the procerus which originates from the fascia of the lower nasal bone and inserts on the skin between the eyebrows. Overall, the glabellar complex requires one injection site into the body of the procerus and 1 to 2 injection sites on each side for the corrugator supercilii muscle [Figure 2]. The more complex treatment of the corrugator supercilii involves a deeper injection at the medial portion to capture the depressor supercilii and a more superficial injection laterally when the muscle approaches the dermis<sup>[10]</sup>. The treatment of glabellar rhytids was the first approved aesthetic use of BoNT-A, and since then, several follow up studies have shown effective treatment for these specific rhytids<sup>[12]</sup>.

A major side effect from improper injection of the glabellar complex, as well as all other neurotoxin injections of the upper face, is eyelid ptosis. For the glabellar complex specifically, it is necessary to inject above the orbital rim to prevent diffusion of the neurotoxin to the levator palpebrae superioris muscle, which would result in eyelid ptosis<sup>[13]</sup>. Ptosis can occur up to 2 weeks after injection. If eyelid ptosis from migration to the levator palpebrae superioris occurs, it can be treated with Apraclonidine (Lopidine) eye drops, which is an alpha-2-adrenergic agonist that causes the superior tarsal muscle to contract and elevate the upper eyelid<sup>[14]</sup>.



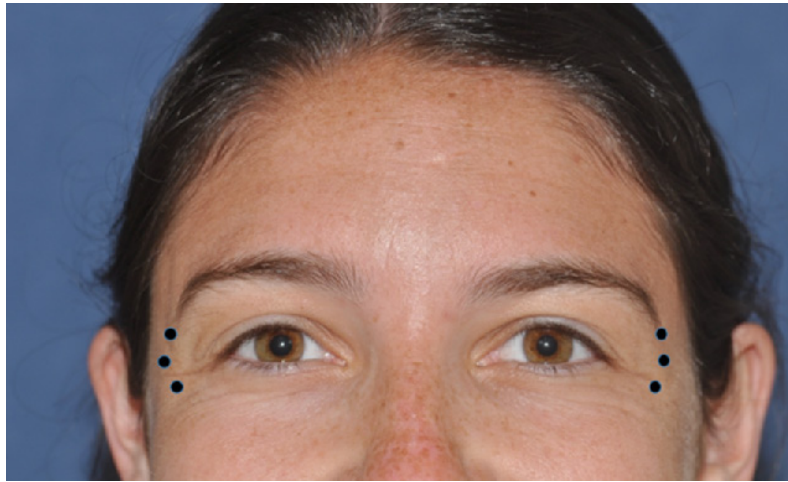
**Figure 1.** Injection sites for forehead rhytids into the frontalis muscle



**Figure 2.** Injection sites for glabellar rhytids into the procerus and corrugator supercilii muscle

### *Lateral orbit*

The main muscle of the lateral orbit is the orbicularis oculi muscle, the muscle involved in closure of the eye and depression of the eyebrow. This muscle is divided into three parts: the orbital, the preseptal, and the pretarsal portions. The muscle extends beyond the orbital rim and creates lateral rhytids known as crow's feet. Treatment of these rhytids can be done with one or three injections on either side [Figure 3]. In a two-center randomized study by Fabi *et al.*<sup>[15]</sup>, no statistically significant difference was seen in crow's feet treated with one injection per side *vs.* crow's feet treated with three injections per side. In addition, there was no difference in adverse events based on injection number. With the use of three injections per side, the middle injection should be in line with the lateral canthus and the remaining should be 8 to 10 mm away. It is critical to inject at least 1 cm lateral to the lateral orbital rim in order to avoid complications, such as diplopia or strabismus, from diffusion into the extraocular muscles resulting in the paralysis of the lateral rectus muscle<sup>[13]</sup>. In addition to the crow's feet rhytids, hypertrophy of the pretarsal portion of the orbicularis oculi can cause what is known as the "jelly roll" appearance around the lower eyelid. When a person is smiling, the contraction of the pretarsal portion diminishes the side of the palpebral aperture



**Figure 3.** Injection sites for lateral orbit (crow's feet) rhytids into the orbicularis oculi muscle

which causes these “jelly roll” lines. It is recommended that 2 units of ONA be injected into the pretarsal portion of the orbicularis oculi to counteract the contraction causing the diminished palpebral aperture<sup>[16]</sup>.

One specific consideration regarding patient satisfaction that should be considered when using BoNT for crow's feet rhytids is the interaction between the dynamic lines, treated by the neurotoxin, and the static lines in this region. The junction of these static and dynamic lines creates rhytids that the patient may not be satisfied with after neurotoxin injection. These static rhytids should then be treated with fillers, such as hyaluronic acid injections, which increase the volume of the skin in this area and remove the lines created by the junction of static and dynamic rhytids<sup>[10]</sup>.

## Midface

### *Nasal sidewalls*

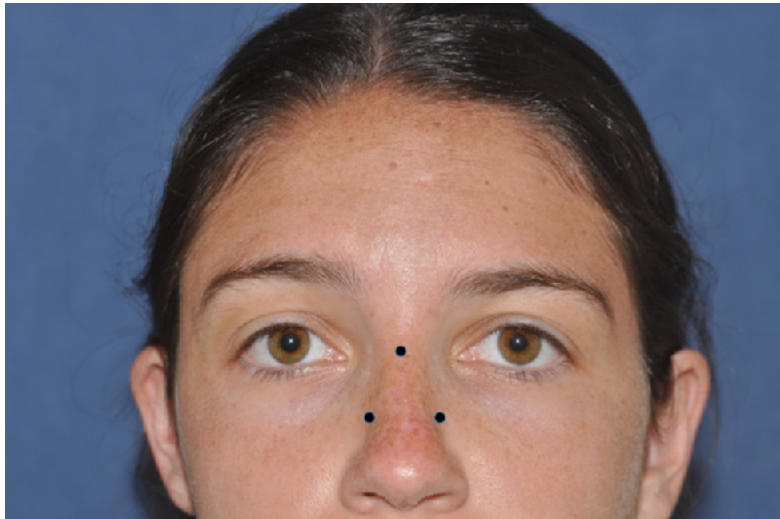
Rhytids in the region of the upper nose, known as “bunny lines,” are a result of contraction of the upper, transverse portion of the nasalis muscle. Recommended injection for treating these “bunny lines” is 2 to 4 units of ONA directly into the nasalis muscle on each side of the nose or one central injection at the midline [Figure 4]. The location of injection is of extreme importance in this region. Injections placed too low on the nose, below the nasofacial groove, can cause relaxation of the levator labii superioris alaeque nasi and levator anguli oris, resulting in both lip ptosis and smile asymmetry. On the other hand, injections placed too high could cause medial rectus paresis and epiphora<sup>[17]</sup>.

One other usage of BoNT in the nose region is to treat the effects of nasal flare, which is when a patient repeatedly involuntarily dilates their nostrils in social situations. This dilation makes the nasal septum visible and causes an undesired appearance that results in lack of self-esteem and confidence in social settings. It is recommended that injection of 5 to 10 units of ONA into the portion of the nasalis muscle over the nasal ala will help to inhibit this involuntary nasal flare<sup>[16]</sup>.

### *Upper lip*

A person's smile is thought to be one of the most meaningful facial expressions in today's society; therefore, it is important to ensure patient satisfaction of the aesthetic appearance of their smile. One of the most desired changes to one's smile is removal of excessive gingival exposure, more commonly known as “gummy smile.” The “gummy smile” is defined as greater than or equal to 2 mm of gingival exposure while smiling<sup>[18]</sup>. Gingival exposure may need surgical intervention based on the specific cause, such as from





**Figure 4.** Injection sites for nasal sidewall rhytids into the nasalis muscle

delayed dental eruption or vertical maxillary excess; however, gingival exposure due to muscle hypertrophy can be treated with BoNT. The “gummy smile” in this case is caused by overactivation of the muscles that elevate the central lip, including the levator labii superioris, levator labii superioris alaeque nasi, and the zygomaticus minor. It is recommended to inject 2 to 4 sites of the lip elevator muscles with 4 to 6 units of ONA [Figure 5]; studies show that a higher dosage is necessary for more extreme gingival exposure<sup>[19]</sup>. While an asymmetric smile can be corrected with the use of BoNT-A on the levator labii superioris alaeque nasi, it is important to be cautious of dosage and location of injection so as to not create an asymmetric smile with the paralysis of the depressor labii inferioris muscle.

#### *Vertical perioral rhytids*

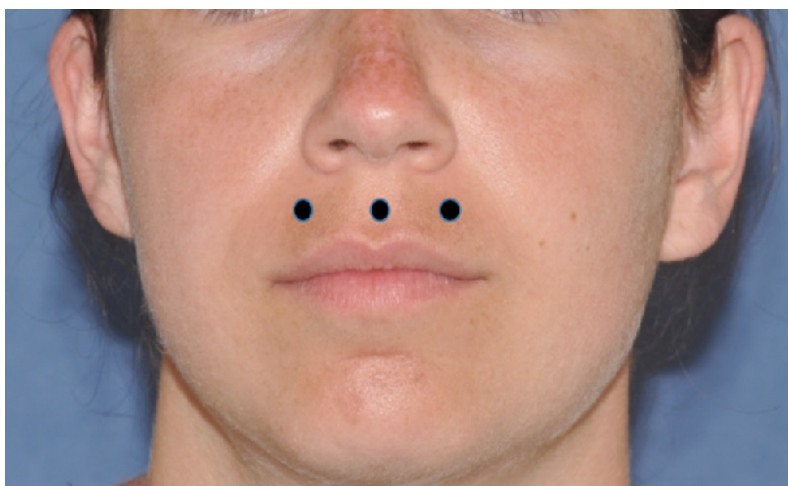
Vertical perioral rhytids are caused by the activation of the orbicularis oris muscle, which encircles the mouth, inserting directly on the lips and controlling movements of the mouth and the lips. It is recommended that 3 to 7 units of ONA are injected over three sites in the upper lip. Because it is more difficult to assess patient response with the orbicularis oris muscle than muscles of the forehead and upper face, careful consideration of dosage is important to avoid complete paralysis of the muscle, resulting in the patient’s inability to purse their lips, drink from a straw, or phonate clearly<sup>[16]</sup>. It is advised to start with lower doses so the patient has time to determine their satisfaction of the treatment response.

#### **Lower face**

##### *Melomental folds*

The activity of the depressor anguli oris muscle on either side of the mouth is responsible for depressing the corner of the mouth. Hyperactivity of these muscles can result in the appearance of being sad, tired or angry in some patients. Treatment with BoNT-A can be used to relax these hyperactive muscles and achieve desired facial appearance.

Injection of BoNT-A into the depressor anguli oris muscle is difficult because of its fan shape with its medial portion overlapping with the depressor labii inferioris muscle and its lateral portion located in close proximity to the risorius, zygomaticus major and platysma muscles. Choi *et al.*<sup>[20]</sup> showed that the best location for injection to avoid unwanted side effects with diffusion of the neurotoxin to other muscle groups is the inferior portion of the muscle located at the mandible, enclosed in the fan shaped area [Figure 6].



**Figure 5.** Injection sites for upper lip rhytids into the lip elevator muscles



**Figure 6.** Injection sites for melomental folds into the depressor anguli oris muscle

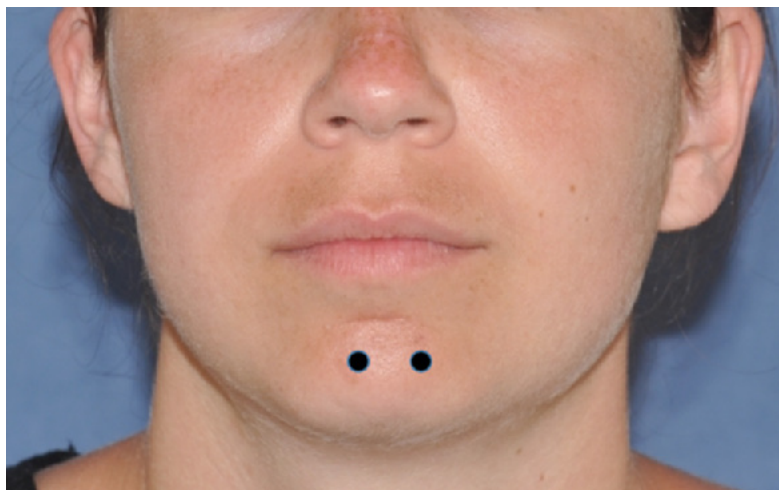
### *Chin*

One of the most common areas of the face frequently injected with BoNT for aesthetic reasons is the chin, with the main muscle of injection being the mentalis muscle. The mentalis has two muscle bellies that originate from the mentum and insert onto the chin soft tissue under the lip. Its function is to elevate and protrude the lower lip while wrinkling the skin of the chin, often producing the emotions of doubt or “pouting”<sup>[13]</sup>. Injections to improve the wrinkling of the chin and aesthetic appearance of this area can be performed with a single injection in the midline of the chin with 5 units of ONA or bilaterally in each of the muscle bellies with 2 units of ONA per injection<sup>[3]</sup> [Figure 7]. Patients are often satisfied with the results of decreased wrinkling in the mental area after injection.

## **OTHER USES OF BONT**

### **Masseteric hypertrophy**

The masseter muscle originates from the inferior border of the zygomatic arch and inserts onto the angle and ramus of the mandible, functioning to elevate and protract the mandible to close the jaw. Hypertrophy



**Figure 7.** Injection sites for chin rhytids into the mentalis muscle

of this muscle can cause the lower face to look square, which is often not a desired appearance for female patients. While the goal of treatment for the facial rhytids described above was to induce muscle paralysis, the goal for BoNT-A injection of the masseter muscle is to soften the square appearance of the face by reducing masseter muscle mass, ultimately creating a more desired aesthetic appearance<sup>[3]</sup>. Dosage of BoNT-A for injection is generally around 15 to 20 units of ONA, but the specific dosage depends on the degree of hypertrophy. The effects of this injection can last six to nine months, but, as with other BoNT-A injections, patients often follow up multiple weeks after injection to determine the need for more injections. Factors such as bruxism and clenching habits can increase masseter muscle thickening and require more frequent BoNT-A injections<sup>[21]</sup>. In addition to BoNT-A's aesthetic effects on the masseter muscle, patients with temporomandibular joint dysfunction (TMJ) symptoms that have masseter hypertrophy report a relief of their TMJ symptoms with BoNT-A injections<sup>[10]</sup>.

The injector must be careful to avoid injecting the risorius muscle, which can result in the undesired side effect of an asymmetric smile. It is important to inject inferiorly and laterally along the masseter muscle to avoid this negative effect. In addition, overdosage of BoNT-A could result in inability to chew<sup>[3]</sup>.

### **Platysmal bands**

In addition to the upper, middle, and lower face, the neck is another region of the body where BoNT treatment as a non-invasive procedure can be beneficial. The platysma muscle is the main muscle of the neck. Unlike most muscles in the body, the platysma is located just below the epidermis and dermis in the superficial layer of fascia. This location in relation to the skin is one of the reasons why this muscle is an important muscle in aging. The platysma is a muscle of facial expression with many roles, including depression of the mandible, depression of the corners of the mouth in frowning, and tensing of the skin of the neck. Treatment with BoNT-A can counteract this downward pull in order to soften the appearance of the neck and provide jawline definition<sup>[10]</sup>.

Since the platysma muscle is located in the superficial layer of fascia, it is important to ensure that the BoNT-A does not spread to the deeper muscles when injected. To prevent this spread, it is recommended that the patient contract their platysma muscle in order for the physician to grasp the muscle with his/her fingers and inject the neurotoxin at a superficial level. Injections not performed this way could result in serious complications such as dysphagia<sup>[22]</sup>.

## Facial paralysis

Facial paralysis is a cause for patient concern both aesthetically and functionally. Aesthetically, patients develop facial asymmetry from this flaccid paralysis, while functionally, patients can develop difficulty with speaking, eating, breathing, and ocular problems<sup>[3]</sup>. In addition to this flaccid paralysis, patients can experience secondary effects of facial palsy healing including hyperkinesis, which consists of static and dynamic asymmetry of the face due to hypertonia, and synkinesis, which is involuntary movement of certain groups of mimic muscles<sup>[23]</sup>. These problems not only affect the patient's ability to function in their daily activities but can also result in psychological and social problems due to their inability to clearly communicate with others.

Facial paralysis has been treated with minor operations such as brow lifts, medial canthoplasty, and orbicularis myectomy, as well as major procedures such as facial-hypoglossal anastomosis, vascular free muscle grafts, and cross-facial surgery<sup>[23]</sup>. BoNT, therefore, can be used as a less invasive method to treat facial nerve paralysis by blocking presynaptic release of acetylcholine resulting in temporary denervation. There is no standard of dosing or location for BoNT-A injection for facial paralysis; treatment is determined based on the clinical evaluation of the individual patient.

## Brow ptosis

Brow ptosis is often related to incorrect injection of BoNT in an unwanted area, leading to muscle weakness and the aesthetic appearance of an eye brow droop<sup>[24]</sup>. However, BoNT, used correctly, can actually help to relieve brow ptosis and even out the eyebrows aesthetically. Injection of 2 units of ONA at 2 to 3 mm below the lateral brow and another injection of 2 units of ONA into the corrugator at the medial brow results in elevation of the brow because of the reduced action of the depressor muscles<sup>[24]</sup> [Figure 8]. The other alternative treatment, if that eyebrow is not able to be raised, is to lower the opposite eyebrow by injecting BoNT in the brow elevators above the eyebrow. This works to achieve symmetry of the eyebrows, but should be done only if elevating the affected eyebrow is not possible<sup>[25]</sup>.

## Process of healing

BoNT has been shown in many studies to improve appearance of scar formation with a normal healing wound through the mechanism of relieving the tension of the skin at the scar region by blocking muscle contraction in that area<sup>[26]</sup>. In addition to effects on normal wound healing, there have been interesting advancements in the usage of BoNT-A for the treatment of pathological scarring, including hypertrophic scars and keloids. Pathologic scars can form from defects in any of the three overlapping processes of healing: the initial inflammatory phase, the proliferative phase with formation of scar tissue, and finally, the tissue remodeling phase<sup>[27]</sup>. Both hypertrophic scars and keloids are firm, erythematous plaques or nodules, but a main difference is that hypertrophic scars are confined to the margin of the wound, while keloids often grow beyond the boundaries of the wound<sup>[28]</sup>.

The exact mechanism of BoNT treatment for pathologic scars is still unclear, but multiple theories have been reviewed in the current literature. One of the important factors in pathologic scar formation where BoNT-A could be of benefit is the tension on the wound caused by the contraction of muscles on the wound edges, which is the same mechanism found in normal healing and scar formation<sup>[29]</sup>. Another mechanism of pathologic scarring where BoNT-A treatment could help is the increased collagen synthesis and increased metabolic activity that results due to local inflammation in the pathologic scarring processes. The injection of BoNT-A is thought to minimize the tension on the wound, allowing the maturation of collagen<sup>[30]</sup>. A final theory is fibroblast hyperproliferation in the formation of both hypertrophic scars and keloids. One study by Austin *et al.*<sup>[31]</sup> showed that BoNT-A can directly decrease fibroblastic activity through alteration of apoptotic, migratory, and fibrotic pathways in scar formation to improve the pathologic scar appearance. Although the exact mechanism for the treatment of hypertrophic scars



**Figure 8.** Injection sites above the elevated eyebrow or below the depressed eyebrow in brow asymmetry from brow ptosis

and keloids by BoNT is currently not completely understood, several studies have pointed to a potential expansion the off-label use of BoNT-A for pathologic scarring.

## COMPLICATIONS

Although there are specific complications and contraindications with usage of BoNT for specific muscle groups that were discussed above, there are general complications to be aware of regardless of where the injection is located. Locally, the complications are most commonly related to the injection site. These complications include development of pain, erythema, ecchymosis, or hematoma at the site of injection. It is often helpful to use local anesthetics and inject the neurotoxin at a slower rate to avoid these injection site complications. It is important to inform your patients that ecchymosis is common and can take up to two weeks to resolve<sup>[32]</sup>. As discussed in detail above, another common side effect is diffusion of the neurotoxin to adjacent muscle groups resulting in paralysis of those muscle groups and unwanted aesthetic as well as functional side effects. Care must be taken to ensure the toxin is injected directly into the desired muscle<sup>[33]</sup>.

Some less common complications of BoNT injection include development of a mild and self-resolving headache with facial injections, paresthesia in the area of the injection site caused by local nerve trauma, and vasovagal episodes associated with anxiety during the injection procedure<sup>[32]</sup>. It is important to be aware of these side effects in order to avoid further complications. Finally, two extremely rare complications to note are the formation of antibodies against the BoNT, which would make the treatment ineffective, and the possible immediate allergic reactions to the toxin, which would present with itching, swelling, and possible anaphylaxis<sup>[34]</sup>. For the formation of antibodies, medications that inhibit neuromuscular signaling can be used to increase the toxin's effect by offsetting the antibody reaction. In the case of an allergic reaction, standard allergy protocol is recommended, but diphenhydramine is not recommended due to its anticholinergic effects.

## CONCLUSION

BoNT has been proven to be safe and efficacious for many clinical indications in the field of facial plastic surgery. The use of BoNT for rhytids of the face has become one of the most common non-invasive procedures in facial plastic surgery with an extremely high patient satisfaction rate. In addition to the FDA approved usage of BoNT for rhytids, off-label uses, such as those described above, are becoming



increasingly common. Although there are some possible complications to be aware of, most patients do extremely well with this treatment. New uses for BoNT in this field are constantly arising and are worth exploring further.

## DECLARATIONS

### Authors' contributions

Conception and design of the work: Sherris D

Analysis and interpretation of data for the work: Favre N, Sherris D

Drafting the work and revising it critically for important intellectual content: Favre N, Sherris D

Final approval of the version to be published: Favre N, Sherris D

### Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

I (Nicole Favre) consent to use my pictures.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. Clark RP, Berris CE. Botulinum toxin: a treatment for facial asymmetry caused by facial nerve paralysis. *Plast Reconstr Surg* 1989;84:353-5.
2. Carruthers JD, Carruthers JA. Treatment of glabellar frown lines with C. botulinum-A exotoxin. *J Dermatol Surg Oncol* 1992;18:17-21.
3. Ong AA, Sherris DA. Neurotoxins. *Facial Plast Surg* 2019;35:230-8.
4. American Society of Plastic Surgeons News Release. Plastic Surgery Statistics Report. Available from: <https://www.plasticsurgery.org/news/plastic-surgery-statistics>. [Last accessed on 10 Nov 2020]
5. Berwick S, Humble Á. Older women's negative psychological and physical experiences with injectable cosmetic treatments to the face. *J Women Aging* 2017;29:51-62.
6. Huang W, Foster JA, Rogachefsky AS. Pharmacology of botulinum toxin. *J Am Acad Dermatol* 2000;43:249-59.
7. Jankovic J, Brin MF. Therapeutic uses of botulinum toxin. *N Engl J Med* 1991;324:1186-94.
8. Hexsel D, Brum C, do Prado DZ, et al. Field effect of two commercial preparations of botulinum toxin type A: a prospective, double-blind, randomized clinical trial. *J Am Acad Dermatol* 2012;67:226-32.
9. Hexsel D, Soirefmann M, Porto MD, Siega C, Schilling-Souza J, Rodrigues TC. Fields of muscular and anhidrotic effects of 2 botulinum toxin-A commercial preparations: a prospective, double-blind, randomized, multicenter study. *Dermatol Surg* 2015;41 Suppl 1:S110-8.
10. Cohn JE, Greco TM. Advanced techniques for the use of Neurotoxins in non-surgical facial rejuvenation. *Aesthetic Plast Surg* 2020;44:1788-99.
11. Rohrich RJ, Ghavami A, Crosby MA. The role of hyaluronic acid fillers (Restylane) in facial cosmetic surgery: review and technical considerations. *Plast Reconstr Surg* 2007;120:41S-54.
12. Carruthers JD, Lowe NJ, Menter MA, Gibson J, Eadie N, Botox Glabellar Lines II Study Group. Double-blind, placebo-controlled study of the safety and efficacy of botulinum toxin type A for patients with glabellar lines. *Plast Reconstr Surg* 2003;112:1089-98.
13. Beer JI, Sieber DA, Scheuer JF 3rd, Greco TM. Three-dimensional facial anatomy: structure and function as it relates to injectable

- neuromodulators and soft tissue fillers. *Plast Reconstr Surg Glob Open* 2016;4:e1175.
14. Scheinfeld N. The use of apraclonidine eyedrops to treat ptosis after the administration of botulinum toxin to the upper face. *Dermatol Online J* 2005;11:9.
  15. Fabi SG, Sundaram H, Guiha I, Goldman MP. A two-center, open-label, randomized, split-face study to assess the efficacy and safety of one versus three intradermal injection sites of abobotulinumtoxinA in the treatment of lateral periocular rhytides. *J Drugs Dermatol* 2013;12:932-7.
  16. Carruthers J, Carruthers A. Aesthetic botulinum A toxin in the mid and lower face and neck. *Dermatol Surg* 2003;29:468-76.
  17. Loos BM, Maas CS. Relevant anatomy for botulinum toxin facial rejuvenation. *Facial Plast Surg Clin North Am* 2003;11:439-43.
  18. Suber JS, Dinh TP, Prince MD, Smith PD. OnabotulinumtoxinA for the treatment of a “gummy smile”. *Aesthet Surg J* 2014;34:432-7.
  19. Sucupira E, Abramovitz A. A simplified method for smile enhancement: botulinum toxin injection for gummy smile. *Plast Reconstr Surg* 2012;130:726-8.
  20. Choi YJ, Kim JS, Gil YC, et al. Anatomical considerations regarding the location and boundary of the depressor anguli oris muscle with reference to botulinum toxin injection. *Plast Reconstr Surg* 2014;134:917-21.
  21. Kim NH, Park RH, Park JB. Botulinum toxin type A for the treatment of hypertrophy of the masseter muscle. *Plast Reconstr Surg* 2010;125:1693-705.
  22. Matarasso A, Matarasso SL. Botulinum A exotoxin for the management of platysma bands. *Plast Reconstr Surg* 2003;112:138S-40.
  23. Filipo R, Spahiu I, Covelli E, Nicastrì M, Bertoli GA. Botulinum toxin in the treatment of facial synkinesis and hyperkinesis. *Laryngoscope* 2012;122:266-70.
  24. King M. Management of Ptosis. *J Clin Aesthet Dermatol* 2016;9:E1-4.
  25. Olson JJ. Balanced botox chemodenervation of the upper face: symmetry in motion. *Semin Plast Surg* 2007;21:47-53.
  26. Gassner HG, Brissett AE, Otley CC, et al. Botulinum toxin to improve facial wound healing: A prospective, blinded, placebo-controlled study. *Mayo Clin Proc* 2006;81:1023-8.
  27. Li J, Chen J, Kirsner R. Pathophysiology of acute wound healing. *Clin Dermatol* 2007;25:9-18.
  28. Ghazawi FM, Zargham R, Gilardino MS, Sasseville D, Jafarian F. Insights into the pathophysiology of hypertrophic scars and keloids: How do they differ? *Adv Skin Wound Care* 2018;31:582-95.
  29. Kasyanjanu Carrero LM, Ma WW, Liu HF, Yin XF, Zhou BR. Botulinum toxin type A for the treatment and prevention of hypertrophic scars and keloids: updated review. *J Cosmet Dermatol* 2019;18:10-5.
  30. Xiao Z, Qu G. Effects of botulinum toxin type a on collagen deposition in hypertrophic scars. *Molecules* 2012;17:2169-77.
  31. Austin E, Koo E, Jagdeo J. The cellular response of keloids and hypertrophic scars to Botulinum toxin A: a comprehensive literature review. *Dermatol Surg* 2018;44:149-57.
  32. Small R. Botulinum toxin injection for facial wrinkles. *Am Fam Physician* 2014;90:168-75.
  33. Batniji RK, Falk AN. Update on botulinum toxin use in facial plastic and head and neck surgery. *Curr Opin Otolaryngol Head Neck Surg* 2004;12:317-22.
  34. Naumann M, Carruthers A, Carruthers J, et al. Meta-analysis of neutralizing antibody conversion with onabotulinumtoxinA (BOTOX®) across multiple indications. *Mov Disord* 2010;25:2211-8.

Review

Open Access



# A review of nonsurgical facial rejuvenation

Stephanie E. Farber<sup>1</sup>, Mathew T. Epps<sup>2</sup>, Emily Brown<sup>2</sup>, Julie Krochonis<sup>2</sup>, Rena McConville<sup>2</sup>, Mark A. Codner<sup>2</sup>

<sup>1</sup>Department of Plastic Surgery, University of Pittsburgh, Pittsburgh, PA 15261, USA.

<sup>2</sup>Department of Plastic Surgery, Mark Codner MD Plastic Surgery, Atlanta, GA 30318, USA.

**Correspondence to:** Dr. Stephanie E. Farber, Department of Plastic Surgery, University of Pittsburgh, 3350 Scaife Hall Suite 6B, Pittsburgh, PA 15261, USA. E-mail: stephanie.farber.md@gmail.com

**How to cite this article:** Farber SE, Epps MT, Brown E, Krochonis J, McConville R, Codner MA. A review of nonsurgical facial rejuvenation. *Plast Aesthet Res* 2020;7:72. <http://dx.doi.org/10.20517/2347-9264.2020.152>

**Received:** 16 Jul 2020 **First Decision:** 25 Aug 2020 **Revised:** 29 Aug 2020 **Accepted:** 25 Nov 2020 **Published:** 10 Dec 2020

**Academic Editor:** James E. Zins **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

As the demand for noninvasive facial rejuvenation continues to grow, it is imperative that plastic surgeons maintain a mastery of nonsurgical techniques for restoring a youthful facial appearance. In this article, noninvasive interventions for skin resurfacing, tissue tightening, rhytid reduction and volume restoration are discussed with an emphasis on technical outcomes and potential complications. Overall, this review should serve as a primer for the aesthetic plastic surgeon who aims to offer safe, effective facial rejuvenation to patients who desire maximal results with minimal downtime.

**Keywords:** Nonsurgical, noninvasive, facial rejuvenation, laser, injectable, radiofrequency skin tightening, ultrasonic skin tightening, skin care

## INTRODUCTION

Over the past decade, plastic surgeons have seen a dramatic increase in the demand for noninvasive rejuvenation procedures. According to American Society of Plastic Surgeons statistics, there were 15.9 million minimally invasive cosmetic procedures performed in 2018 - a 2% increase from 2017 and a 228% increase from 2000<sup>[1]</sup>. In contrast, there were 1.8 million cosmetic surgical procedures performed in 2018 - a 5% decrease from 2000. These numbers highlight the fact that the growth of noninvasive procedures outpaces that of surgical procedures. For that reason, it is critical that plastic surgeons are prepared to offer safe and effective nonsurgical rejuvenation to respond to this growing demand.



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



Part of the increase in noninvasive procedures can be attributed to the rise in new injectables and technologies offered to patients. Many of the soft tissue fillers, laser treatments, and radiofrequency- or ultrasound-based skin tightening methodologies used today were introduced within the last decade<sup>[1]</sup>. With the growing availability of novel techniques, plastic surgeons are charged with maintaining a masterful knowledge of how to deliver these interventions to optimize outcomes and minimize complications.

This article focuses on the four key age-related facial changes - diminished skin quality, increased soft tissue laxity, formation of rhytids and volume deflation or redistribution - and how each of these can be countered using noninvasive technology - skin resurfacing, soft tissue tightening, rhytid reduction, and volume restoration, respectively. The aim of this review is to arm the aesthetic plastic surgeon with the knowledge and techniques to obtain the best results in the safest, nonsurgical fashion.

## SKIN RESURFACING

### Skin care

#### *Sunscreen*

Sun exposure accelerates intrinsic skin aging by damaging dermal collagen and elastin and interfering with normal skin metabolism. Together, these derangements promote irregular pigmentation, telangiectasias, elastosis, and rhytids<sup>[2]</sup>. However, multiple studies have demonstrated that sunscreen effectively combats further progression of photoaging, while not reversing existing effects<sup>[3]</sup>. Inorganic sunscreens, specifically zinc oxide and titanium dioxide, provide superior, broad-spectrum ultraviolet light protection, increased photostability, and reduced allergenic potential compared to organic sunscreens like aminobenzoates, cinnamates, and salicylates<sup>[4]</sup>. The ideal sunscreen to offer to plastic surgery patients contains the highest percentage of nano- or micro-sized titanium dioxide and zinc oxide to allow for a cosmetically elegant application without the white appearance characteristic of traditional inorganic sunscreens<sup>[5]</sup>. Patients should be instructed to apply sun protection thirty minutes before sun exposure, reapply frequently, and use in conjunction with other sun avoidance habits<sup>[6]</sup>.

#### *Antioxidants*

Antioxidants are intended to protect skin from oxidative stress and to restore skin subjected to environmental damage<sup>[7]</sup>. Vitamin C enhances collagen production and epidermal turnover to improve skin surface appearance, while vitamin E synergistically neutralizes reactive oxygen species<sup>[8]</sup>. The addition of ferulic acid stabilizes these two antioxidants and increases their efficacy in retarding and reversing cutaneous signs of aging<sup>[9]</sup>. The ideal antioxidant should contain a blend of these ingredients and others, such as polyphenols, flavonoids, and carotenoids, in a stable formulation and in a high enough concentration to be effective without causing irritation<sup>[4]</sup>. Patients should be encouraged to use these antioxidants as part of their daily skincare routines.

#### *Retinoids*

Retinoids increase dermal collagen content, epidermal thickness, and glycosaminoglycan deposition. These histologic effects lead to increased skin smoothness and wrinkle reduction<sup>[10]</sup>. The prescription formulation, tretinoin, is limited by compliance issues due to problematic side effects such as redness, irritation, and desquamation that may cause patients to stop treatment if they are not fully educated on how to manage these issues such as with reduction in frequency of application or concentration of medication. However, while these side effects have been shown to be dose dependent, the skin rejuvenating effects are not. Therefore, patients can be prescribed the lowest concentration (0.025%) of tretinoin and still expect maximal benefit<sup>[10]</sup>. Retinol, a tretinoin precursor, must be converted to its active counterpart in the skin and is, therefore, 20-fold less effective and has 1000-fold lower dermal concentration than tretinoin<sup>[11]</sup>. Retinaldehyde, another tretinoin precursor, has similar efficacy to tretinoin with fewer side effects. However, its long-term benefits have not yet been compared to tretinoin<sup>[12]</sup>. For these reasons, tretinoin



**Figure 1.** Skincare regimen. 53-year-old Hispanic female treated with a skincare regimen which includes topical retinoid, vitamin C, and hydroquinone. Before (top) and following 5 weeks (bottom) of daily skincare treatment

remains the gold standard retinoid for skin rejuvenation and should be recommended as an adjunct to other aesthetic treatments.

An example of a patient treated with a combination regimen of a retinoid, vitamin C, and hydroquinone is included in [Figure 1](#).

#### *Acids*

Alpha hydroxy acids, such as glycolic, lactic, and citric acids, work to exfoliate the skin and interfere with epidermal cohesion, creating a more youthful skin appearance. One study demonstrated an increase in skin thickness with improved elastin quality and collagen density in patients using a combined alpha hydroxy acid formulation<sup>[13]</sup>. Another study showed a dose-dependent improvement in skin smoothness and reduction in rhytids with use of topical lactic acid<sup>[14]</sup>. Patient should be encouraged to use the topical alpha hydroxy acid with the highest effective concentration that they can tolerate without significant redness or desquamation. They should be instructed that acids should not be used with antioxidants as the combination can destabilize vitamin C and reduce its efficacy<sup>[15]</sup>.

#### *Medical facials*

Dermal infusion facials (such as those offered by HydraFacial®, Diamond Glow®, and SilkPeel®) include a combination of mechanical exfoliation, suction-based extraction, and active ingredient infusion. The exfoliation technique associated with dermal infusion facials is termed hydradermabrasion and combines microdermabrasion with pressurized application of an ingredient-containing serum. In one study comparing combined hydradermabrasion and antioxidant serum infusion with topical application of the same antioxidant serum, the treatment group was found to have increased epidermal and dermal thickness, increased antioxidant levels, decreased fine lines, decreased pore size, and decreased hyperpigmentation<sup>[16]</sup>.



Though further study is needed to support these conclusions, dermal infusion facials are a low-risk, appealing adjunct to other nonsurgical rejuvenation techniques.

## Chemical peels

### *Pretreatment*

Chemical peels are a cost-effective means of skin resurfacing that can be tailored to various patients. Prior to treatment, a Fitzpatrick analysis is critical as patients with a Fitzpatrick type of IV through VI are at highest risk of pigmentary abnormalities. These patients should be treated with extreme caution or not at all<sup>[17]</sup>.

Pretreatment increases the effectiveness of chemical peels and should be initiated four to six weeks before treatment. Tretinoin pretreatment results in more uniform frosting and more rapid reepithelialization<sup>[18]</sup>. Hydroquinone should be recommended to patients with Fitzpatrick type III or greater to reduce the risk of post-inflammatory hyperpigmentation<sup>[19]</sup>. Glycolic acid at a concentration of 10% or less is also administered daily prior to treatment in order to accelerate exfoliation. These are all stopped one week prior to treatment to avoid irritation prior to the peel. Antiviral therapy should also be given to patients with a history of herpes simplex starting two days before and continuing for ten days after<sup>[20]</sup>.

Immediately before treatment, the skin should be de-greased with acetone or rubbing alcohol. Following this, the peeling agent can be applied by facial subunit with gauze or a cotton-tipped applicator and feathered at the edges to blend with untreated areas<sup>[21]</sup>. Application to the eyes, mouth, and alar facial groove should be avoided due to the risk of erosion<sup>[22]</sup>.

### *Superficial*

Superficial chemical peels are indicated for mild skin texture abnormalities and dyschromias. They have the advantage of minimal to no downtime and are usually used serially to maximize effect. The appropriate superficial peel depth through epidermis only is achieved when a cloudy white frost on a pink background is noted. When this frosting is achieved, the peel should be neutralized if needed. Glycolic acid at a concentration of 30%-50% is superficial peeling agent that must be neutralized with water or a weak base. Jessner solution is another superficial peeling agent that does not require neutralization<sup>[23]</sup>.

### *Medium*

Medium depth peels are indicated for fine wrinkles and dyschromias. Depth of peel is appropriate when a white frost with erythematous strikethrough is observed, indicating penetration to or through the papillary dermis. At this time, glycolic acid 70% is neutralized as above and TCA 30%-50% does not require neutralization. These peels require between three to seven days of downtime during which patients should expect swelling and erythema<sup>[23]</sup>.

### *Deep*

Deep chemical peels are indicated for coarse wrinkles and deeper acne scars. Due to their depth of penetration, these peels should be administered with intravenous sedation or regional blocks. Phenol based peels, including Baker-Gordon formula must be performed with cardiac monitoring and intravenous fluids. Depth of peel through the papillary dermis into the reticular dermis is achieved when solid white frosting without background erythema is noted. A grey hue should be avoided as this indicates excessive depth of peeling. Deep chemical peels require ten to fourteen days of downtime and are associated with a more painful recovery<sup>[23]</sup>.

Hetter's solution - the combination of phenol and croton oil to increase penetration - can be performed as a superficial, intermediate or deep peel. The depth is varied by adjusting the number of swipes, amount of solution applying, or concentration of the phenol and croton oil.

Peel depth will be evident in the nature of the frost, with pink frost indicating an epidermal injury, pink-white a papillary dermal injury, and white a reticular dermal injury.

#### *Posttreatment*

Following medium and deep peels, patients are advised to moisturize frequently with petroleum-based cream. Sun avoidance should begin immediately, but application of sunscreen can resume ranging from immediately after a superficial peel to two weeks after a deep peel when re-epithelialization has occurred. Hydroquinone should be initiated by the treating physician immediately at the first sign of hyperpigmentation<sup>[23]</sup>. Milia typically respond to treatment with topical tretinoin<sup>[22]</sup>.

In addition to oral acyclovir in patients with a known history of herpes simplex, patients with prolonged, painful erythema should be treated with two doses of fluconazole out of concern for a yeast infection<sup>[24]</sup>.

### **Laser**

#### *Resurfacing*

Laser resurfacing induces an epidermal or dermal injury and regeneration, resulting in improved skin tone, effaced wrinkles, and reduced dyspigmentation. Resurfacing should be performed with caution in patients with history of scarring and Fitzpatrick type of V or greater. The pretreatment regimen is similar to the regimen outlined for chemical peels above, including tretinoin and hydroquinone, as well as acyclovir which may be used selectively for patients with a history of herpes infection or may be used for all patients. Superficial resurfacing can be performed with topical anesthetic, while deeper treatments should be performed under nerve blocks, intravenous sedation, or general anesthesia<sup>[25]</sup>. Resurfacing lasers can be categorized as ablative or non-ablative and fractionated or non-fractionated.

Non-ablative lasers have reduced downtime and lower risk, while ablative lasers have a more dramatic rejuvenating effect<sup>[21]</sup>. Ablative lasers, such as the carbon dioxide (CO<sub>2</sub>) laser and erbium:YAG (Er:YAG) laser, use water as a chromophore and vaporize treated zones, inducing collagen remodeling. CO<sub>2</sub> lasers are limited by their prolonged recovery time - occasionally up to six months - and their risk of hypopigmentation. The Er:YAG laser is more specifically absorbed by water-containing tissue and results in less collateral damage. However, because of these characteristics, its skin rejuvenating effect may be less pronounced<sup>[21]</sup>. Non-ablative lasers and non-ablative non-coherent light sources, such as intense pulsed light (IPL), pulsed-dye, and neodymium:YAG (Nd:YAG), generate heat to induce dermal injury and improve rhytids without creating open wounds. The thermal energy is hypothesized to stimulate dermal fibroblasts while keeping the epidermis cool and protecting it from injury<sup>[21]</sup>.

Lasers can also be fractionated or non-fractionated. While fractionated lasers create small columns of injury with unaffected areas between, non-fractionated lasers injure the entire treated area. Fractionated lasers can extend to a deeper level of injury while still having reduced downtime due to the uninjured skin between injured columns. However, for the same reason, multiple treatments between 2-4 weeks apart may be required to achieve the desired result<sup>[26]</sup>.

Of the combinations of laser resurfacing treatments, ablative non-fractionated lasers such as 10,600 nm CO<sub>2</sub> and 2,940 nm Er:YAG have the most dramatic effect with the greatest downtime. On the other end of the spectrum, non-ablative fractionated lasers such as 1,440 nm Nd:YAG are the lowest risk with the least downtime, but also have the most modest effect.

Non-ablative non-fractionated lasers such as 1,319 nm pulsed dye, 1,320 nm Nd:YAG and 1,450 nm diode, as well as ablative fractionated lasers such as 10,600 nm fractionated CO<sub>2</sub> and 2,940 nm fractionated Er:YAG, serve as a middle ground in terms of effectiveness, safety, and downtime<sup>[26]</sup>. Examples of patients



**Figure 2.** Skincare regimen in combination with pulsed-laser phototherapy [intense pulsed light (IPL)]. 46-year-old Fitzpatrick II Italian-portuguese female seeking perioral rejuvenation before (left) and after (right) a combination of daily skincare regimen (topical retinoid, vitamin C, and hydroquinone), hyaluronic acid filler to treat the upper lip rhytids, IPL for three sessions, microneedling to a 2 mm depth

who underwent pulsed-dye laser resurfacing using IPL, along with a combination of other noninvasive treatments, are shown in [Figures 2 and 3](#). When choosing the correct laser for an individual patient, it is important to consider the patients expectations in terms of desired effect, social downtime, and willingness to undergo multiple procedures.

Posttreatment skincare consists of petrolatum or occlusive dressings until epithelialization is complete, which is between 24-48 h for fractional treatments or a few weeks for full field treatments, at which point a nonocclusive moisturizer can be initiated. Sunblock should also be started as soon as re-epithelialization is complete. Other skin care should be initiated judiciously to prevent irritation<sup>[25]</sup>.

The most common untoward effect of laser resurfacing is erythema, which is an expected part of the healing process. Skin eruptions, such as milia or acne, can occur and usually respond to discontinuation of occlusive agents. Hypopigmentation cannot be treated but can sometimes be camouflaged by treating and reducing pigmentation in other areas. For hyperpigmentation, it can be an expected sequelae of laser treatments in patients with higher Fitzpatrick types or who have early sun exposure, as in [Figure 4](#). In these cases, it is self-limiting. If hyperpigmentation persists, it can be treated with IPL treatments. In rarer situations, burns can occur due to noncompliance with preoperative skincare and sun avoidance, as seen in [Figure 5](#). Other infections rarely occur without the use of prolonged occlusive dressings. If these do occur, they should be treated with the appropriate antiviral, antibacterial or antifungal agents<sup>[25]</sup>.

#### *Other laser indications*

In addition to resurfacing, lasers can also be employed to specifically treat pigmentary and vascular lesions, which are very common sequelae of skin aging. Flat pigmented lesions can be treated using a diode laser at 805 nm or IPL at 515 nm with melanin as a chromophore. Lesions with any concern for malignancy should not be treated with laser as this interferes with or prevents pathologic diagnosis. Vascular lesions such as telangiectasias or cutaneous angiomas can be treated with a diode at 805 nm or IPL at 560 nm using oxyhemoglobin as a chromophore. These lesions often require multiple treatments<sup>[21]</sup>.

### **Dermabrasion**

#### *Microdermabrasion*

Microdermabrasion is a technique that employs an abrasive component, usually a solid crystalline material, and a vacuum component. Though it is touted as an effective treatment for rhytids, dyspigmentation, and superficial scarring, its effects have been shown to be modest. However, it has been proven to effectively





**Figure 3.** Skincare regimen, pulsed-dye laser resurfacing [intense pulsed light (IPL)], high-intensity focused ultrasound (Ultherapy), and recurrent Botox injections to the glabella, forehead, and crow's feet. 43 yo Caucasian Fitzpatrick II presented with effects of long-term sun damage including dyschromia, rhytids, and mild skin laxity. The patient was initiated on a skincare regimen consisting of topical retinoid, vitamin C, and hydroquinone for four weeks prior to undergoing two sessions of full-face and neck high-intensity focused ultrasound followed by three treatments of pulsed-laser phototherapy (IPL), and recurrent neuromodulator injections to the glabella, forehead, and crow's feet at 3-4 month intervals. Total treatment time was approximately 19 months

increase transdermal delivery of active skincare ingredients when these modalities are combined. The main advantage of microdermabrasion is that, unlike lasers and chemical peels, it is safe in most patients including patients with higher Fitzpatrick types or who are otherwise at higher risk of pigmentary changes or scarring<sup>[27]</sup>.

The procedure begins with facial cleansing and degreasing, followed by three passes in different directions over the treated skin. The degree of dermabrasion depends on the rate of handpiece movement and strength of flow. No anesthetic is required. Following treatment, sunscreen is applied, and there is typically no social downtime. Patients are advised that they will likely undergo multiple treatments. Complications are mostly self-limiting and include erythema, irritation, and petechiae<sup>[26]</sup>.



**Figure 4.** Phototherapy-induced inflammatory hyperpigmentation (PIH). 46-year-old Fitzpatrick II Female pre-treated with our skincare regimen (Tretinoin, Vitamin C, and Hydroquinone 4%) 1 month prior to intense pulsed light (IPL) phototherapy (left) with a normal physiological hyperpigmentation response to phototherapy (right). PIH normally occurs within 6 h of IPL treatment and typically resolves at 5-7 days



**Figure 5.** Pulsed-laser phototherapy [intense pulsed light (IPL)] complication. Patient presented for IPL therapy without compliance to preoperative skincare regimen and with extended sunlight exposure over a period of 72 h prior to IPL therapy. Prior sunlight exposure resulted in a cumulative excess of UV light which resulted in a superficial burn. The burn fully resolved with a short course of topical hydrocortisone cream



### *Dermabrasion*

Dermabrasion uses an abrasive motorized wheel to create a mid-dermal wound. It is less frequently used since the advent of laser resurfacing given its increased risk of aerosolized viral particles, dyspigmentation and scarring. It is still an effective option in the treatment of deeper acne scars, but its indications in facial rejuvenation are limited<sup>[21]</sup>.

## **SOFT TISSUE TIGHTENING**

### **Radiofrequency**

#### *Mechanism*

Radiofrequency skin tightening works by transmission of thermal energy to the reticular dermis, triggering a remodeling cascade with subsequent collagen, elastin, and vascular formation. It is a non-ablative technology that specifically treats the dermis and spares the epidermis, thereby reducing scarring or pigmentary complications and speeding recovery<sup>[28]</sup>.

#### *Indications and contraindications*

Radiofrequency treatments have been shown to be beneficial for the treatment of facial wrinkles, brow lifting, periorbital wrinkles, nasolabial folds, jowls, marionette lines, jawline contouring, and neck laxity<sup>[28]</sup>. Radiofrequency skin tightening is contraindicated in patients with implantable medical devices and those with underlying healing disorders. Multiple studies have shown it to be less effective in older patients, either due to decreased capacity for dermal healing, decreased tissue hydration, or increased severity of preoperative deformity<sup>[29]</sup>.

#### *Procedure*

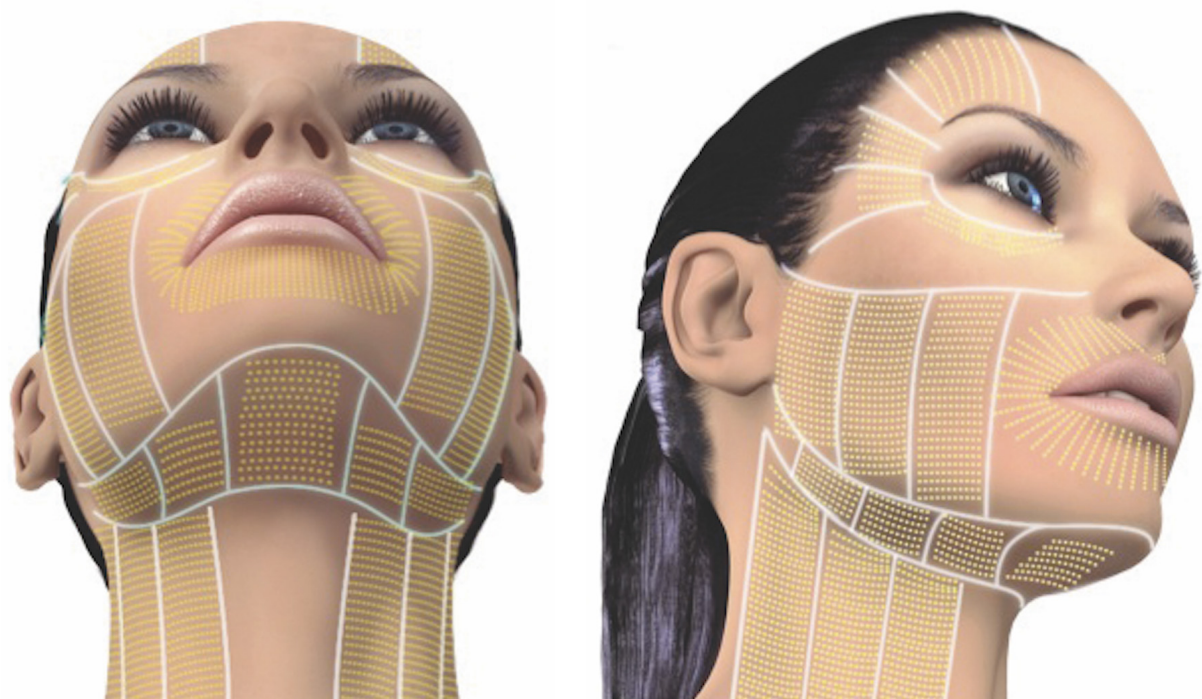
The administering practitioner must determine whether to treat in one session with higher energy or in multiple sessions with lower energy. One study showed that using lower energy over more sessions was more effective for remodeling of collagen and improvement in skin elasticity<sup>[30]</sup>.

These modalities can be administered through monopolar devices (one electrode with body grounded for unidirectional current flow through the skin such as Thermage<sup>®</sup>), bipolar devices (two electrodes - one subcutaneous and one percutaneous - with current flowing between such as Body-Tite<sup>®</sup>), and unipolar devices (one electrode without a grounding pad for omnidirectional field of effect administered subcutaneously such as Exilis<sup>®</sup>)<sup>[31]</sup>. While monopolar radiofrequency penetrates through all tissue layers and creates deeper tissue injury, bipolar radiofrequency is more localized and offers more superficial treatment, while also being less painful<sup>[28]</sup>. Furthermore, monopolar radiofrequency can be administered under topical anesthetic, while unipolar and bipolar usually require tumescent or general anesthesia<sup>[31]</sup>.

Fractional radiofrequency treatment or radiofrequency microneedling (such as Profound RF<sup>®</sup> and Morpheus<sup>®</sup>) uses microneedle electrodes to deliver fractional radiofrequency energy directly to the reticular dermis, while protecting adnexal structures<sup>[28]</sup>. The ablative effect of the microneedling allows for both a resurfacing and tightening effect and can be beneficial in patients who require treatment of both these signs of aging and are willing to accept a longer recovery than traditional, non-ablative radiofrequency treatments.

#### *Outcomes and complications*

Patients should be instructed that full results of treatment can be expected within six months to one year. The most common adverse events are swelling, numbness, and bruising which generally resolve within 1-2 months and can be treated expectantly<sup>[32]</sup>.



**Figure 6.** Ultherapy treatment areas. Markings demonstrating the areas and individual yellow ultrasound energy points treated to induce lifting and tightening for the neck, submental region, and cheeks

## Ultrasound

### *Mechanism*

Ultrasound-based skin tightening (such as Ultherapy<sup>®</sup>) uses a lower energy, microfocused ultrasound to heat tissue through vibration and friction. This heating produces small coagulation points in the reticular dermis to induce remodeling, while sparing the overlying papillary dermis and epidermis. Like radiofrequency skin tightening, this epidermal sparing reduces the risk and recovery time associated with this procedure. Unlike radiofrequency skin tightening, however, microfocused ultrasound energy can heat deeper tissues without heating the skin, allowing for transmission of higher energies and penetration to the superficial muscular aponeurotic system and platysma<sup>[33]</sup>. It also allows for direct visualization of treated tissues during treatment, adding an additional level of safety<sup>[34]</sup>.

### *Indications and contraindications*

Ultrasound skin tightening is approved for the eyebrows, chin, neck and chest. The ideal patient has mild to moderate skin laxity and does not have any underlying condition that would inhibit wound healing<sup>[34]</sup>. Because treatment in higher Fitzpatrick types has not been thoroughly studied and because two cases of hypopigmentation have been reported, ultrasound based skin tightening should be used with caution in patients with Fitzpatrick type V or VI<sup>[35]</sup>. Like radiofrequency skin tightening, microfocused ultrasound has been shown to be less effective in older patients with more severe age-related deformities<sup>[33]</sup>.

### *Procedure*

The procedure is performed following application of topical anesthetic. Given the moderate to severe pain associated with this treatment, some options for additional anesthetic include nerve blocks, nitrous oxide gas, and oral anxiolytics. Focal depth is visualized on the monitor and calibrated to correspond to the layer intended to be treated before delivering a linear array of ultrasound pulses. Most therapy platforms have pre-programmed protocols for specific treatment areas, as shown in [Figure 6](#), and treatment times vary from 30-60 min per region<sup>[34]</sup>.



**Figure 7.** High-intensity focused ultrasound (Ultherapy). 64-year-old African American female before (left) and 2 months following (right) High-intensity focused ultrasound therapy to the neck, cheeks

#### *Outcomes and complications*

Following treatment, patients should expect swelling, erythema, and transient bruising, all of which are self-limiting. Complete results can usually be expected within six months<sup>[33]</sup>. Studies report that greater than 80% of patients and providers note an improvement following treatment<sup>[34]</sup>. An example of a patient treated with high-intensity focused ultrasound is included in Figure 7. Other examples of patients who underwent a combination of ultrasonic skin tightening and skin resurfacing is shown in Figures 8 and 9.

Rarely, patients can develop linear patterns of skin discoloration that resolve within a few weeks. There have been reports of motor nerve paresis, specifically in the temporal and marginal mandibular branches of the facial nerve, but these have all resolved within six weeks<sup>[36]</sup>.





**Figure 8.** Fractionated bipolar radiofrequency sublative skin resurfacing (eMatrix) and high-intensity focused ultrasound (Ultherapy). 56 yo Caucasian Fitzpatrick III with a history of lower lid blepharoplasty and full-face TCA peel presented with fine facial rhytids and mild facial ptosis. Before (top) and after (bottom) full face fractionated bipolar radiofrequency skin resurfacing (eMatrix) and high-intensity focused ultrasound (Ultherapy) to the neck, face, and brow

## Infrared light energy

### *Mechanism*

Of the noninvasive skin tightening modalities, infrared technology (such as SkinTyte<sup>®</sup>) is the least well studied. The procedure works by delivering infrared energy via broadband light to create heat in the dermis and initiate collagen remodeling.

### *Indications*

Unlike ultrasound-based skin tightening, infrared energy does not penetrate to the deeper subcutaneous tissues. Therefore, it is indicated only in mild forms of skin laxity that do not involve the underlying tissue. Similar to other modalities, it should be used with caution in patients with higher Fitzpatrick types.

### *Procedure*

Typically, infrared skin tightening is not painful enough to require any form of anesthetic. It is performed by heating individual treatment zones to a particular temperature by performing a series of treatment cycles and skin temperature measurements. The skin is then allowed to cool or cool compresses are applied<sup>[37]</sup>.



**Figure 9.** Skincare regimen, fractionated bipolar radiofrequency subablative skin resurfacing (eMatrix) and high-intensity focused ultrasound (Ultherapy). 48 yo caucasian Fitzpatrick II, before (upper top, lower top) and after (upper bottom, lower bottom), a series of three full-face fractionated bipolar radiofrequency subablative skin resurfacing treatments, a single full-face high-intensity focused ultrasound treatment, 1 mL Hyaluronic acid filler to the nasolabial folds, and recurrent injections of neuromodulator to the glabella, forehead, and crow's feet. Marked improvement in skin texture, dyschromia, and fine rhytids are seen. Treatments occurred for a period of 12-months

### *Outcomes and complications*

Usually patients experience mild discomfort post-treatment. Full results should be expected after three to six treatments performed at two- to four-week intervals. Results are variable and outcome and complication data is not well published<sup>[37]</sup>.

## **Microneedling**

### *Mechanism*

Microneedling (such as with Skinpen<sup>®</sup> and Dermapen<sup>®</sup>) is performed by breaching the epidermis with micron-sized needles to create an injury to the outermost layer of the skin. These micro-punctures are touted as a means of injuring the skin and causing elastin and collagen production and deposition<sup>[38]</sup>.





**Figure 10.** Skincare regimen and microneedling (x3). 61 yo Caucasian female Fitzpatrick I skin before (left) and after (second from left) skincare regimen (topical retinoid, vitamin C, hydroquinone) with 1 mL hyaluronic acid filler to the lips, followed by microneedling procedures. Shown at one-week post-needling (middle), two-week post-needling (second from right), and three-week post-needling (right). Total course of treatment was approximately 12 months

### Indications

Because the treatment is so superficial, it has only been shown to treat very superficial rhytids<sup>[39]</sup>. A newer application of microneedling is for drug delivery, which will be discussed in a later section.

### Procedure

The procedure is performed by cleansing and applying a topical anesthetic. The microneedling device is applied to the skin in a perpendicular fashion and the device is moved in multiple directions to evenly distribute passes. Uniform, pinpoint bleeding is the endpoint of the procedure, at which point in time a moisturizing sunblock is applied routinely for at least forty-eight hours or indefinitely<sup>[40]</sup>.

### Outcomes and complications

Treatments can be repeated every two to four weeks until desired effect is achieved<sup>[40]</sup>. As seen in [Figure 10](#), effects are modest secondary to the superficial level of injury. However, this superficial penetration also reduces the downtime and risk profile associated with this treatment, with the primary adverse effects being bruising, erythema, and irritation that are self-limiting<sup>[41]</sup>.

## RHYTID RELAXATION OR REDUCTION

### Neuromodulators

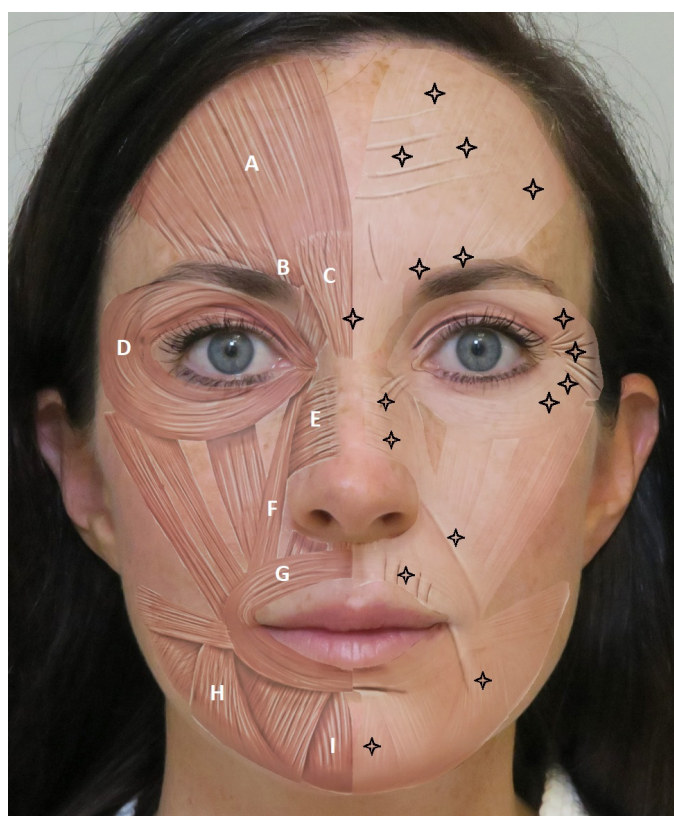
Botulinum toxin is the most commonly used nonsurgical facial rejuvenation modality in the United States<sup>[1]</sup>. The toxin works to temporarily paralyze facial muscles, thereby eliminating dynamic rhytids and reducing static ones. Multiple formulations of the toxin are commercially available currently (e.g., Botox<sup>®</sup>, Dysport<sup>®</sup>, Xeomin<sup>®</sup>, Javeau<sup>®</sup>), with additional preparations set to be released (e.g., Daxi). The principle difference in these products is their content of proteins that may cause sensitivity or resistance over time. A meta-analysis of studies comparing three formulations of botulinum toxin failed to find any significant difference in treatment response but concluded that further comparison study is needed<sup>[42]</sup>.

Botulinum toxin treatment is generally safe, but is contraindicated in patients who have allergies to eggs or albumin, who have an infection at the injection site, and who are pregnant or breastfeeding. Relative contraindications include use of anticoagulation given the presumed elevated bleeding risk, underlying neuromuscular disorder given the possibility of increasing severity, or blepharoptosis given the risk of worsening with frontalis paralysis<sup>[43]</sup>.

A general schematic of botulinum toxin injection sites is included in [Figure 11](#) with additional detail below<sup>[44,45]</sup>.

### Upper face

Forehead and periorbital botulinum toxin injections should be performed with careful attention to brow morphology, as each injection will have a resulting impact on brow position. An illustration of how each muscle impacts brow position is shown in [Figure 12](#).

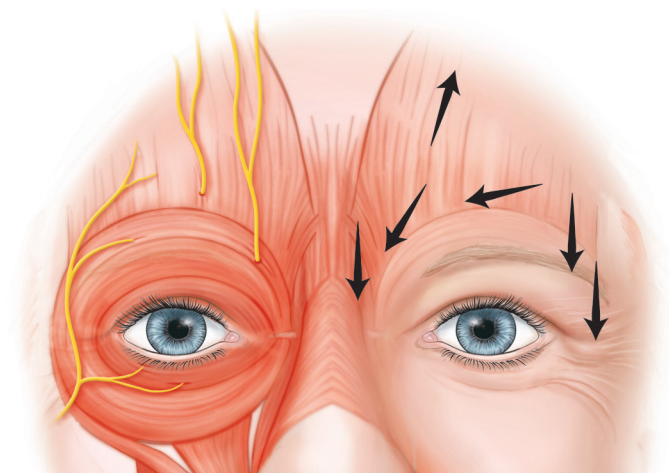


Muscle/muscle group	Muscle action	Static/dynamic rhytids
Frontalis (A)	Elevation brows	Horizontal forehead lines
Glabellar muscles: Corrugator supercilii (B) Procerus (C)	Medial Elevation of brows Depressor of brows	Frown lines, glabellar creases
Orbicularis Oculi (D)	Elevation of eyelids, concentric tightening of periorbital tissue	Crow's feet
Nasalis (E)	Medial elevation of nasal skin	Bunny lines
Levator labii superioris alaeque nasi (F)	Elevation of the central lip	Gummy smile, nasolabial folds
Orbicularis oris (G)	Lip pursing, lower lip and corner of mouth depressor	Radial lip lines, smoker's lines
Depressor anguli oris (H)	Corner of mouth depressor	Down turned smile, marionette lines
Mentalis (I)	Elevation of chin and lower lip	Dimpled chin, Chin lines
♦Common Neuromodulator Injection Sites		

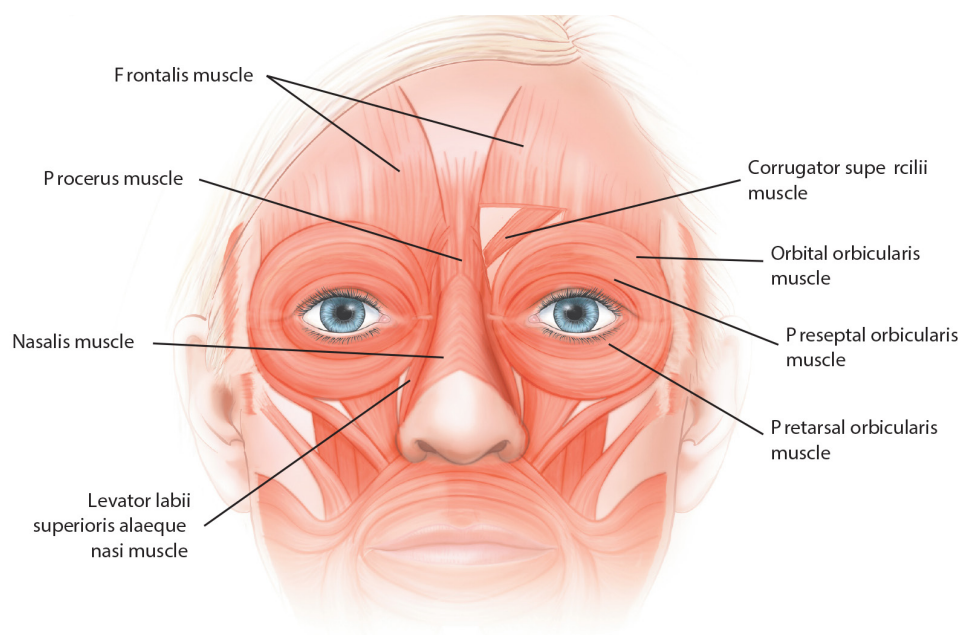
**Figure 11.** Muscles of facial expression, their actions, corresponding wrinkles, and points of neuromodulator injection<sup>[44]</sup>

The frontalis, which creates transverse forehead rhytids, as seen in [Figure 13](#), should be injected in 1.25-2 unit aliquots, depending on the injector's preferred dilution. Injections should be deep given the anatomy of the frontalis muscle and should be planned based on the individual patient's forehead rhytid pattern. To maintain an aesthetic brow shape, injections should not extend too inferiorly or laterally to preserve brow height and arch.

The glabellar muscles, which create mid-brow rhytids, as seen in [Figure 14](#), include the corrugator and procerus. These can be injected in a V pattern with 4-5 units of botulinum toxin per injection. The plane of injection should be deep in order to preserve frontalis in this area. The specific injection pattern should be titrated to the individual patient's rhytid morphology when animating.

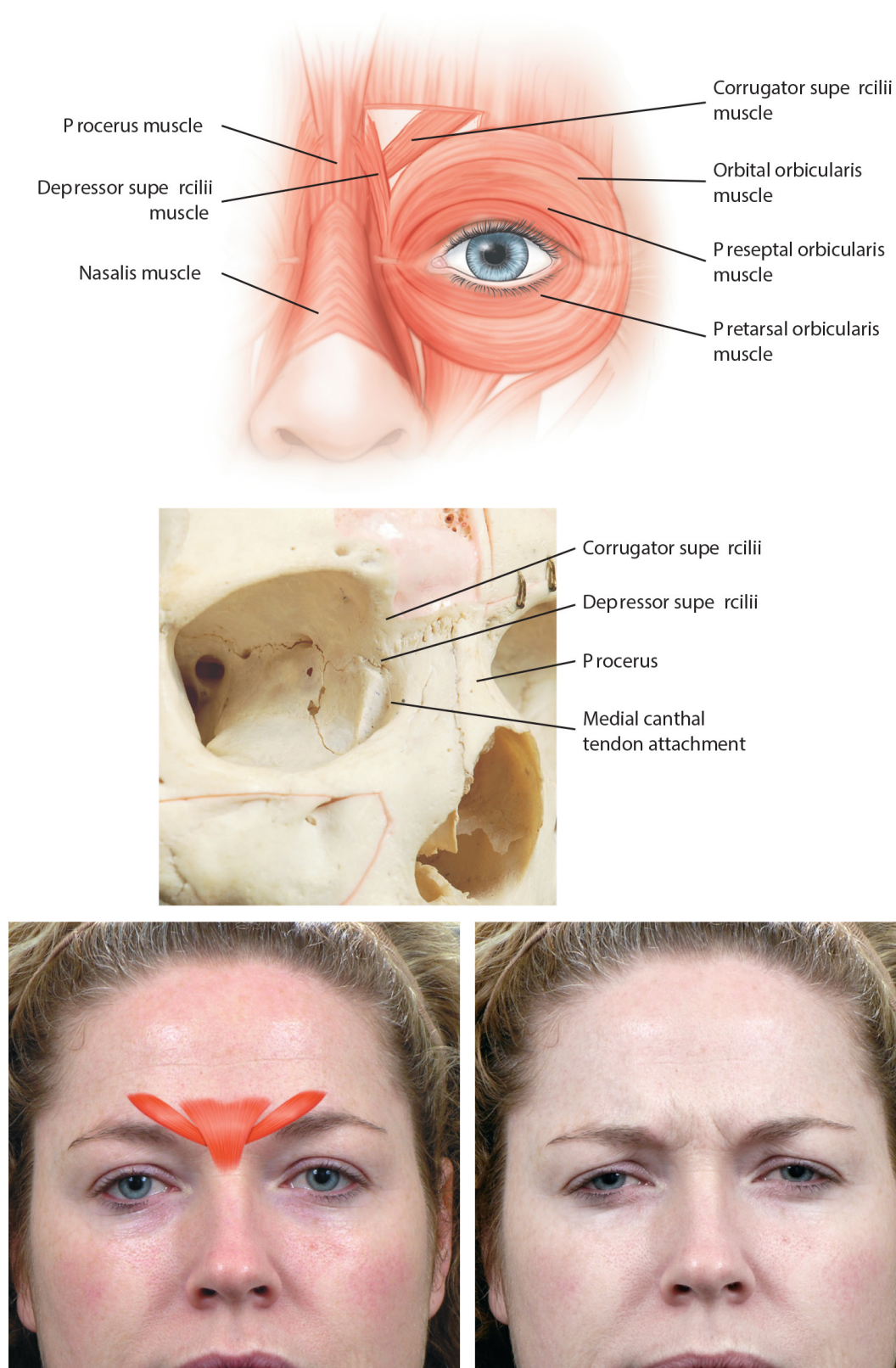


**Figure 12.** Upper face muscle pull on eyebrow. An illustration of the direction that each upper facial muscle pulls on the eyebrow, showing the medial glabellar muscles exerting a downward force, the frontalis exerting an upward force, and the orbicularis oculi exerting a downward force

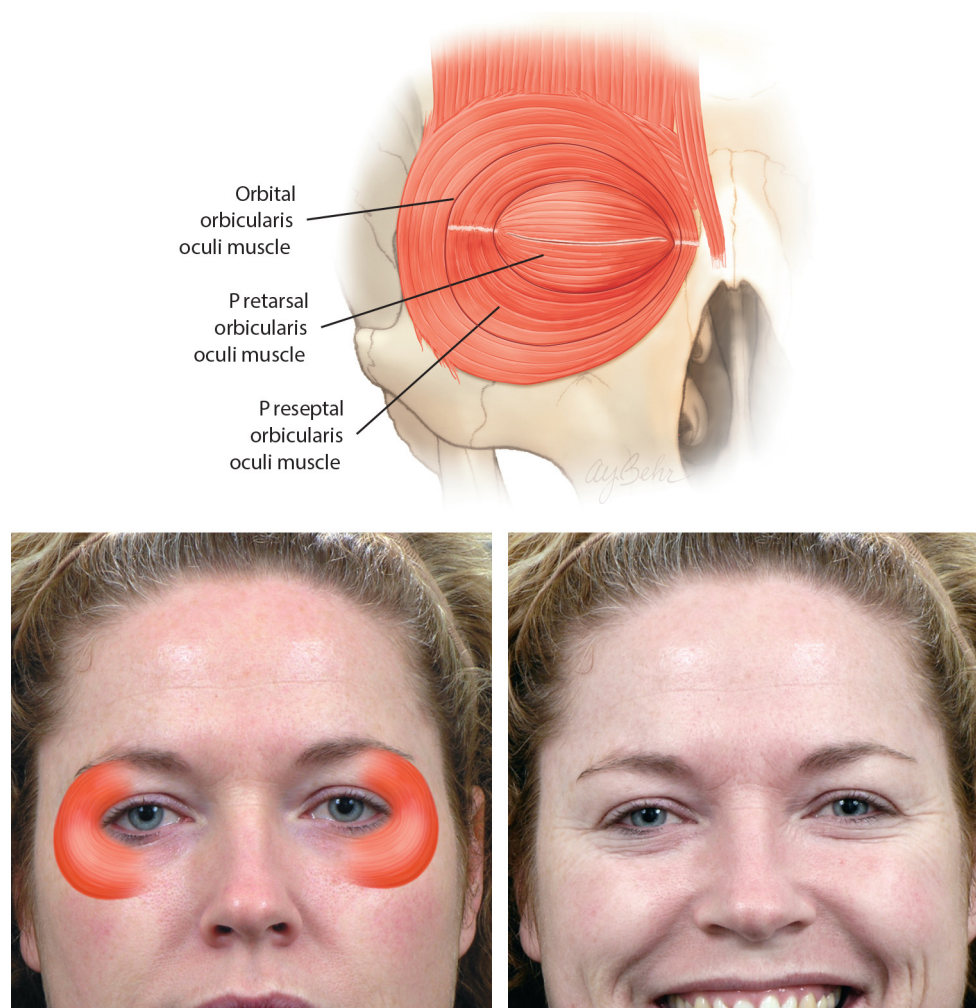


**Figure 13.** Frontalis muscle anatomy. Facial muscle anatomy (top) and morphology, as seen with animation (bottom)





**Figure 14.** Glabellar muscle anatomy. The corrugator and procerus are seen anatomically (top) with their bony attachments illustrated (middle) and their functional rhytid pattern (bottom)



**Figure 15.** Orbicularis oculi anatomy. Anatomic drawing of the orbicularis oculi muscle (top) and functional anatomy showing dynamic rhytids (bottom)

The orbicularis oculi, [Figure 15](#), should be injected very superficially to target the muscle and avoid spread to the levator palpebrae superioris. These injections serve to obliterate lateral orbital rhytids and to raise the lateral brow. Injections should be performed in 1-2 unit aliquots in three to five sites. Patients with very active muscles may require a second row of injections<sup>[46]</sup>.

Complications of upper facial botulinum toxin injections are undesirable but reversible based on the temporary effect of treatment. Brow asymmetries or overly peaked brows can be managed with additional, judicious injections. Blepharoptosis, however, is not reversible for the duration of treatment effect and can only be mitigated with alpha-adrenergic activation of Muller's muscle to temporarily elevate the eyelid (e.g., with apraclonidine drops). Other adverse effects such as swelling, bruising, or redness are self-limiting<sup>[43]</sup>.

#### *Lower face and neck*

Botulinum toxin injection in the lower face and neck is more difficult and less reliable than in the upper face. Therefore, knowledge of anatomy is critical for using this treatment safely and effectively.

Treatment of the orbicularis oris softens fine vertical perioral rhytids that occur with animation. It is performed by injecting very superficially in two points on the superior and inferior lip at the vermilion



border. Ideally, a small amount and volume of product should be injected to minimize diffusion (e.g., less than 0.1 mL of concentrated toxin). It will not treat the static rhytids that occur due to chronological aging or smoking as these are better managed with resurfacing treatments.

Injection into the depressor anguli oris raises the oral commissures. Injections should be performed a few millimeters above the mandibular border just anterior to the jawl at two sites per side, each with 2-2.5 units.

The mentalis muscle can be addressed to reduce its cobblestoning effect on the chin. It is injected at one point per side, five millimeters lateral to midline and one centimeter above the tip of the chin using 2-2.5 units per injection.

Injection of the platysma muscle softens platysmal bands. Usually several (up to ten) superficial injections of 2-2.5 units are required in a vertical line on each side at the anterior border of the muscle with one to two centimeters between injection sites. This technique is facilitated by pinching the muscle between the injector's fingers. Treatment of this area can also help to elevate the oral commissures.

Adverse effects of botulinum toxin in the lower face can be debilitating and are difficult to treat. These can include smile asymmetries, speaking difficulties, drooling, and dysphagia. Safe injection technique and mastery of the relevant anatomy will help to reduce the risk of complications<sup>[47]</sup>.

## VOLUME RESTORATION OR REDISTRIBUTION

### Restoration

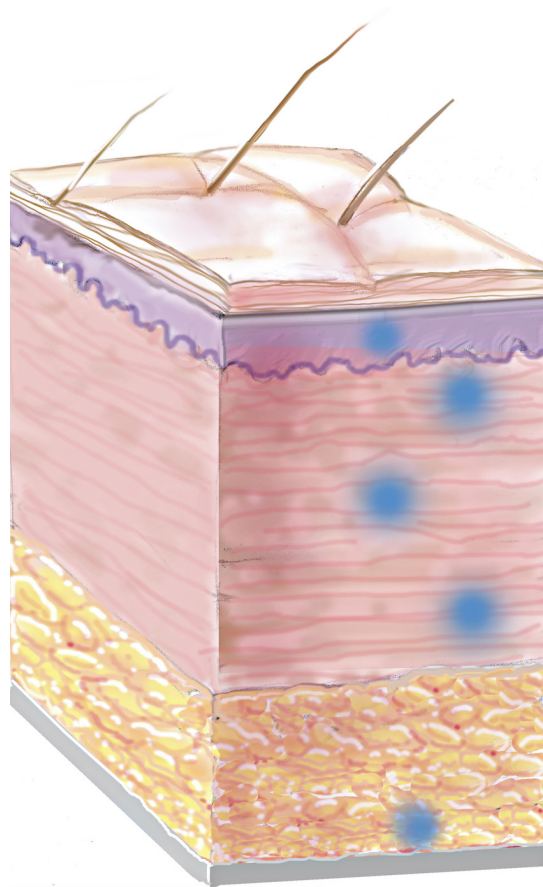
#### *Fillers*

Injectable soft tissue fillers can be either resorbable (hyaluronic acid and poly-L-lactic acid) or non-resorbable (calcium hydroxyapatite and polymethylmethacrylate). Due to its enzymatic reversibility in the face of adverse outcomes, hyaluronic acid is the most commonly used filler. Its degree of cross-linking determines its viscosity and indications. Specifically, the more highly cross-linked formulations are more viscous and are indicated for deeper injections, whereas the less cross-linked formulations are thinner and indicated for more superficial injections<sup>[48]</sup>. A schematic of the layer of injection for higher and lower degrees of cross-linking is shown in [Figure 16](#).

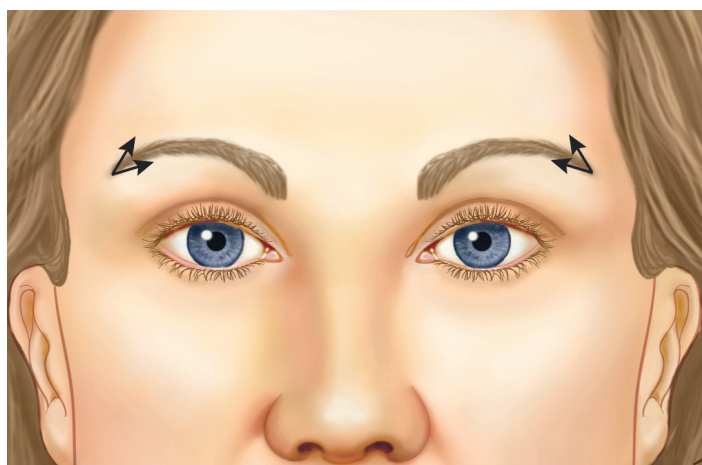
In the upper face, filler can be used to elevate the lateral brow by injecting into the deep tissue of the lateral eyebrow at the inferior aspect of the supraorbital rim using 0.25-0.5 mL of product [\[Figure 17\]](#). It can also be injected into the upper lid sulcus deep to the orbicularis to correct an A-frame deformity<sup>[49]</sup>. The tear trough is more difficult to correct due to the thin skin and high risk of discoloration or contour irregularities. The safest method to treat this area is to place a 0.25 mL aliquot of a low cross-linked filler along the preperiosteal infraorbital rim rather than injecting directly into the tear trough. Various injection strategies for correction of the tear trough deformity are shown in [Figure 18](#). All areas are massaged following injection to create a smooth, even contour<sup>[48]</sup>.

In the midface, cheek augmentation can be achieved by performing small volume injections along the zygoma at the level of the periosteum beginning lateral to the midpupillary line with subsequent massage to smooth the filler. In this area, a more highly-crosslinked filler should be used given the depth of injection. Nasolabial folds are treated by injecting the mid to deep dermis with a medium cross-linked filler<sup>[49]</sup> [\[Figure 19\]](#).

In the lower face, the lips can be injected to create a more full, everted youthful appearance. Many patients require small aliquots of local anesthesia or nerve blocks for this procedure. Lip augmentation can be performed using many techniques, and the best is one that is tailored to the patient's unique anatomy and asymmetries. A lower viscosity filler should be used and aesthetic lip proportions should be respected,

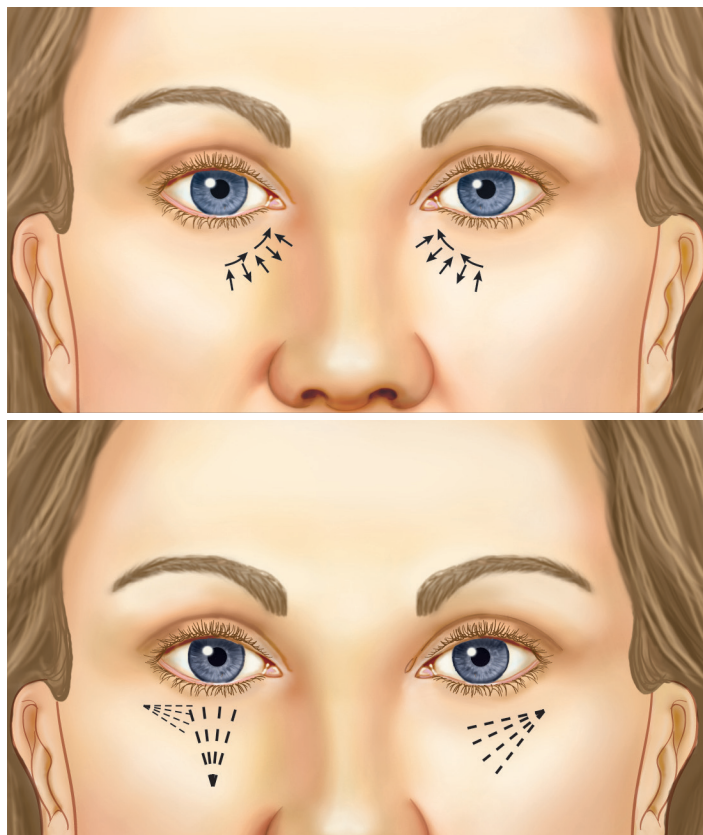


**Figure 16.** Filler injection depths. Blue areas illustrate depths of filler injection from intradermal (top), to various levels of intramuscular (middle three), and preperiosteal (bottom)

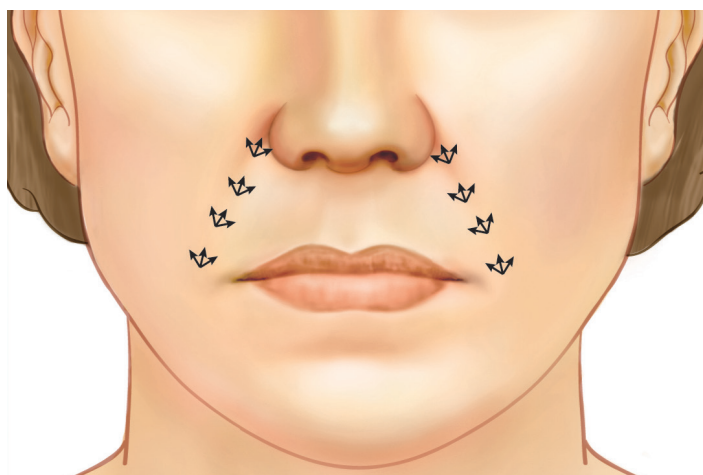


**Figure 17.** Filler for lateral brow elevation. Location and direction of lateral brow filler injections for brow elevation

injecting along the white roll, within the vermillion and below the commissure. Some patients also benefit from philtral injections. Marionette lines and the pre-jowl sulcus can be treated by a superficial subcutaneous injection of a medium cross-linked filler material. Finally, a recessed chin can be augmented using a supraperiosteal bolus of a highly cross-linked filler material at three sites across the lower chin<sup>[50]</sup>.

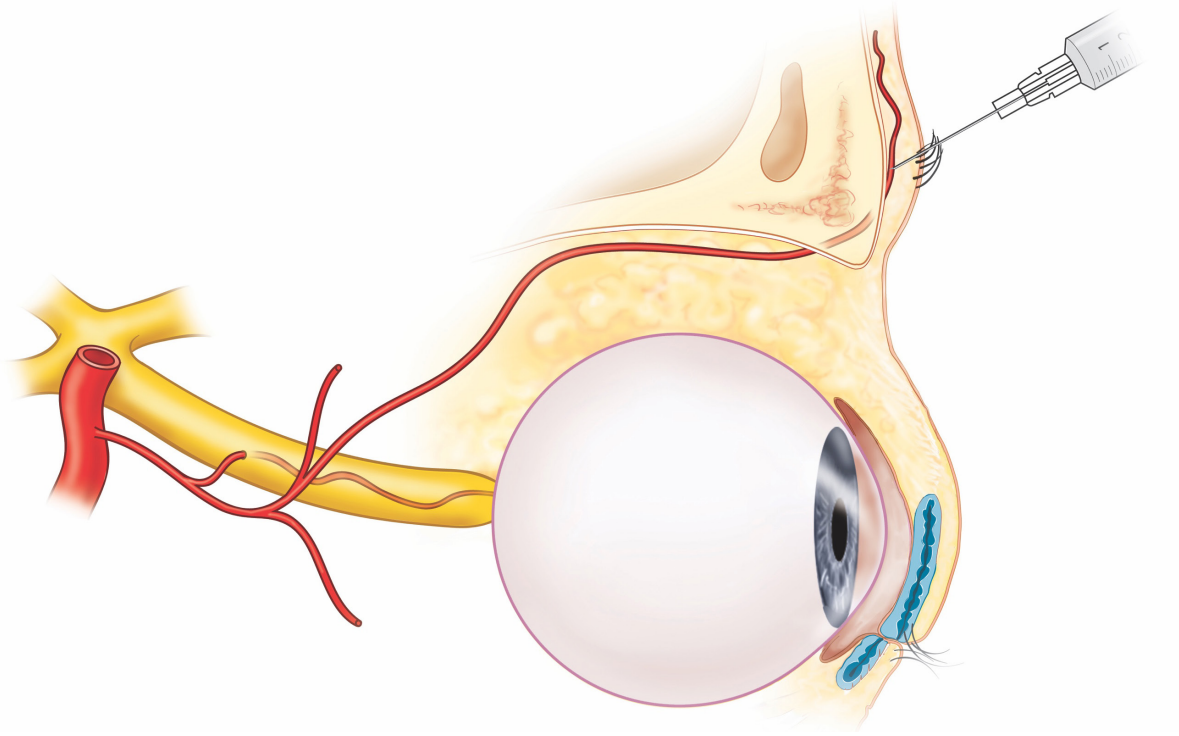


**Figure 18.** Tear trough filler injection strategies. Various strategies for using filler to correct the tear trough deformity are shown including injection along the tear trough and along the infraorbital rim (top) and within the volume defect in the midcheek (bottom)



**Figure 19.** Nasolabial fold filler injection. The directions and pattern of injection for correcting nasolabial fold

Mild adverse events following filler injection include bruising, edema, and erythema that are self-limiting. A herpes flare is a more concerning reaction, which highlights the importance of thorough preoperative history taking to determine the need for pre- and postprocedural prophylaxis. Nodules may form if too much filler is injected in one area or if highly cross-linked fillers are placed too superficially. A trial of massage can be initiated followed by dissolution of the nodule with hyaluronidase (20 units of enzyme for every 0.1 mL of filler). Most of the dissolution should occur within a few minutes of injection. Vascular



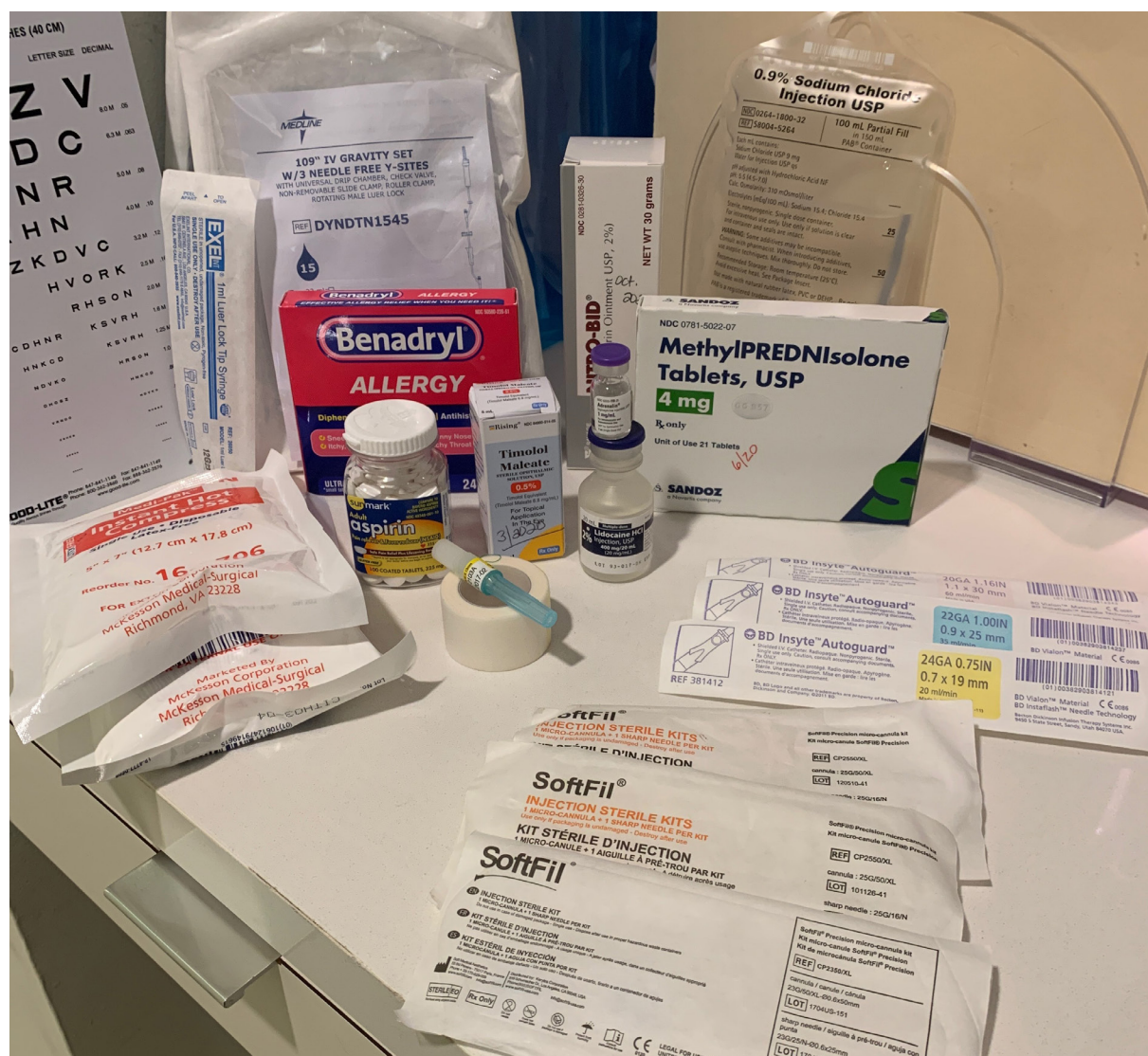
**Figure 20.** Mechanism of filler-induced blindness. An example of how retrograde arterial flow after injection into an ophthalmic artery branch can cause blindness. This phenomenon can also occur with injection of other periorbital vessels

occlusion resulting in either soft tissue loss or blindness is a rare but known complication of filler injection. This devastating outcome is minimized by safe injection techniques (aspiration prior to injection, avoidance of anatomic areas that risk vascular compromise via compression or embolization, and avoidance of injecting large volumes). However, if this complication does occur, early detection is key with immediate hyaluronidase injection if a hyaluronic acid filler was used at the first sign of color or sensory change or onset of pain. The treatment algorithm involves 500 units per anatomic unit every hour until resolution of soft tissue changes are noted. Blindness is the most feared complication of fillers and occurs due to retrograde arterial flow after injection into an ophthalmic artery branch [Figure 20]. If this should occur, there is no known consistent means of reversing filler induced visual changes. Retrobulbar hyaluronidase has been suggested, but it is a controversial means of treatment, and its use in unpracticed hands is ill-advised. Yet, immediate ophthalmologic evaluation to reduce intraocular pressure and encourage return of blood flow is recommended<sup>[48]</sup>. A photo of the senior author's in-office filler emergency kit is included [Figure 21]. Because the success of hyaluronidase injection for clearing an intravascular occlusion is not certain, prevention of embolism is critical. The careful injector should aspirate prior to injection to ensure the needle is not intraarterial, move the needle during injection to avoid injecting a large volume in one area, and apply only gentle pressure to avoid forcing filler into a false, undesired plane.

#### *Fat grafting*

Fat grafting is another tool for facial rejuvenation and volume restoration. It has the advantages of availability and cost effectiveness, but is limited by unpredictable ultimate retention. Many of the injection techniques are similar to those used for fillers. However, special consideration must be given to the harvesting and processing techniques that optimize fat graft retention and to the methods of minimizing complications specific to fat grafting.





**Figure 21.** Emergency Vascular Occlusion/Ischemic Event Kit. Having both a protocol and Emergency Vascular Occlusion Kit (above) is a necessity in any practice utilizing injectable fillers. Staff should be familiarized with the protocol(s) and location of emergency supplies. Listed are the contents, at a minimum, included in the senior author's vascular occlusion emergency kit: hot pack(s), nitroglycerin paste, aspirin (325 mg), medrol dose pack, hyaluronidase (750 units), and a 25-gauge two-inch microcannula

When harvesting fat for grafting, a careful technique should be used to minimize adipocyte trauma and maximize viability. Although harvest location has not been shown to impact viability, ultrasound- or power-assisted liposuction should be avoided due to the trauma inflicted upon adipocytes. Fat is then prepared by gravity separation, centrifugation or Telfa rolling - none of which have been shown to more or less effective for increasing ultimate graft retention as long as centrifugation is limited to less than 3000 rpm and less than three minutes. Fat should be injected using 18-20 gauge blunt-tipped cannulas and in small 0.1 mL aliquots to reduce the risk of fat necrosis or nodule formation<sup>[51]</sup>. In the periorbital region, injection should be performed deep to the orbicularis and overcorrection should be avoided. Suborbicularis injections are critical as injection just deep to the skin will result in visible contour abnormalities and nodule formation<sup>[52]</sup>.

The most common complication of fat grafting, particularly periorbital fat grafting, is contour abnormality that is visible or palpable. Liposuction is rarely a successful management strategy. Triamcinolone injections



can be attempted but direct excision may be necessary. Vascular compromise can occur, similar to synthetic fillers, but is more difficult to treat as no enzymatic digestion is available<sup>[52]</sup>. Importantly, patients should be counseled that multiple treatments may be needed to achieve the desired effect.

## Reduction

### *Deoxycholic acid*

When performing facial rejuvenation, volume reduction is most commonly indicated in the submental region. Deoxycholic acid (Kybella<sup>®</sup>) is an injectable agent that disrupts adipocyte cell membranes and is used for noninvasive fat reduction.

Deoxycholic acid injections are indicated for the pre-platysmal fat, but should not be injected deep to the platysma. They can be performed in men or women and do not require anesthesia. The ideal patient has good skin elasticity in order to expect skin retraction following the procedure<sup>[53]</sup>.

When injected into the submental fat, an average of three to four rounds of injections are needed with decreasing amounts of product and a reduction of 92.8% of submental fat volume is achieved<sup>[53]</sup>. Injections are performed in a pre-marked grid with approximately two milligrams of product per square centimeter, amounting to up to fifty injection sites.

Risks of the procedure include swelling, which occurs within 48 h of the procedure, lasts an average of 2 weeks, and results in an average 8.68% volume increase. In spite of this post-procedure swelling, patient satisfaction was significantly improved with deoxycholic acid injections. Other reported risks include nerve paralysis, dysphagia, dysphonia, and wound formation, but these are rare and reversible<sup>[53]</sup>.

### *Cryolipolysis*

Submental fat reduction can also be achieved through cryolipolysis. Cryolipolysis (CoolSculpting<sup>®</sup>) works by controlled cooling to selectively damage adipocytes while sparing surrounding tissue.

Prior to treatment, the area is cleaned and a gel is applied. The procedure is then performed using a small applicator intended for the submental region and lasts approximately forty-five minutes. Massage is required following treatment to rewarm and reshape the tissue which will be significantly swollen immediately upon probe removal<sup>[54]</sup>.

Studies of submental cryolipolysis demonstrated a volume reduction of 20.3% that was stable by 6 weeks following treatment. Seventy-six percent of patients were satisfied with the results of their treatment<sup>[54]</sup>. Patient selection is critical in this regard as patients with significant overlying skin laxity will have worsened laxity following fat dissolution.

Adverse events following cryolipolysis most commonly include erythema, edema, and paresthesias. All of these are self-limiting. There have been cases of paradoxical fat hypertrophy following treatment, but these cases are rare, affecting approximately 1% of patients. Risk factors for this complication include male gender, history of cryolipolysis, and Hispanic origin. Other risk factors, such as abdominal region and large handpiece, do not apply to the submental region<sup>[55]</sup>.

## OTHER TECHNIQUES

### **Platelet rich plasma**

#### *Mechanism*

Platelet rich plasma (PRP) is an autogenous solution derived from a sample of a patient's own blood. It is theorized to work to promote wound healing by releasing secretory granules that contain growth factors to induce tissue regeneration and collagen formation that, altogether, increase skin thickness<sup>[56]</sup>.

### *Indications*

PRP has been used in facial rejuvenation to reverse skin aging and to supplement fat grafting for increased retention<sup>[57]</sup>. There have been seven studies of PRP for rejuvenation of aging facial skin. These studies varied in their number of treatments (one treatment or three treatments every two to four weeks) and in their sites of intradermal injection. Findings included improved skin texture and elasticity, decreased rhytids, improved skin color, improved fullness, and normalization of pigmentation<sup>[57]</sup>. Given the heterogeneity of these studies, variable samples sizes, and short follow up duration, additional studies are needed.

There have also been seven studies of PRP supplementation of fat grafting. The ratio of fat to PRP in these studies varied widely from 2:1 to 10:1. Injection sites included nasolabial folds, malar region and temporal region. Most of these studies evaluated results after only one treatment. Results were variable with some reporting aesthetic improvement and others reporting increased resorption. Of note, these studies did not have a control group so results should be interpreted with caution<sup>[57]</sup>.

### *Procedure*

If planning to offer PRP for either of these indications in one's practice, it is important to realize that standardized, evidence-based protocols have yet to be developed. Of the available articles on PRP in aesthetic surgery, the volume of blood drawn, PRP isolation procedure, and PRP yield varied widely. Therefore, each surgeon is encouraged to determine the protocol that is most efficient and effective in his or her practice<sup>[57]</sup>.

In terms of complications, patients may experience self-limiting redness, swelling, or bruising, but there were no reported serious complications secondary to PRP harvest or injection<sup>[57]</sup>.

## **Microinjection devices**

### *Mechanism*

Microinjection (e.g., Aquagold<sup>®</sup>) is the combination of microneedling, which was discussed previously, and the delivery of active ingredients such as hyaluronic acid, peptides, and other active ingredients. By injuring the stratum corneum, this technique is theorized to allow for more efficient drug delivery into the deeper layers of the dermis where they can exert their intended effect.

### *Indications*

Hyaluronic acid microinjection is intended to increase dermal thickness and skin quality. Peptide microinjection is beneficial as peptides cannot naturally permeate the skin barrier. These peptides have various applications including stimulation of collagen production. However, both these indications are theoretical and have yet to be fully studied<sup>[38]</sup>. Other skincare ingredients, such as antioxidants and retinols, have also been hypothesized to benefit from microinjection. Early studies have confirmed their effective delivery. However, the benefit of this system is yet to be confirmed<sup>[58]</sup>.

### *Outcomes*

While this technique requires further study, the use of microneedling with drug delivery or with subsequent application of active skincare ingredients has been shown to increase the penetration of these agents and is a relatively safe procedure with risks including irritation, erythema, swelling and bruising that are self-limiting.

## **CONCLUSION**

With the growing demand for noninvasive rejuvenation procedures, it is imperative that plastic surgeons remain experts in the safest and most effective ways in which to use these emerging technologies. This review article provides an overview of the modalities available for skin resurfacing, skin tightening, rhytid

reduction, and volume restoration, as well as novel modalities to combat facial aging. For most patients, a combination of these interventions will deliver optimal outcomes. Our goal is to prepare plastic surgeons to continue to offer the best and safest results to patients who desire appreciable results with minimal downtime.

## DECLARATIONS

### Authors' contributions

Manuscript drafting: Farber SE

Manuscript outlining: Brown E, Krochonis J, McConville R

Figure compilation and manuscript revisions: Epps MT

Manuscript revisions, figures, and videos: Codner MA

### Availability of data and materials

Not applicable.

### Financial support and sponsorship

None.

### Conflicts of interest

All authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for Publication

Written consent for publication was obtained for all images and videos.

### Copyright

© The Author(s) 2020.

## REFERENCES

1. American Society of Plastic Surgeons Release. 2018 Plastic surgery statistics report. Available from: <https://www.plasticsurgery.org/documents/News/Statistics/2018/plastic-surgery-statistics-full-report-2018.pdf>. [Last accessed on 2 Dec 2020]
2. Sambandan DR, Ratner D. Sunscreens: an overview and update. *J Am Acad Dermatol* 2011;64:748-58.
3. Hughe MCB, Williams GM, Baker P, Green AC. Sunscreen and prevention of skin aging: a randomized trial. *Ann Intern Med* 2013;158:781-90.
4. Wang SQ, Balaqula Y, Osterwalder U. Photoprotection: a review of the current and future technologies. *Dermatol Ther* 2010;23:31-47.
5. Xu S, Kwa M, Agarwal A. Sunscreen product performance and other determinants of consumer preferences. *JAMA Dermatol* 2016;152:920-7.
6. Mancuso JB, Maruthi R, Wang SQ, Lim HW. Sunscreens: an update. *Am J Clin Dermatol* 2017;18:643-50.
7. Verschoore M, Nielson M. The rationale of anti-aging cosmetic ingredients. *J Drugs Dermatol* 2017;16:94-7.
8. Nusgens BV, Humbert P, Rougier A, et al. Topically applied vitamin C enhances the mRNA level of collagens I and II, their processing enzymes and tissue inhibitor matrix metalloproteinase 1 in the human dermis. *J Invest Dermatol* 2001;116:853-9.
9. Murray JC, Burch JA, Steilen RD, et al. A topical antioxidant solution containing vitamins C and E stabilized by ferulic acid provides protection for human skin against damage caused by ultraviolet radiation. *J Am Acad Dermatol* 2008;59:418-25.
10. Hubbard BA, Unger JG, Rohrich RJ. Reversal of skin aging with topical retinoids. *Plast Reconstr Surg J* 2014;133:481-90e.
11. Chiu A, Kimball AB. Topical vitamins, minerals and botanical ingredients as modulators of environmental and chronological skin damage. *Br J Dermatol* 2003;149:681-91.
12. Leyden J. Adapalene in clinical practice. *Cutis* 2001;68:7-9s.
13. Ditre CM, Griffen TD, Murphy GF, et al. Effects of alpha-hydroxy acids on photoaged skin: a pilot clinical, histologic, and ultrastructural study. *J Am Acad Dermatol* 1996;34:187-95.
14. Smith WP. Epidermal and dermal effects of topical lactic acid. *J Am Acad Dermatol* 1996;35:388-91.

15. Graf J. Antioxidants and skin care: the essentials. *Plast Reconstr Surg J* 2010;125:378-83.
16. Freedman BM. Hydradermabrasion: an innovative modality for nonablative facial rejuvenation. *J Cosmet Dermatol* 2008;7:275-80.
17. Brody HJ. Medium-depth peeling. In: Chemical Peeling and Resurfacing. St. Louis: Mosby; 1997. pp. 109-10.
18. Hevia O, Nemeth AJ, Taylor JR. Tretinoin accelerates healing after trichloroacetic acid chemical peel. *Arch Dermatol* 1991;127:678-82.
19. Grimes PE. A microsphere formulation of hydroquinone 4% and retinol 0.15% in the treatment of melasma and postinflammatory hyperpigmentation. *Cutis* 2004;74:362-8.
20. Monheit GD. Skin preparation: an essential step before chemical peeling or laser resurfacing. *Cosmet Dermatol* 1996;9:13-4.
21. Hirsch RJ, Dayan SH, Shah AR. Superficial skin resurfacing. *Facial Plast Surg Clin N Am* 2004;12:311-21.
22. Krochonis JB, McConville RL, Kosowski TR. Nonsurgical periorbital rejuvenation. In: Codner MA, McCord DC, editors. Eyelid and periorbital surgery. 2nd ed. New York: Thieme; 2017. pp. 483-510.
23. Pathak A, Mohan R, Rohrich RJ. Chemical peels: role of chemical peels in facial rejuvenation today. *Plast Reconstr Surg J* 2020;145:58-66e.
24. Herbig K, Trussler AP, Khosla RK, Rohrich RJ. Combination Jessner's solution and trichloroacetic acid chemical peel: technique and outcomes. *Plast Reconstr Surg J* 2009;124:955-64.
25. Pozner JN, DiBernardo BE. Laser resurfacing: full field and fractional. *Clin Plast Surg* 2016;43:515-25.
26. Preissig J, Hamilton K, Markus R. Current laser resurfacing technologies: a review that delves beneath the surface. *Semin Plast Surg* 2012;26:109-16.
27. Karimipour DJ, Karimipour G, Orringer JS. Microdermabrasion: an evidence-based review. *Plast Reconstr Surg J* 2010;125:372-7.
28. Shin JM and Kim JE. Radiofrequency in clinical dermatology. *Medical Lasers* 2013;2:49-57.
29. Bonjorno AR, Gomes TB, Pereira MC, et al. Radiofrequency therapy in esthetic dermatology: a review of clinical evidences. *J Cosmet Dermatol* 2020;19:278-81.
30. Zelickson BD, Kist D, Bernstein E, et al. Histological and ultrastructural evaluation of a radiofrequency-based nonablative dermal remodeling device: a pilot study. *Arch Dermatol* 2004;140:204-9.
31. Beasley KL and Weiss RA. Radiofrequency in cosmetic dermatology. *Dermatol Clin* 2014;32:79-90.
32. Dendle J, Wu DC, Fabi SG, Melo D, Goldman MP. A retrospective evaluation of subsurface monopolar radiofrequency for lifting of the face, neck, and jawline. *Dermatol Surg* 2016;42:1261-5.
33. Gutowski KA. Microfocused ultrasound for skin tightening. *Clin Plast Surg* 2016;43:577-82.
34. Minkis K, Alam M. Ultrasound skin tightening. *Dermatol Clin* 2014;32:71-7.
35. Suh DH, Shin MK, Lee SJ, et al. Intense focused ultrasound tightening in asian skin: clinical and pathologic results. *Dermatol Surg* 2011;37:1-8.
36. MacGregor JL, Tanzi EL. Microfocused ultrasound for skin tightening. *Semin Cutan Med Surg* 2013;32:18-25.
37. Bunin LS, Carniol PJ. Cervical facial skin tightening with an infrared device. *Facial Plast Surg Clin North Am* 2007;15:179-84.
38. McCrudden MT, McAlister E, Courtenay AJ, et al. Microneedle applications in improving skin appearance. *Exp Dermatol* 2015;24:561-6.
39. Ablon G. Safety and effectiveness of an automated microneedling device in improving the signs of aging skin. *J Clin Aesthet Dermatol* 2018;11:29-34.
40. Alster TS, Graham PM. Microneedling: a review and practical guide. *Dermatol Surg* 2018;44:397-404.
41. Kim SE, Lee JH, Kwon HB, Ahn BJ, Lee AY. Greater collagen deposition with the microneedle therapy system than intense pulsed light. *Dermatol Surg* 2011;37:336-41.
42. Bonaparte JP, Ellis D, Quin JG, et al. A comparative assessment of three formulations of botulinum toxin type a for facial rhytides: a systematic review with meta-analyses. *Plast Reconstr Surg J* 2016;127:1125-40.
43. McConville RL, Krochonis JB, Popp ME, Kosowski TR. Cosmetic uses of botulinum toxin. In: Codner MA and McCord DC editors. Eyelid and Periorbital Surgery. 2nd ed. New York: Thieme; 2017. pp. 539-60.
44. Small R. Botulinum toxin injection for facial wrinkles. *Am Fam Physician* 2014;90:168-75.
45. Lamilla GC, Ingallina FM, Poulain B, Trevidic P. Anatomy and botulinum toxins injections. 2nd ed. Paris, FR: E2e Medical Publishing; 2010.
46. Jabbour S, Awaida CJ, Elkhoury JS, et al. The impact of upper face botulinum toxin injections on eyebrow height and forehead lines: randomized controlled trial and an algorithmic approach to forehead injection. *Plast Reconstr Surg J* 2018;142:1212-7.
47. Trevidic P, Sykes J, Criollo-Lamilla G. Anatomy of the lower face and botulinum toxin injections. *Plast Reconstr Surg J* 2015;136:84-91s.
48. Krochonis JB, McConville RL, Kosowski TR. Injectable fillers. In: Codner MA, McCord DC editors. Eyelid and Periorbital Surgery. 2nd ed. New York: Thieme; 2017. pp. 511-38.
49. De Maio M, DeBouille K, Braz A, Rohrich RJ. Facial assessment and injection guide for botulinum toxin and injectable hyaluronic acid fillers: focus on the midface. *Plast Reconstr Surg J* 2017;140:540-51e.
50. De Maio M, Wu WTL, Goodman GJ, Monheit G. Facial assessment and injection guide for botulinum toxin and injectable hyaluronic acid fillers: focus on the lower face. *Plast Reconstr Surg J* 2017;140:393-405e.
51. Donofrio LM. Techniques in facial fat grafting. *Aesthet Surg J* 2008;28:681-7.
52. DiFrancesco LM. Fat grafting and complications in the periorbital region. In: Codner MA, McCord DC, editors. Eyelid and Periorbital Surgery. 2nd ed. New York: Thieme; 2017. pp. 561-86.
53. Grow JN, Holding J, Korentager R. Assessing the efficacy of deoxycholic acid for the treatment of submental fat: a three-dimensional study. *Aesthet Surg J* 2019;39:1400-11.
54. Jain M, Savage NE, Spiteri K, Snell BJ. A 3-dimensional quantitative analysis of volume loss following submental cryolipolysis. *Aesthet*

- Surg J* 2020;40:123-32.
55. Stroumza N, Gauthier N, Senet P, et al. Paradoxical adipose hypertrophy (PAH) after cryolipolysis. *Aesthet Surg J* 2018;38:411-7.
  56. Puri N. Platelet rich plasma in dermatology and aesthetic medicine. *Our Dermatol* 2015;6:207-11.
  57. Motosko C, Khouri K, Poudrier G, Sinno S, Hazen A. Evaluating platelet-rich therapy for facial aesthetics and alopecia: a critical review of the literature. *Plast Reconstr Surg J* 2018;141:1115-23.
  58. Kim M, Yang H, Kim H, et al. Novel cosmetic patches for wrinkle improvement: retinol retinoate- and ascorbic acid-loaded dissolving microneedles. *Int J Cosmet Sci* 2014;36:207-12.



Review

Open Access



# Nonsurgical rhinoplasty using soft tissue fillers

Yuyang Chu<sup>1</sup>, Jonathan Bacos<sup>2</sup>, Sasha Becker<sup>1</sup>

<sup>1</sup>Northwestern University, Feinberg School of Medicine, Chicago, IL 60611, USA.

<sup>2</sup>Department of Plastic Surgery, Medical College of Wisconsin, Milwaukee, WI 53226, USA.

**Correspondence to:** Mr. Yuyang Chu, Northwestern University, Feinberg School of Medicine, 420 East Superior Street, Chicago, IL 60611, USA. E-mail: yuyang.chu@northwestern.edu

**How to cite this article:** Chu Y, Bacos J, Becker S. Nonsurgical rhinoplasty using soft tissue fillers. *Plast Aesthet Res* 2020;7:73. <http://dx.doi.org/10.20517/2347-9264.2020.169>

**Received:** 24 Aug 2020 **First Decision:** 12 Nov 2020 **Revised:** 16 Nov 2020 **Accepted:** 24 Nov 2020 **Published:** 10 Dec 2020

**Academic Editor:** Wen-Guo Cui **Copy Editor:** Cai-Hong Wang **Production Editor:** Jing Yu

## Abstract

Nonsurgical rhinoplasty, also known as liquid rhinoplasty, is a filler-based approach to treating deformities of the nose. Despite the potential for serious complications such as tissue necrosis and blindness, patients' desires for rapid results with minimal downtime and low costs have served as an impetus for rhinoplasty surgeons to become skilled injectors. Additionally, many physicians that are less skilled in rhinoplasty may be emboldened to perform a simpler procedure. While soft tissue filler is not always a viable alternative to rhinoplasty, it can be a useful adjunct or stand-alone treatment for managing a drooping nasal tip, minor asymmetries, or a dorsal hump. This article provides an overview of liquid rhinoplasty and how to best obtain the patient's desired aesthetic result.

**Keywords:** Nonsurgical rhinoplasty, hyaluronic acid, calcium hydroxyl apatite, injectables

## INTRODUCTION

The application of soft tissue filler is the second most common minimally invasive cosmetic procedure reported in the 2018 plastic surgery statistics report<sup>[1]</sup>. Overall, fillers provide an enticing alternative to surgical procedures for patients seeking an aesthetic improvement. This is due to the minimal downtime, low cost, and low risk profile associated with fillers. While soft tissue fillers initially made their debut in the 1970s, it was not until the mid 1980s that physicians began reporting contouring noses with injectables<sup>[2,3]</sup>. Recently, the popularity of injectable fillers has increased significantly. Filler use rose 274% between 2000 and 2015<sup>[4]</sup>. Initial reports consisted of using bovine collagen and silicone, but a transition to more reliable and safe formulations of hyaluronic acid (HA) and calcium hydroxylapatite (CaHa) have allowed clinicians



© The Author(s) 2020. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, sharing, adaptation, distribution and reproduction in any medium or format, for any purpose, even commercially, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.



to obtain remarkable results with nonsurgical rhinoplasties. Nonetheless, the most important factor in providing optimal treatment is patient selection.

Nasal reshaping with HA filler is often most helpful as a post rhinoplasty adjunct. However, many authors have reported the use of HA filler in an unoperated nose. When treating the virgin nose, injectors must be cognizant that the use of large amounts of HA filler to correct a deformity may lead to vascular compromise, thus making liquid rhinoplasty a poor solution for significant nasal deformities. The most appropriate candidates for nonsurgical rhinoplasty are often those with mild cosmetic defects. Patients who tend to show the best results are those with a mildly deviated nose, mild dorsal hump, slight imbalances from previous surgery, and high nasal tip with flat radix<sup>[5,6]</sup>. Following a physical examination, the clinician must determine whether a reasonable improvement in the defect can be achieved, and inform the patient of alternatives such as surgical rhinoplasty as well as risks and benefits in order to improve patient understanding and set reasonable expectations.

For some patients, there is a contraindication to using fillers. This group of patients includes individuals with a history of bleeding disorders, autoimmune disorders, and hypersensitivity to filler composition. Patients who are pregnant or are breastfeeding should avoid filler injections. In addition, patients who show signs of inflammation or infection near injection sites should not receive the procedure. Extreme caution should be taken with patients that have undergone a recent surgical rhinoplasty. Additionally, all patients are advised to avoid substances that impair hemostasis for one week to prevent bruising and bleeding<sup>[6]</sup>. Further, it is suggested that patients with suspected body dysmorphic disorder (BDD) see a psychiatrist before undergoing liquid rhinoplasty. This is done in an effort to avoid patient dissatisfaction, as there is an increased occurrence of BDD in people seeking a rhinoplasty<sup>[7]</sup>.

Successful application of filler also relies on choosing the appropriate product. Permanent fillers are generally not used due to their irreversible nature and the risk of granuloma formation. Currently, HA filler is the most common filler used in liquid rhinoplasty, with up to 80% of liquid rhinoplasty procedures using HA<sup>[6]</sup>. Many clinicians prefer HA because it is soft, provides a natural feel and can be reversed and quickly dissolved with hyaluronidase<sup>[8]</sup>. However, some clinicians opt for CaHa. While CaHa cannot be easily reversed, it provides a longer lasting effect and is more durable over time. CaHa fillers usually last between 12 and 18 months, while HA fillers last between 6 and 12 months<sup>[9]</sup>. Therefore, HA fillers may require more repeated injections in order to maintain the desired form or shape. However, while CaHa requires less product, more stiffness can cause increased discomfort after the procedure<sup>[10]</sup>.

## TECHNIQUE

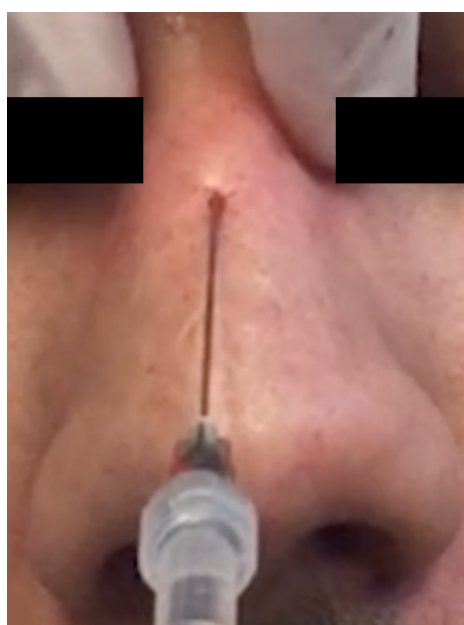
Prior to injection, topical anesthetic ointment is applied 15-30 min before the procedure.

Fillers can be reconstituted with the addition of 1% lidocaine with epinephrine (1:100,000) to reduce discomfort, viscosity, and ultimately result in less postprocedural ecchymosis. As the average injection volume for nasal reshaping is 0.4 mL of HA per injection site, addition of 0.004 mL of lidocaine is suggested<sup>[11]</sup>.

Injections can be delivered with a needle or cannula. Previously, it has been shown that cannulas provide improved patient comfort, with decreased side effects such as edema and ecchymosis, especially in highly vascular regions<sup>[12]</sup>. Additionally, Pavicic *et al.*<sup>[12]</sup> demonstrated that filler placement with cannulas provides improved precision. Nonetheless, injection technique is highly variable and often based on prior training and comfort<sup>[13]</sup>. Due to the reduced risk of vascular complications and bruising, the use of microcannulas has become more popular [Figures 1 and 2].

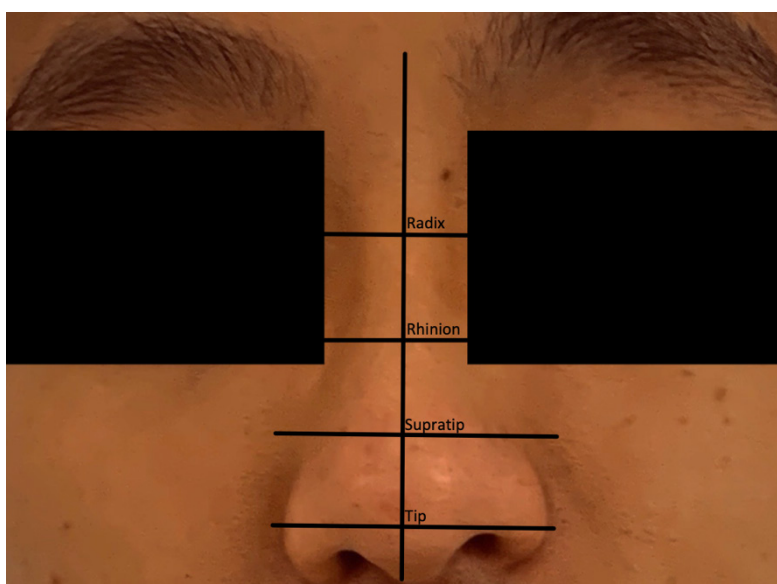


**Figure 1.** Injection site is created with a 23-gauge needle



**Figure 2.** 22-gauge microcannula insertion through the injection site

Understanding the anatomy of the nose is critical to minimizing the risk of complications and optimizing the aesthetic outcome. From superficial to deep, the four layers that comprise the nose are the superficial fatty layer, the SMAS (superficial musculo-aponeurotic system), the deep fatty layer, and periosteum<sup>[14]</sup>. The superficial fatty and SMAS layers contain major blood vessels of the nose. To minimize and avoid vascular occlusion, skin necrosis, and blindness, filler injections should be delivered into the deep fatty layer, which is between the SMAS and the periosteum. In regards to the dorsal nasal artery, it is imperative that the injector verifies that the tip of the cannula is in the preperiosteal plane. This helps to avoid accidental arterial cannulation which can often lead to serious ocular complications<sup>[15]</sup>.



**Figure 3.** Anatomic injection sites



**Figure 4.** Initial treatment of the radix

Additionally, from superior to inferior, the nose can be broken down into four segments starting with the radix, followed by the rhinion, supratip and tip [Figure 3]. A top down approach is often preferred to address the nose [Figures 4 and 5].

Patients may also benefit from improved nasal tip definition and may stand to benefit from enhancement of the nasolabial angle. Nasal tip contouring can involve injections into the alar margin, nasal spine, dome area, and columellar space<sup>[16]</sup>. Injecting into the alar margin can help correct alar retraction. Injecting into the nasal spine elevates the nasal tip, which can help contribute to a more obtuse nasolabial angle<sup>[17]</sup>. Filling



**Figure 5.** Treatment of the rhinion following treatment of the radix



**Figure 6.** Treatment of the nasal tip

the columellar space can help correct columellar retraction by providing additional support for the nasal tip. Many injectors prefer injection of the tip prior to treatment of the supratip because the tip has relatively weaker supporting structures, which makes it difficult to ascertain the final amount of projection that can be achieved at the desired location [Figure 6].

Overall, general safe injection technique involves injecting below the SMAS plane into the deep fatty layer and staying midline whenever possible to decrease the likelihood of asymmetries. Consider the use of a blunt cannula to decrease the chance of vascular complications. When injecting, a two-hand approach is recommended: one hand is responsible for the action of injecting while the other hand helps to stabilize and guide the needle into the skin. After injection, light massaging of the area is recommended to help form a smooth contour. Following the above technique can help achieve safe, reproducible results.



## Novel techniques

We recommend the usage of a 50-70 mm 22-gauge blunt cannula on the nose. With this needle size, the nose can be treated through one port, reducing the number of additional needle sticks. The large size of the needle also decreases injection pressure and the risk of penetrating a blood vessel, and because the nose is a highly vascular region, this increases the safety of the procedure. We also recommend blood aspiration before injection to diminish the risk of vascular complications. A 2015 article reports that the aspiration was reliable in 53% of cases in predicting accidental entry into a vessel<sup>[18]</sup>. When augmenting the nasal dorsum with HA filler injections, we suggest compressing vessels against the nose bilaterally with one hand, while holding the cannula in the other hand and injecting up and down the nose. These injections can also lift the nasal tip and the upper lip. If also correcting for ala depression, ports can be inserted bilaterally to the nasal tip to augment the depression.

Moreover, while we recommend the use of HA, it is of note that Radiesse injections have been increasing in popularity in recent years. Radiesse injections contain calcium hydroxylapatite and are preferred by some physicians due to specific properties of calcium hydroxylapatite, including its long duration of hold, moldability, high viscosity, high elasticity, and low immunogenicity<sup>[19]</sup>. Along with the changing materials of liquid rhinoplasty, the applications of liquid rhinoplasty have been expanding to become a corrective treatment post-surgical rhinoplasty. A 2016 study found that 27% of patients undergoing liquid rhinoplasty chose to as a secondary correction for surgical rhinoplasty<sup>[20]</sup>.

Finally, it is also critical to acknowledge that there is a significant gap in data on filler injections in Chinese women of the Han nationality, despite liquid rhinoplasty being popular among this patient population. Technique can be highly modified to match patient preference and need. In a 2015 study of 280 Chinese women of Han nationality, a 5-step process using a blunt and sharp needle was found to be most effective<sup>[21]</sup>. The first step includes using a 26-gauge sharp needle to inject HA into the periosteum of the nasal spine in order to augment the nasolabial angle. The second step is to use the 26-gauge needle to create an injection site on the nasal tip subcutaneous to the nasal process. Filling should then be performed several times (3-4) in order to lengthen the columella. Third, nasal tip filling is completed using 0.1-0.2 mL of HA with the 26-gauge needle. However, it is suggested to do a bilateral injection rather than a midline injection in this patient population as it achieves a more natural aesthetic appearance. The next step, while injecting the nasal dorsum, is to make several injections of 0.2-0.6 mL of HA starting at the nasal root and ending at the nasal tip. This is done first in the supraperiosteal and supraperichondrial layers and then in the intramuscular and subcutaneous regions. The fifth and final step is to use a 26-gauge needle on the inner part of the eyebrow to enhance the nasal root appearance<sup>[21]</sup>. Of note, a 23-gauge needle can be used for any of the above steps if there is concern for vascular complications.

## COMPLICATIONS

Complications of liquid rhinoplasty can be divided into two categories: early and delayed onset. Early onset complications are characterized by an appearance after several hours, while delayed onset complications are characterized by an appearance after weeks or years. The most common early complication is asymmetry, which can be prevented by staying as close to the midline as possible. There can also be hypersensitivity reactions to the filler content leading to fever, pruritis, and pain. Surface nodules and unevenness can also occur along with erythema, edema, ecchymosis, and pain at the sites of the injection<sup>[22]</sup>. Another early complication is the Tyndall effect, which occurs due to superficial filling and is characterized by a bluish tint on the skin. Delayed complications include granuloma formation, scarring, and skin color change.

Some of the more serious complications are infection, which has a complication rate of 0.04% and skin necrosis, which has a complication rate of 0.06%<sup>[23]</sup>. Skin necrosis is caused by vascular occlusion, which can be a result of direct intravascular injection, or from a compressive effect on local vessels. The main

arteries at risk of vascular complications in the nose are the lateral nasal artery, which predominately supplies the nasal tip, and the dorsal nasal artery, which supplies the upper dorsum of the nose. There are several anastomoses in the nasal region between the external and internal carotid systems, whose blood flow can be reversed with injections, creating a potential retrograde embolization and subsequent stroke or blindness<sup>[24]</sup>. To diminish the risk of vascular complications, injectors should aspirate before injection, inject slowly with minimal pressure, use products with vasoconstrictors, and use blunt, large microcannulas in the avascular deep plane below the level of the SMAS<sup>[25]</sup>.

In addition to complications and safety concerns, novel research has shown that liquid rhinoplasty can have an insufficient effect in some patient populations. A 2016 study notes that 10% of 250 patients reported that the filler injection lasted only a short duration of less than 6 months or did not achieve the desired aesthetic result<sup>[20]</sup>. This requires patients to receive repeat injections, which increases the risk of complications and may cause potential financial burden.

### **Novel treatments of complications**

Recent literature highlights the treatment for the complications explained above. Immediate adverse effects of HA, such as ischemia, can be corrected in a variety of ways. Hyaluronidase is the standard treatment, and there are several supplemental treatments available to help spread the hyaluronidase effectively. These supplemental treatments include topical nitropaste, oral acetylsalicylic acid, warm compresses, and massage. Nitropaste and warm compresses promote vasodilation, which promotes spread of the hyaluronidase through tissues. The pressure caused by massage helps to distribute the hyaluronidase rapidly, and acetylsalicylic acid provides an anti-clotting effect<sup>[26]</sup>.

Additionally, a 2019 study elucidates a procedural strategy to reduce complications. In the study, researchers injected a colored filler into the dorsum of a cadaver. In three of six cadavers, the filler was injected into the superficial layer and in the other three cadavers, the filler was injected by direct percutaneous injection into the deep, avascular layer. Injection through the glabella into the deep layer allowed for more accurate injection. This can decrease the chance of vascular injury, which can reduce the risk of necrosis and vision loss<sup>[27]</sup>.

## **CONCLUSION**

Liquid rhinoplasty with HA fillers is a safe procedure with positive aesthetic results when performed by a trained professional. Post procedure, many patients will have self-limiting side effect such as mild erythema and swelling. Very rarely, some patients will suffer infections and local tissue necrosis. Using correct injection technique such as filling only in the deep fatty layer and staying in the midline can help reduce the chance of adverse effects. Further, having a targeted plan on the location and order of injections can help achieve the desired aesthetic result with safety, consistency, and reproducibility.

## **DECLARATIONS**

### **Authors' contributions**

All contributed equally to the research, writing, and editing of the manuscript: Chu Y, Bacos J, Becker S

### **Availability of data and materials**

Not applicable.

### **Financial support and sponsorship**

None.

**Conflict of interest**

All authors declared that there are no conflicts of interest.

**Ethical approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Copyright**

© The Author(s) 2020.

**REFERENCES**

1. American Society of Plastic Surgeons Release. 2018 Plastic surgery statistics report. Available from: <https://www.plasticsurgery.org/documents/News/Statistics/2018/plastic-surgery-statistics-full-report-2018.pdf>. [Last accessed on 24 Jun 2020]
2. Knapp TR, Vistnes LM. The augmentation of soft tissue with injectable collagen. *Clin Plast Surg* 1985;12:221-5.
3. Webster RC, Hamdan US, Gaunt JM, Fuleihan NS, Smith RC. Rhinoplastic revisions with injectable silicone. *Arch Otolaryngol Head Neck Surg* 1986;112:269-76.
4. Wang LL, Friedman O. Update on injectables in the nose. *Curr Opin Otolaryngol Head Neck Surg* 2017;25:307-13.
5. Kontis TC. Nonsurgical rhinoplasty. *JAMA Facial Plast Surg* 2017;19:430-1.
6. Raggio BS, Asaria J. Filler rhinoplasty. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020.
7. Sarwer DB, Wadden TA, Pertschuk MJ, Whitaker LA. Body image dissatisfaction and body dysmorphic disorder in 100 cosmetic surgery patients. *Plast Reconstr Surg* 1998;101:1644-9.
8. Betemps JB, Marchetti F, Lim T, et al. Projection capacity assessment of hyaluronic acid fillers. *Plast Aesthet Res* 2018;5:19.
9. Sundaram H, Voigts B, Beer K, Meland M. Comparison of the rheological properties of viscosity and elasticity in two categories of soft tissue fillers: calcium hydroxylapatite and hyaluronic acid. *Dermatol Surg* 2010;36 Suppl 3:1859-65.
10. Kablik J, Monheit GD, Yu L, Chang G, Gershkovich J. Comparative physical properties of hyaluronic acid dermal fillers. *Dermatol Surg* 2009;35 Suppl 1:302-12.
11. Hedén P. Nasal reshaping with hyaluronic acid: an alternative or complement to surgery. *Plast Reconstr Surg Glob Open* 2016;4:e1120.
12. Pavicic T, Frank K, Erbacher K, et al. Precision in dermal filling: a comparison between needle and cannula when using soft tissue fillers. *J Drugs Dermatol* 2017;16:866-72.
13. Bacos JT, Dayan SH. Superficial dermal fillers with hyaluronic acid. *Facial Plast Surg* 2019;35:219-23.
14. Moon HJ. Injection rhinoplasty using filler. *Facial Plast Surg Clin North Am* 2018;26:323-30.
15. Tansatit T, Apinuntrum P, Phetudom T. Facing the worst risk: confronting the dorsal nasal artery, implication for non-surgical procedures of nasal augmentation. *Aesthetic Plast Surg* 2017;41:191-8.
16. Daniel RK, Letourneau A. Rhinoplasty: nasal anatomy. *Ann Plast Surg* 1988;20:5-13.
17. Menick FJ. Anatomic reconstruction of the nasal tip cartilages in secondary and reconstructive rhinoplasty. *Plast Reconstr Surg* 1999;104:2187-98; discussion 2199-201.
18. Casabona G. Blood aspiration test for cosmetic fillers to prevent accidental intravascular injection in the face. *Dermatol Surg* 2015;41:841-7.
19. Rho NK, Chang YY, Chao YY, et al. Consensus recommendations for optimal augmentation of the asian face with hyaluronic acid and calcium hydroxylapatite fillers. *Plast Reconstr Surg* 2015;136:940-56.
20. Rosenberger ES, Toriumi DM. Controversies in revision rhinoplasty. *Facial Plast Surg Clin North Am* 2016;24:337-45.
21. Han X, Hu J, Cheng L, Li F. Multiplane hyaluronic acid (EME) in female Chinese rhinoplasty using blunt and sharp needle technique. *J Plast Reconstr Aesthet Surg* 2015;68:1504-9.
22. Dayan SH, Arkins JP, Brindise R. Soft tissue fillers and biofilms. *Facial Plast Surg* 2011;27:23-8.
23. Harb A, Brewster CT. The nonsurgical rhinoplasty: a retrospective review of 5000 treatments. *Plast Reconstr Surg* 2020;145:661-7.
24. Kim DW, Yoon ES, Ji YH, Park SH, Lee BI, Dhong ES. Vascular complications of hyaluronic acid fillers and the role of hyaluronidase in management. *J Plast Reconstr Aesthet Surg* 2011;64:1590-5.
25. Beleznyay K, Carruthers JD, Humphrey S, Jones D. Avoiding and treating blindness from fillers: a review of the world literature. *Dermatol Surg* 2015;41:1097-117.
26. DeLorenzi C. Complications of injectable fillers, part 2: vascular complications. *Aesthet Surg J* 2014;34:584-600.
27. Jung GS, Chu SG, Lee JW, et al. A safer non-surgical filler augmentation rhinoplasty based on the anatomy of the nose. *Aesthetic Plast Surg* 2019;43:447-52.

# AUTHOR INSTRUCTIONS

---

## 1. Submission Overview

Before you decide to publish with us, please read the following items carefully and make sure that you are well aware of Editorial Policies and the following requirements.

### 1.1 Topic Suitability

The topic of the manuscript must fit the scope of the journal. Please refer to Aims and Scope for more information.

### 1.2 Open Access and Copyright

The journal adopts Gold Open Access publishing model since its establishment and has been distributing contents under Attribution 4.0 International License since October 2017, whereas Attribution-NonCommercial-ShareAlike 3.0 Unported had been adopted by then. Please make sure that you are well aware of these policies.

### 1.3 Publication Fees

Authors are required to pay Article Processing Charges of 499 US Dollars after the manuscript is officially accepted. For more details, please refer to Article Processing Charges.

### 1.4 Language Editing

All submissions are required to be presented clearly and cohesively in good English. Authors whose first language is not English are advised to have their manuscripts checked or edited by a native English speaker before submission to ensure the high quality of expression. A well-organized manuscript in good English would make the peer review even the whole editorial handling more smooth and efficient.

If needed, authors are recommended to consider the language editing services provided by Charlesworth to ensure that the manuscript is written in correct scientific English before submission. Authors who publish with OAE journals enjoy a special discount for the services of Charlesworth via the following two ways.

Submit your manuscripts directly at <http://www.charlesworthauthorservices.com/~OAE>;

Open the link <http://www.charlesworthauthorservices.com/>, and enter Promotion Code “OAE” when you submit.

### 1.5 Work Funded by the National Institutes of Health

If an accepted manuscript was funded by National Institutes of Health (NIH), the author may inform editors of the NIH funding number. The editors are able to deposit the paper to the NIH Manuscript Submission System on behalf of the author.

## 2. Submission Preparation

### 2.1 Cover Letter

A cover letter is required to be submitted accompanying each manuscript. It should be concise and explain why the study is significant, why it fits the scope of the journal, and why it would be attractive to readers, *etc.*

Here is a guideline of a cover letter for authors' consideration:

In the first paragraph: include the title and type (e.g., Original Article, Review, Case Report, *etc.*) of the manuscript, a brief on the background of the study, the question the author sought out to answer and why;

In the second paragraph: concisely explain what was done, the main findings and why they are significant;

In the third paragraph: indicate why the manuscript fits the Aims and Scope of the journal, and why it would be attractive to readers;

In the fourth paragraph: confirm that the manuscript has not been published elsewhere and not under consideration of any other journal. All authors have approved the manuscript and agreed on its submission to the journal. Journal's specific requirements have been met if any.

If the manuscript is contributed to a special issue, please also mention it in the cover letter.

If the manuscript was presented partly or entirely in a conference, the author should clearly state the background information of the event, including the conference name, time and place in the cover letter.

### 2.2 Types of Manuscripts

There is no restriction on the length of manuscripts, number of figures, tables and references, provided that the manuscript is concise and comprehensive. The journal publishes Original Article, Review, Meta-Analysis, Case Report, Commentary, *etc.* For more details about paper type, please refer to the following table.

<b>Manuscript Type</b>	<b>Definition</b>	<b>Abstract</b>	<b>Keywords</b>	<b>Main Text Structure</b>
Original Article	An Original Article describes detailed results from novel research. All findings are extensively discussed.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Review	A Review paper summarizes the literature on previous studies. It usually does not present any new information on a subject.	Unstructured abstract. No more than 250 words.	3-8 keywords	The main text may consist of several sections with unfixed section titles. We suggest that the author includes an "Introduction" section at the beginning, several sections with unfixed titles in the middle part, and a "Conclusion" section in the end.
Case Report	A Case Report details symptoms, signs, diagnosis, treatment, and follows up an individual patient. The goal of a Case Report is to make other researchers aware of the possibility that a specific phenomenon might occur.	Unstructured abstract. No more than 150 words.	3-8 keywords	The main text consists of three sections with fixed section titles: Introduction, Case Report, and Discussion.
Meta-Analysis	A Meta-Analysis is a statistical analysis combining the results of multiple scientific studies. It is often an overview of clinical trials.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Systematic Review	A Systematic Review collects and critically analyzes multiple research studies, using methods selected before one or more research questions are formulated, and then finding and analyzing related studies and answering those questions in a structured methodology.	Structured abstract including Aim, Methods, Results and Conclusion. No more than 250 words.	3-8 keywords	The main content should include four sections: Introduction, Methods, Results and Discussion.
Technical Note	A Technical Note is a short article giving a brief description of a specific development, technique or procedure, or it may describe a modification of an existing technique, procedure or device applied in research.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Commentary	A Commentary is to provide comments on a newly published article or an alternative viewpoint on a certain topic.	Unstructured abstract. No more than 250 words.	3-8 keywords	/
Editorial	An Editorial is a short article describing news about the journal or opinions of senior editors or the publisher.	None required	None required	/
Letter to Editor	A Letter to Editor is usually an open post-publication review of a paper from its readers, often critical of some aspect of a published paper. Controversial papers often attract numerous Letters to Editor	Unstructured abstract (optional). No more than 250 words.	3-8 keywords (optional)	/
Opinion	An Opinion usually presents personal thoughts, beliefs, or feelings on a topic.	Unstructured abstract (optional). No more than 250 words.	3-8 keywords	/
Perspective	A Perspective provides personal points of view on the state-of-the-art of a specific area of knowledge and its future prospects. Links to areas of intense current research focus can also be made. The emphasis should be on a personal assessment rather than a comprehensive, critical review. However, comments should be put into the context of existing literature. Perspectives are usually invited by the Editors.	Unstructured abstract. No more than 150 words.	3-8 keywords	/



## **2.3 Manuscript Structure**

### **2.3.1 Front Matter**

#### **2.3.1.1 Title**

The title of the manuscript should be concise, specific and relevant, with no more than 16 words if possible. When gene or protein names are included, the abbreviated name rather than full name should be used.

#### **2.3.1.2 Authors and Affiliations**

Authors' full names should be listed. The initials of middle names can be provided. Institutional addresses and email addresses for all authors should be listed. At least one author should be designated as corresponding author. In addition, corresponding authors are suggested to provide their Open Researcher and Contributor ID upon submission. Please note that any change to authorship is not allowed after manuscript acceptance.

#### **2.3.1.3 Abstract**

The abstract should be a single paragraph with word limitation and specific structure requirements (for more details please refer to Types of Manuscripts). It usually describes the main objective(s) of the study, explains how the study was done, including any model organisms used, without methodological detail, and summarizes the most important results and their significance. The abstract must be an objective representation of the study: it is not allowed to contain results which are not presented and substantiated in the manuscript, or exaggerate the main conclusions. Citations should not be included in the abstract.

#### **2.3.1.4 Keywords**

Three to eight keywords should be provided, which are specific to the article, yet reasonably common within the subject discipline.

### **2.3.2 Main Text**

Manuscripts of different types are structured with different sections of content. Please refer to Types of Manuscripts to make sure which sections should be included in the manuscripts.

#### **2.3.2.1 Introduction**

The introduction should contain background that puts the manuscript into context, allow readers to understand why the study is important, include a brief review of key literature, and conclude with a brief statement of the overall aim of the work and a comment about whether that aim was achieved. Relevant controversies or disagreements in the field should be introduced as well.

#### **2.3.2.2 Methods**

Methods should contain sufficient details to allow others to fully replicate the study. New methods and protocols should be described in detail while well-established methods can be briefly described or appropriately cited. Experimental participants selected, the drugs and chemicals used, the statistical methods taken, and the computer software used should be identified precisely. Statistical terms, abbreviations, and all symbols used should be defined clearly. Protocol documents for clinical trials, observational studies, and other non-laboratory investigations may be uploaded as supplementary materials.

#### **2.3.2.3 Results**

This section contains the findings of the study. Results of statistical analysis should also be included either as text or as tables or figures if appropriate. Authors should emphasize and summarize only the most important observations. Data on all primary and secondary outcomes identified in the section Methods should also be provided. Extra or supplementary materials and technical details can be placed in supplementary documents.

#### **2.3.2.4 Discussion**

This section should discuss the implications of the findings in context of existing research and highlight limitations of the study. Future research directions may also be mentioned.

#### **2.3.2.5 Conclusion**

It should state clearly the main conclusions and include the explanation of their relevance or importance to the field.

### **2.3.3 Back Matter**

#### **2.3.3.1 Acknowledgments**

Anyone who contributed towards the article but does not meet the criteria for authorship, including those who provided professional writing services or materials, should be acknowledged. Authors should obtain permission to acknowledge from all those mentioned in the Acknowledgments section. This section is not added if the author does not have anyone to acknowledge.

### **2.3.3.2 Authors' Contributions**

Each author is expected to have made substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of data, or the creation of new software used in the work, or have drafted the work or substantively revised it.

Please use Surname and Initial of Forename to refer to an author's contribution. For example: made substantial contributions to conception and design of the study and performed data analysis and interpretation: Salas H, Castaneda WV; performed data acquisition, as well as provided administrative, technical, and material support: Castillo N, Young V.

If an article is single-authored, please include "The author contributed solely to the article." in this section.

### **2.3.3.3 Availability of Data and Materials**

In order to maintain the integrity, transparency and reproducibility of research records, authors should include this section in their manuscripts, detailing where the data supporting their findings can be found. Data can be deposited into data repositories or published as supplementary information in the journal. Authors who cannot share their data should state that the data will not be shared and explain it. If a manuscript does not involve such issue, please state "Not applicable." in this section.

### **2.3.3.4 Financial Support and Sponsorship**

All sources of funding for the study reported should be declared. The role of the funding body in the experiment design, collection, analysis and interpretation of data, and writing of the manuscript should be declared. Any relevant grant numbers and the link of funder's website should be provided if any. If the study is not involved with this issue, state "None." in this section.

### **2.3.3.5 Conflicts of Interest**

Authors must declare any potential conflicts of interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. If there are no conflicts of interest, please state "All authors declared that there are no conflicts of interest." in this section. Some authors may be bound by confidentiality agreements. In such cases, in place of itemized disclosures, we will require authors to state "All authors declare that they are bound by confidentiality agreements that prevent them from disclosing their conflicts of interest in this work." If authors are unsure whether conflicts of interest exist, please refer to the "Conflicts of Interest" of OAE Editorial Policies for a full explanation.

### **2.3.3.6 Ethical Approval and Consent to Participate**

Research involving human subjects, human material or human data must be performed in accordance with the Declaration of Helsinki and approved by an appropriate ethics committee. An informed consent to participate in the study should also be obtained from participants, or their parents or legal guardians for children under 16. A statement detailing the name of the ethics committee (including the reference number where appropriate) and the informed consent obtained must appear in the manuscripts reporting such research.

Studies involving animals and cell lines must include a statement on ethical approval. More information is available at Editorial Policies.

If the manuscript does not involve such issue, please state "Not applicable." in this section.

### **2.3.3.7 Consent for Publication**

Manuscripts containing individual details, images or videos, must obtain consent for publication from that person, or in the case of children, their parents or legal guardians. If the person has died, consent for publication must be obtained from the next of kin of the participant. Manuscripts must include a statement that a written informed consent for publication was obtained. Authors do not have to submit such content accompanying the manuscript. However, these documents must be available if requested. If the manuscript does not involve this issue, state "Not applicable." in this section.

### **2.3.3.8 Copyright**

Authors retain copyright of their works through a Creative Commons Attribution 4.0 International License that clearly states how readers can copy, distribute, and use their attributed research, free of charge. A declaration "© The Author(s) 2020." will be added to each article. Authors are required to sign License to Publish before formal publication.

### **2.3.3.9 References**

References should be numbered in order of appearance at the end of manuscripts. In the text, reference numbers should be placed in square brackets and the corresponding references are cited thereafter. Only the first five authors' names are required to be listed in the references, other authors' names should be omitted and replaced with "et al.". Abbreviations of the journals should be provided on the basis of Index Medicus. Information from manuscripts accepted but not published should be cited in the text as "Unpublished material" with written permission from the source.

References should be described as follows, depending on the types of works:

Types	Examples
Journal articles by individual authors	Weaver DL, Ashikaga T, Krag DN, Skelly JM, Anderson SJ, et al. Effect of occult metastases on survival in node-negative breast cancer. <i>N Engl J Med</i> 2011;364:412-21. [PMID: 21247310 DOI: 10.1056/NEJMoal008108]
Organization as author	Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. <i>Hypertension</i> 2002;40:679-86. [PMID: 12411462]
Both personal authors and organization as author	Vallancien G, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1,274 European men suffering from lower urinary tract symptoms. <i>J Urol</i> 2003;169:2257-61. [PMID: 12771764 DOI: 10.1097/01.ju.0000067940.76090.73]
Journal articles not in English	Zhang X, Xiong H, Ji TY, Zhang YH, Wang Y. Case report of anti-N-methyl-D-aspartate receptor encephalitis in child. <i>J Appl Clin Pediatr</i> 2012;27:1903-7. (in Chinese)
Journal articles ahead of print	Odibo AO. Falling stillbirth and neonatal mortality rates in twin gestation: not a reason for complacency. <i>BJOG</i> 2018; Epub ahead of print [PMID: 30461178 DOI: 10.1111/1471-0528.15541]
Books	Sherlock S, Dooley J. Diseases of the liver and biliary system. 9th ed. Oxford: Blackwell Sci Pub; 1993. pp. 258-96.
Book chapters	Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. <i>The genetic basis of human cancer</i> . New York: McGraw-Hill; 2002. pp. 93-113.
Online resource	FDA News Release. FDA approval brings first gene therapy to the United States. Available from: <a href="https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm">https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm574058.htm</a> . [Last accessed on 30 Oct 2017]
Conference proceedings	Harnden P, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ Cell Tumour Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer; 2002.
Conference paper	Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. <i>Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming</i> ; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. pp. 182-91.
Unpublished material	Tian D, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. <i>Proc Natl Acad Sci U S A</i> . Forthcoming 2002.

For other types of references, please refer to U.S. National Library of Medicine.

The journal also recommends that authors prepare references with a bibliography software package, such as EndNote to avoid typing mistakes and duplicated references.

### 2.3.3.10 Supplementary Materials

Additional data and information can be uploaded as Supplementary Material to accompany the manuscripts. The supplementary materials will also be available to the referees as part of the peer-review process. Any file format is acceptable, such as data sheet (word, excel, csv, cdx, fasta, pdf or zip files), presentation (powerpoint, pdf or zip files), image (cdx, eps, jpeg, pdf, png or tiff), table (word, excel, csv or pdf), audio (mp3, wav or wma) or video (avi, divx, flv, mov, mp4, mpeg, mpg or wmv). All information should be clearly presented. Supplementary materials should be cited in the main text in numeric order (e.g., Supplementary Figure 1, Supplementary Figure 2, Supplementary Table 1, Supplementary Table 2, *etc.*). The style of supplementary figures or tables complies with the same requirements on figures or tables in main text. Videos and audios should be prepared in English, and limited to a size of 500 MB or a duration of 3 minutes.

## 2.4 Manuscript Format

### 2.4.1 File Format

Manuscript files can be in DOC and DOCX formats and should not be locked or protected.

### 2.4.2 Length

There are no restrictions on paper length, number of figures, or amount of supporting documents. Authors are encouraged to present and discuss their findings concisely.

### 2.4.3 Language

Manuscripts must be written in English.

### 2.4.4 Multimedia Files

The journal supports manuscripts with multimedia files. The requirements are listed as follows:

Videos or audio files are only acceptable in English. The presentation and introduction should be easy to understand. The frames should be clear, and the speech speed should be moderate.

A brief overview of the video or audio files should be given in the manuscript text.

The video or audio files should be limited to a duration of 3 min and a size of up to 500 MB.

Please use professional software to produce high-quality video files, to facilitate acceptance and publication along with the submitted article. Upload the videos in mp4, wmv, or rm format (preferably mp4) and audio files in mp3 or wav format.

### 2.4.5 Figures

Figures should be cited in numeric order (e.g., Figure 1, Figure 2) and placed after the paragraph where it is first cited;

Figures can be submitted in format of tiff, psd, AI or jpeg, with resolution of 300-600 dpi;

Figure caption is placed under the Figure;

Diagrams with describing words (including, flow chart, coordinate diagram, bar chart, line chart, and scatter diagram, *etc.*) should be editable in word, excel or powerpoint format. Non-English information should be avoided;

Labels, numbers, letters, arrows, and symbols in figure should be clear, of uniform size, and contrast with the background; Symbols, arrows, numbers, or letters used to identify parts of the illustrations must be identified and explained in the legend;

Internal scale (magnification) should be explained and the staining method in photomicrographs should be identified;

All non-standard abbreviations should be explained in the legend;

Permission for use of copyrighted materials from other sources, including re-published, adapted, modified, or partial figures and images from the internet, must be obtained. It is authors' responsibility to acquire the licenses, to follow any citation instruction requested by third-party rights holders, and cover any supplementary charges.

### 2.4.6 Tables

Tables should be cited in numeric order and placed after the paragraph where it is first cited;

The table caption should be placed above the table and labeled sequentially (e.g., Table 1, Table 2);

Tables should be provided in editable form like DOC or DOCX format (picture is not allowed);

Abbreviations and symbols used in table should be explained in footnote;

Explanatory matter should also be placed in footnotes;

Permission for use of copyrighted materials from other sources, including re-published, adapted, modified, or partial tables from the internet, must be obtained. It is authors' responsibility to acquire the licenses, to follow any citation instruction requested by third-party rights holders, and cover any supplementary charges.

### 2.4.7 Abbreviations

Abbreviations should be defined upon first appearance in the abstract, main text, and in figure or table captions and used consistently thereafter. Non-standard abbreviations are not allowed unless they appear at least three times in the text. Commonly-used abbreviations, such as DNA, RNA, ATP, *etc.*, can be used directly without definition. Abbreviations in titles and keywords should be avoided, except for the ones which are widely used.

### 2.4.8 Italics

General italic words like *vs.*, *et al.*, *etc.*, *in vivo*, *in vitro*; *t* test, *F* test, *U* test; related coefficient as *r*, sample number as *n*, and probability as *P*; names of genes; names of bacteria and biology species in Latin.

### 2.4.9 Units

SI Units should be used. Imperial, US customary and other units should be converted to SI units whenever possible. There is a space between the number and the unit (i.e., 23 mL). Hour, minute, second should be written as h, min, s.

### 2.4.10 Numbers

Numbers appearing at the beginning of sentences should be expressed in English. When there are two or more numbers in a paragraph, they should be expressed as Arabic numerals; when there is only one number in a paragraph, number < 10 should be expressed in English and number > 10 should be expressed as Arabic numerals. 12345678 should be written as 12,345,678.

### 2.4.11 Equations

Equations should be editable and not appear in a picture format. Authors are advised to use either the Microsoft Equation Editor or the MathType for display and inline equations.

## 2.5 Submission Link

Submit an article via <https://oaemesas.com/par/>.



**OAE Publishing Inc.**

[www.oaepublish.com](http://www.oaepublish.com)

**Plastic and Aesthetic Research  
(PAR)**

Los Angeles Office

245 E Main Street ste122, Alhambra,  
CA 91801, USA

Tel: +1 323 9987086

E-mail: [par\\_editor001@parjournal.net](mailto:par_editor001@parjournal.net)

Website: [www.parjournal.net](http://www.parjournal.net)

