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Systematic Review

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The use of acellular fish skin grafts in burns and complex trauma wounds: a systematic review of clinical data

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Abstract

Aim: In the field of burns and soft tissue reconstruction, skin substitutes have been successfully used for various indications. They allow for conservative treatment as well as temporal coverage through the improvement of wound bed conditions, pathogen control and the formation of new tissue. Fish skin grafts (FSGs) have gained rising attention as a new tool in the skin substitute market. This systematic review aims to provide an update on clinical studies investigating the effects of FSG on healing for the following indications: donor sites of split-thickness skin grafts, superficial and deep partial-thickness burns, full-thickness burns, combat wounds, and other acute wounds.

Methods: A systematic review of the peer-reviewed literature available as of January 2024 was conducted to examine the effects of FSG on wound healing of burn and complex trauma wounds, using the databases PubMed and Web of Science. Only clinical studies published in English were included.

Results: In total, 11 clinical studies were considered eligible and therefore included in the present review**.** According to the available data, the main advantages of the two commonly used types of FSGs (Kerecis® Omega3 Wound Matrix and Nile tilapia) are an acceleration of re-epithelialization time, a reduction in pain intensity and infection

rates, as well as a reduction in the number of required dressing changes.

Conclusion: FSGs represent a safe and promising product for the management of donor sites, partial-thickness and full-thickness burns, as well as complex trauma wounds. However, there is a paucity of high-quality clinical evidence, especially randomized controlled trials. More research is needed to fully understand the product's potential for wound healing and to create a more meaningful treatment algorithm.

Keywords: Fish skin graft, Omega-3 Wound Matrix, xenograft, skin substitutes, burns, trauma, combat injuries

INTRODUCTION

Despite the rapid advancements in burn care therapies since the second half of the 20th century, successful burn wound management still poses a challenge[1]. Even though there is a variety of novel wound dressings designed especially for the conservative treatment of superficial burns, a gold standard has not yet been defined[2]. Unlike superficial burns, deep partial- and full-thickness burns usually need to be addressed surgically in order to prevent infections and consequent complications, such as SIRS and multiple organ failure, and to finally achieve pleasing aesthetic and functional outcomes[3]. Hence, early excision of necrotic tissue followed by autologous split-thickness skin grafting (STSG) is the standard of care for deep partial- and full-thickness burns[3]. Extensive deep burns with > 35% total body surface area (TBSA) are especially challenging to treat, not only due to the frequent systemic consequences, but also due to the restricted availability of donor skin for autologous grafting[4]. Therefore, surgeons often must rely on skin substitutes for temporary coverage in order to reduce water, protein and heat loss and to prevent wound colonization[4]. Temporary wound coverage can be achieved with fully synthetic skin substitutes, with allografts derived from human cadaveric skin, or with xenografts from mammals, such as bovine, porcine, or fish skin[5-7]. However, there are some disadvantages in using allografts and mammalian xenografts, including the transmission of bacterial and viral diseases and the risk of an auto-immune response[7]. In light of these disadvantages, fish skin presents itself as superior. Zoonotic spillover is known to be highest in domesticated mammals due to their phylogenetic proximity to humans and opportunity for human contact[8]. To fight aquatic pathogens, the body surface of fish is covered by a layer of epidermal mucus, a viscous colloid containing antibacterial enzymes[9]. Hence, this layer serves as a physical barrier to prevent pathogen invasion, but it also contains factors of innate immunity, such as lysozyme, immunoglobulin, complement proteins, C-reactive protein (CRP), proteolytic enzymes, and various other antibacterial proteins and peptides[9]. As marine pathogens tend to flourish in warm water, warm-water fish are exposed to a higher pathogen load[10]. The mucus composition and the antimicrobial component of mucus vary between different species of fish[9]. What is more, fish skin naturally contains Omega-3 polyunsaturated fatty acids, eicosapentaenoic acid, and docosahexaenoic acid, which might suppress inflammation[11,12]. *In vitro*, these bioactive molecules exerted antimicrobial capacity against bacteria, fungi, and viruses[13]. Thus, they play an important role in the acceleration of wound healing in the clinical setting.

Currently, there are two very different products derived from two distinct species of fish that are commonly being used as wound healing matrices: Fish skin grafts (FSGs) derived from Nile tilapia (Oreochromis niloticus) and Kerecis® Omega3 Wound Matrix (Kerecis, Isafjordur, Iceland), derived from north Atlantic cod fish (Gadus morhua)[14-16]. Nile tilapia-derived fish skin grafts (NTG) are widely used, especially in Brazil, and mainly for superficial partial-thickness burns (SPTB)[17-19]. According to the Food and Agriculture Organization (FAO) of the United Nations, Nile tilapia is the most cultivated fish globally[14]. Its skin is obtained from local farmers and usually processed by the burn units themselves[17]. In preclinical studies, the morphology of NTG exhibited resemblances to human skin, featuring a thick dermis comprised of organized collagen fibers that are arranged both horizontally and vertically. Additionally, a higher

proportion of type I collagen, along with elevated resistance and tensile strength, was observed compared to human skin[14]. Being a warm-water fish, it requires harsher processing than cold-water fish, including chemical sterilization, glycerolization, and gamma irradiation[17]. Until today, this product has not been commercially available. However, it acts as an important cost-effective tool in burn care management, especially in developing countries, where 90% of burn injuries occur[18].

Kerecis® Omega3 Wound Matrix, on the other hand, is Food and Drug Administration (FDA)-approved and available worldwide. As it is a cold-water fish, there is a negligible risk of transmitting diseases to humans, which allows for gentler processing, without any harsh chemicals that dissolve the soluble components of the tissue[11]. The result is a decellularized matrix with otherwise preserved dermal microarchitecture. The structural integrity and the molecular components of the skin including the proteoglycans, glycoproteins, soluble collagen, elastin, laminin, fibronectin, lipids, and the Omega-3 polyunsaturated fatty acids are retained[11,20]. Additionally, the product demonstrates a heterogeneous pore structure to efficiently endure external tensile stress and allow for vascular ingrow[6]. Yoon *et al.* conducted an *in vitro* study to compare cell growth under the influence of the Kerecis® Omega3 Wound Matrix and a bovine collagen wound matrix (ProHeal®, MedSkin Solutions, Germany)[6]. The cell density after days 1 and 3 was significantly higher with the Kerecis® Omega3 Wound Matrix, which indicates a faster creation of new tissue and, consequently, better skin regeneration[6]. Lastly, the Kerecis® Omega3 Wound Matrix is easy to use and can be preserved up to three years after production, which is beneficial when the cold chain is interrupted, which is often the case in military contexts[20].

Due to their numerous beneficial properties, FSGs are commonly used to treat chronic wounds, as well as complex acute wounds, such as combat injuries, and burns across various countries worldwide[16,18,20,21]. Nonetheless, a systematic review from 2022 found only a scarce body of literature on the effects of FSGs on wound healing in burns[7]. In the present systematic review, we aimed to give an update on clinical studies investigating the effects of the two above-mentioned FSGs (Kerecis® Omega3 Wound Matrix and NTG) on healing for the following indications: donor sites from split-thickness skin grafts, superficial and deep partial-thickness burns, full-thickness burns, combat wounds, and other acute wounds.

METHODS

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (PROSPERO ID: CRD42024500798)[22].

Search strategy and article selection

A systematic search of two databases, PubMed and Web of Science, was performed in January 2024 by two authors (A.H. and A.L.P.) for literature published in any year related to clinical studies that used acellular fish skin in the context of burns or complex trauma wounds. The following search terms were used: ("fish skin" OR "fish skin graft" OR "acellular fish skin" OR "acellular fish skin graft" OR "marine graft" OR "North Atlantic Cod" OR "Nile Tilapia") AND ("trauma*" or "burn*" or "injur*" or "war" or "militar*" or "explosion*").

Based on the search terms, both authors independently screened the study titles, abstracts, and, if available, full-text articles. Only human-based clinical studies published in English that involved the use of acellular fish skin for treating burns or complex trauma wounds were included. Articles including preclinical studies (e.g., laboratory work, animal experiments), reviews, commentaries, or letters were excluded. Both authors independently recorded all search results and available clinical data in an Excel spreadsheet (Microsoft Excel 2016, Microsoft Office [16.44] 32-bit). Afterwards, the extracted data were compared and reconciled. In

cases of discrepancies between the two authors, respective articles were then reviewed by a third author (L.P.K.).

RESULTS

A total of 673 studies were identified through our search from two databases, with 501 studies identified from PubMed and 172 from Web of Science. After the exclusion of 78 duplicates, 558 studies out of 595 studies were excluded due to the non-applicability of the exact study purpose. After removing duplicates and non-applicable studies, the remaining 37 studies were analyzed regarding the inclusion and exclusion criteria. Articles were excluded based on the previously mentioned exclusion criteria, which included reviews, letters or commentaries (n = 12), and preclinical studies (n = 14). After application of the inclusion and exclusion criteria, eleven clinical studies have been identified that used fish skin for clinical applications in burns, acute or complex trauma wounds [Figure 1]. Out of these eleven studies, four were randomized controlled trials (RCT), three were cohort studies, and four were case reports. The effects of FSG derived from Nile Tilapia were evaluated in four publications, whereas Kerecis® Omega3 Wound Matrix was investigated in seven publications [Table 1].

DISCUSSION

Fish skin is beneficial for the conservative treatment of SPTB

Complete wound healing for SPTB is expected within two weeks after injury, without the need to be surgically addressed[14]. However, SPTB can easily progress into deep partial-thickness burns within the first few days, necessitating surgical attention. Therefore, adequate wound therapy that prevents infection and maintains a moist wound environment can help to prevent the burn wound from deteriorating[26]. The most commonly used dressings for SPTB involve silver-impregnated dressings and silver-containing creams[26-28]. Recently, FSGs have gained increased popularity in burn care[7]. Here, we have identified four clinical studies focusing on the re-epithelialization duration and number of dressing changes for the treatment of SPTB with FSGs. In all of these studies, NTGs had been used[14,17,18,20].

Enhanced healing time

One of the most essential parameters in evaluating the effectiveness of wound dressings is the time until complete wound healing (> 95% re-epithelialization), which is commonly assessed via clinical judgment by a consultant. A clinical study by Lima Júnior *et al.* investigated the healing time of superficial and deep partial-thickness burns treated with NTG, comparing it to silver sulfadiazine cream (10 mg/g)[14]. Burns treated with NTG showed a faster re-epithelialization time for both indications. However, the mean difference in time until re-epithelialization between the treatment and the control group was found to be more pronounced in the deep partial-thickness group (3.2 days) than in the other two groups of superficial burns (1.4 and 1.1 days)[14]. In a third study phase, the difference in the number of days until complete wound healing of the burns dressed with NTG was confirmed lower (9.7 ± 0.6) than those treated with silver sulfadiazine cream (10.2 ± 0.9). However, the difference was not relevant from a clinical standpoint, with an average treatment duration reduction of merely a half day[18]. In a different study conducted in a pediatric population, no statistically significant difference emerged between the re-epithelialization time of SPTB treated with NTG (10.1 ± 0.5) and those treated with silver sulfadiazine cream 1% (10.5 ± 0.7)[23]. Although differences between groups were minor, NTG performed slightly better.

Reduced number of dressing changes

In the analyzed studies, the number of dressing changes was assessed when using NTG. In the control groups, a change of dressing was defined as replacing the primary cover (cream or dressing) and cleaning the wound. The frequency of dressing changes was predefined depending on the extent and depth of the

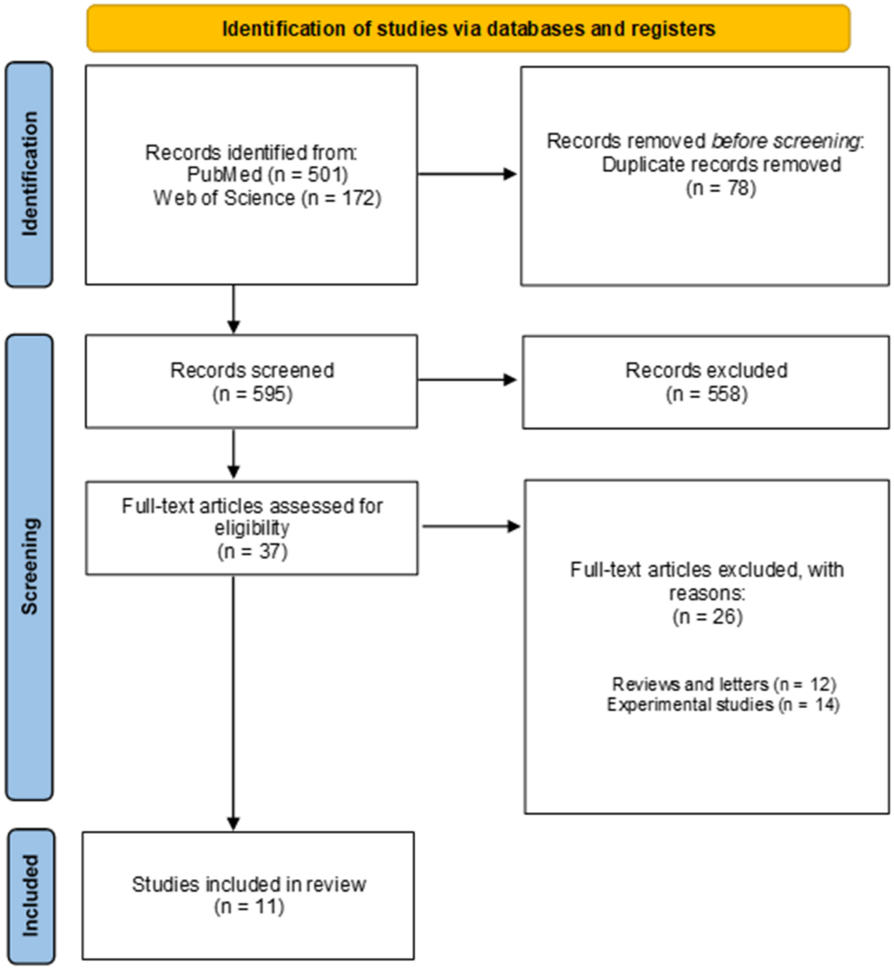

Figure 1. PRISMA Flow Diagram. In total, 673 clinical publications elucidating the effects of FSG were identified. After elimination of duplicates and non-eligible articles, eleven publications remained to be included. PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; FSG: Fish skin grafts.

burn wound[14,18,19,23]. In the intervention groups, on the other hand, dressing changes of the NTG were only performed when the product did not adhere properly to the wound and/or the secondary dressing was soaked in exudate.

In a pilot study conducted by Lima Júnior *et al.*, patients with SPTB up to 10% of TBSA were included[19]. In the control group, treated with an absorbent silver dressing (Aquacel® Ag, ConvaTec, Skillman, NJ), dressing changes were mandatory, following the manufacturer's recommendation[19]. The total number of dressings was significantly reduced ($P < 0.001$) in the NTG-treated group compared to the control group[19].

In a phase two clinical trial, Lima Júnior *et al.* divided the patients into three groups: Group A consisted of out-patients with SPTB covering less than 10% of TBSA; Group B included in-patients with SPTB covering 10%-20% of TBSA; Group C comprised in-patients with deep partial-thickness burns covering 5%-15% of TBSA[14]. In the control group (silver sulfadiazine cream 1%), a dressing change was performed every 24 h in groups B and C, and every 48 h in group A[14]. In the NTG-treated groups, a significantly lower number of dressing changes was observed ($P < 0.0001$)[14].

Table 1. Clinical studies evaluating the use of acellular fish skin in burn and complex trauma wounds

Study	Study type	*N* (total)	Fish skin product	Comparison to	Condition	Treatment period	End points
Lima Júnior *et al.*, 2020[14]	Randomized, monocentric, open-label clinical study phase II	62	Nile Tilapia FSG	Silver sulfadiazine cream (1%)	Superficial and deep partial-thickness burns	Until > 95% re-epithelialization)	Re-epithelialization
Lima Júnior *et al.*, 2020[18]	Randomized, monocentric, parallel, open-label, controlled pilot clinical study phase III	115	Nile Tilapia FSG	Silver sulfadiazine cream (1%)	SPTB	Until full re-epithelialization	Re-epithelialization
Lima Júnior *et al.*, 2020[23]	Randomized, monocentric open-label, controlled clinical study phase II	30	Nile Tilapia FSG	Silver sulfadiazine cream (1%)	SPTB	Until > 95% re-epithelialization	Re-epithelialization
Costa *et al.*, 2019[17]	Case report	1	Nile Tilapia FSG	/	SPTB in a child	Until full re-epithelialization	Re-epithelialization
Wallner *et al.*, 2022[16]	Controlled retrospective cohort study	12	Kerecis® Omega3 Wound Matrix	STSG	Deep partial-thickness burns	Not stated	Not stated
Yoon *et al.*, 2022[6]	Controlled cohort study	52	Kerecis® Omega3 Wound Matrix	No treatment + bovine collagen matrix	Donor site	Until full re-epithelialization	Re-epithelialization
Badois *et al*, 2019[24]	Controlled cohort study	21	Kerecis® Omega3 Wound Matrix	Paraffin gauze	Donor site	Until full re-epithelialization	Re-epithelialization
Alam *et al.*, 2019[15]	Case series	10	Kerecis® Omega3 Wound Matrix	/	Donor site	Until full re-epithelialization	Re-epithelialization
Reda *et al.*, 2023[20]	Case series	3	Kerecis® Omega3 Wound Matrix	/	Combat injuries	Not consistently stated	Not stated
Smolle *et al.*, 2023[25]	Case report	1	Kerecis® Omega3 Wound Matrix	/	Epifascially debrided burn wound	Until suitable wound conditions for STSG	Infection control and sufficient granulation for STSG
Kirsner *et al.*, 2020[11]	Prospective, double-blinded RCT	170	Kerecis® Omega3 Wound Matrix	dHACM allograft	Acute full-thickness wound	Until full re-epithelialization	Re-epithelialization

STSG: Split-thickness skin graft; dHACM: dehydrated human amnion/chorion membrane.

In contrast, in a phase III study, which was conducted in patients with SPTB of < 15% TBSA, Lima Júnior *et al.* re-applied the sulfadiazine cream in the control group every 48 h[18]. Still, a significantly lower ($P < 0.001$) number of dressing changes (1.6 ± 0.7) was observed in the NTG group, with a mean difference of 3.3 days[18]. Similar results were achieved in another study conducted in a pediatric population ($P < 0.001$)[23].

A reduction in the number of dressing changes represents not only a decrease in the individual cost of treatment but also a reduction in the overall workload for healthcare staff.

Reduction in pain intensity

In the above-mentioned study by Lima Júnior *et al.*, pain was assessed before and after dressing changes using the visual analog scale (VAS) and the Electronic von Frey to measure changes in mechanical pain

threshold[19]. No statistically significant difference was demonstrated between the pain levels before dressing changes in the intervention group treated with NTG and the control group treated with Aquacel® Ag. Pain levels after dressing changes, on the other hand, were significantly lower when NTG was used[19].

In their second study phase, Lima Júnior *et al.* analyzed pain intensity throughout the treatment using the VAS[14]. In study arm A (SPTB covering less than 10% of TBSA), no notable difference in pain intensity between the NTG and silver sulfadiazine cream groups was observed. In study arm B (SPTB covering 10% to 20% of TBSA), patients treated with NTG reported significantly lower pain intensity during the second, third, fourth, and fifth evaluation visits. Concerning study arm C (deep partial-thickness burns), the results are discussed separately in the next chapter[14].

In the third phase of their study, Lima Júnior *et al.* measured pain intensity after the first application of the dressing and at the beginning of each visit using the VAS and Electronic von Frey[18]. A significant reduction in pain intensity (measured with VAS) was observed in the intervention group treated with NTG compared to the control group treated with silver sulfadiazine cream. It was found that VAS and Burns Specific Pain Anxiety Scale were significantly lower in the NTG group[18].

Fish skin may serve as an alternative to autologous grafting in deep partial-thickness burns

The normal healing period for deep partial-thickness burns (DPTB) lasts at least three weeks[29]. Optimal therapy is based on the early tangential excision of necrotic tissue followed by wound coverage to avoid excessive inflammation and improve aesthetic and functional outcomes[30]. According to the standard of care, wound coverage is usually achieved by STSGs[30]. However, various skin substitutes have been proposed as an alternative to autografting in deep partial-thickness burns[31,32]. In a different approach, skin substitutes have been used prior to autografting to prevent infections and water loss, enhance wound bed conditions, and improve graft take rates[33,34]. There is evidence that the early use of dermal substitutes prior to autografting might improve skin graft quality, functional and cosmetic outcomes and contribute to the prevention of contractures[33]. Currently, there are three studies (one case report, one RCT, and one retrospective controlled cohort study) that reported the use of Kerecis® Omega3 Wound Matrix as an alternative to autografting in deep partial-thickness burns[14-16] With our search strategy, we did not find any published data on the use of FSG in deep partial-thickness burns before autografting.

Wallner *et al.* performed a retrospective cohort study involving twelve patients with multiple burn wounds exhibiting mixed burn depth patterns[16]. All burn wounds in this study were treated with enzymatic debridement (NexoBrid™, MediWound Germany GmbH, Germany) instead of surgical excision on the second day after the patient's admission. Subsequent wound coverage in deep dermal burns was performed either with Kerecis® Omega3 Wound Matrix (intervention group; twelve wounds) or a STSG (0.2 mm, meshed 1:1.5; control group; seven wounds). SPTB were covered with an alloplastic epidermal substitute (Suprathel®, PolyMedics Innovations GmbH, Germany; eight wounds) after the enzymatic debridement. Due to the lack of comparability, the latter are not further discussed here[16]. Wound healing, scar quality-associated parameters (skin elasticity, skin thickness, hydration, pigmentation, vascularity and sebum production), pain and itch levels were investigated 12 months after the burn injury[16]. Compared to burns managed with STSG, those treated with Kerecis® Omega3 Wound Matrix demonstrated faster wound healing properties, a significantly higher water-storage capacity (similar to healthy skin), reduced pain levels, as well as ameliorated functional and cosmetic outcomes, such as improved scar quality[16]. Sebum production and skin elasticity, however, showed no statistically significant superiority compared to STSG-treated wounds[16].

In their case report, Alam *et al.* included two cases of patients who were - after the debridement - treated with Kerecis® Omega3 Wound Matrix for small-size deep partial-thickness burns of the thigh and thumb[15]. Both patients already showed complete re-epithelialization at a two-week follow-up. Moreover, both patients reported the FSG to be comfortable and noticed reduced pain levels during the treatment period[15].

Similarly, in a clinical study performed by Díaz-Puertas *et al.,* the authors used NTG to treat patients with deep partial-thickness burns (study arm C, explained in more detail above) covering 5%-15% of TBSA and compared the product to topical silver sulfadiazine cream treatment. Patients receiving the NTG exhibited significantly lower pain intensity[13].

Fish skin reduces donor site morbidity after autologous grafting

STSGs are widely used to treat various types of wounds such as ulcers or deep partial- and full-thickness burns[35]. The donor site resembles a partial-thickness burn wound, necessitating 2-3 weeks for healing[35]. This process can be negatively impacted by the wound size and patient-related factors, including age and comorbidities[36]. The ideal dressing for donor sites should be inexpensive, easy to apply, prevent infections, and above all, allow for rapid re-epithelialization[37]. The standard of care is non-adherent meshed gauze dressings infused with different salves[38]. Those products are cheap and easy to apply, but they can stick to the wound bed and cause pain during dressing changes. According to Barnea *et al.*, the primary burden for patients during the initial ten days post grafting is pain at the donor site[36]. While there is a variety of dressing options for donor sites, the use of FSG is supported by promising results from clinical studies[6,15,24]. In all included studies for this indication, Kerecis® Omega3 Wound Matrix was used. In total, only one case report and two comparative cohort studies investigated the use of Kerecis® Omega3 Wound Matrix for donor sites.

Alam *et al.* performed a case series with ten included patients using Kerecis® Omega3 Wound Matrix after STSG harvests on the upper thigh[15]. The first dressing change was performed seven days after surgery and every three days thereafter until fully epithelialized[15]. The study focused on pain, signs of infection, and days until epithelialization (assessed only visually). Badois *et al.* compared the pain intensity, signs of infection, and time until re-epithelialization (remaining wound area measured at their widest and longest point) between Kerecis® Omega3 Wound Matrix (intervention group) and the standard of care treatment with fatty gauze (control group)[24]. Donor site defects were 0.4 mm (16/1,000 inch) thick and located at the medial aspect of the upper arm. Kerecis® Omega3 Wound Matrix was re-applied if it broke down in the first few days after surgery. The product was covered with moist compresses, followed by a semi-permeable film dressing. Evaluations were performed from five days after surgery until epithelialization was completed[24]. Yoon *et al.*, on the other hand, compared Kerecis® Omega3 Wound Matrix to a bovine collagen skin graft (ProHeal® Collagen Wound Dressing, MedSkin Solutions, Germany) and to no treatment on donor sites on the thigh with a thickness of 0.2-0.23 mm (8-9/1,000 inch). Polyurethane foam dressings were used on top[6]. The dressings were applied one to two days after split-thickness skin harvest and were not re-applied thereafter[6]. The main findings of those three publications are discussed below.

Enhanced healing time

Within 11.5 days (range 10-16 days) after application of Kerecis® Omega3 Wound Matrix, Alam *et al.* observed 100% re-epithelialization in their case series of ten patients[15]. Here, re-epithelialization was assessed solely visually without using objective assessing methods. In their comparative cohort study, Badois *et al.* measured the remaining wound area at their widest and longest point between Kerecis® Omega3 Wound Matrix and the standard of care treatment with fatty gauze[24]. The average re-epithelialization time after application of Kerecis® Omega3 Wound Matrix was nearly three times longer (31.5 ± 24.7 days) than

reported by Badois *et al.* Despite not yielding statistical significance, donor sites dressed with the standard of treatment (fatty gauze) healed remarkably slower (67.9 ± 66.2 days) than the Kerecis® Omega3 Wound Matrix -treated (31.5 ± 24.7 days) donor sites[24].The time until re-epithelialization (over 95% of wound area) was assessed for donor sites treated with Kerecis® Omega3 Wound Matrix, ProHeal® Collagen Wound Dressing and those left untreated[6]. The inter-group differences were less drastic than in the above-mentioned studies. Nonetheless, Kerecis® Omega3 Wound Matrix-treated donor sites still healed faster by four days compared to ProHeal® Collagen Wound Dressing. The average wound healing time using Kerecis® Omega3 Wound Matrix was 9.1 ± 1.0 days, compared to 11.9 ± 1.4 days with no wound treatment and 13.1 ± 1.4 days using ProHeal® Collagen Wound Dressing ($P < 0.001$, respectively)[6].

Reduction in pain intensity

Two clinical studies investigated pain intensity at the donor site after the application of Kerecis® Omega3 Wound Matrix[15,24]. Alam *et al.* investigated pain levels using the Verbal Rating Scale (VRS) with a scale between 0 and 10. The authors reported an average pain score of 2.3 (1-4 range) after seven days of product use[15]. Badois *et al.* compared the pain intensity using the VAS (scale 0-10) five days after surgery between Kerecis® Omega3 Wound Matrix and fatty gauze (control group). In the control group, 40% of patients claimed a VAS score ≥ 3, which represents the threshold for clinically relevant pain[24,39]. That was not the case in the Kerecis® Omega3 Wound Matrix-treated group, where no patient reported a VAS score of ≥ 3 after the fifth day post-surgery[24]. This emphasizes and supports the positive effect of FSGs on pain management. The underlying mechanism of action for pain relief is not yet entirely understood.

No evidence of infections at donor sites

Alam *et al.* and Badois *et al.* observed no signs of infection at donor sites treated with Kerecis® Omega3 Wound Matrix[15,24]. In contrast, 60% of the patients dressed with fatty gauze did show signs of infection[24].

Fish skin promotes regeneration and reduces microbial burden in combat injuries and full-thickness burns

The conditions on and around a battlefield present a unique set of challenges for wound treatment. Limitations in logistics and supply, lacking facilities, and a high case rate are some of the factors favoring less-than-optimal treatment, with the injuries themselves often presenting as complicated, affecting deep tissues, covering a large surface area, and necessitating adequate early care to expedite recovery and reduce the risk of serious infection[20,25]. Civilian casualties have more and more emerged as major victims in war. In World War I, the rate of nonmilitant casualties reached approximately 20%, with the estimated rate in recent conflicts around 80%[25]. A good product should have the following qualities: help condition wounds currently unfit for coverage; help stabilize wounds that are fit for coverage but cannot currently be covered; help reduce or prevent wound infection. Two studies were identified focusing on the treatment of combat injuries and/or full-thickness burns[20,25].

Reda *et al.* described a case series and reported on the forward deployment of Kerecis® Omega3 Wound Matrix in the setting of the 2020 Nagorno-Karabakh War, with the primary goal of using Kerecis® Omega3 Wound Matrix in war victims where skin grafting required prior wound stabilization and conditioning[20]. Secondary goals included reduced healing time, reduced time to skin grafting, and improved aesthetic results[20]. Wounds included expansive full-thickness burns and blast wounds, predominantly treated 3 to 5 days post injury. The wounds received additional debridement where needed, followed by Kerecis® Omega3 Wound Matrix and negative pressure wound therapy (NPWT) application[20]. The formation of granulation tissue several days to weeks earlier was described in all cases treated with Kerecis® Omega3 Wound Matrix, as well as an absolute lack of infection, enabling a step down in the reconstructive ladder and earlier skin grafting. However, this study lacked a control group[20]. Kerecis® Omega3 Wound Matrix was able to provide

favorable characteristics for use in a front-line environment, including low weight, ease of transport, ruggedness, and easy handling. The authors noted that provided with sterile conditions for debridement, sterile saline solution, and room temperature dry storage, Kerecis® Omega3 Wound Matrix can easily be utilized[20].

Smolle *et al.* reported on a 17-year-old female injured by a landmine in the War in Ukraine with mostly deep dermal to full-thickness burns of a TBSA of 17 % and associated acute kidney injury (acute kidney injury I stage 3) on continuous hemodialysis, with prior craniotomy and intracerebral shrapnel removal in Ukraine and a longitudinal tracheal rupture[25]. The patient was transferred to the author's institution 13 days after trauma[25]. With the remaining burn wounds already partially covered by STSG 19 days after injury, wounds of the epifascially debrided right upper extremity were still coated by a meaningful amount of debris and therefore debrided and treated with NPWT. Wound swabs of the right arm revealed multi-resistant gram-negative (4MRGN) *Acinetobacter baumannii*, 4MRGN *Pseudomonas aeruginosa*, *Fusarium species*, and mold fungus[25]. The empiric antimicrobial therapy of voriconazole, linezolid, and meropenem was swapped for a focused regimen consisting of polymyxin B, cefiderocol, and isavuconazole. 26 days after injury, the wounds of the right upper extremity, despite improvement, continued to lack conditions suitable for skin grafting and still tested positive for 4MRGN *Pseudomonas aeruginosa*. To facilitate wound conditioning and suppress wound colonization, Kerecis® Omega3 Wound Matrix was applied[25]. Six days after application, the wounds showed sufficient granulation and were able to be covered using split-thickness skin grafts, with a subsequent excellent graft take[25]. The authors argued that the omega-3 fatty acids contained in Kerecis® Omega3 Wound Matrix possess anti-inflammatory and antiseptic qualities[25]. Both studies report promising findings on the use of Kerecis® Omega3 Wound Matrix in conflict areas or with victims of armed conflict, but lack any kind of control group. While their findings seem to be supported by literature, further clinical trials in the field are necessary. It is worth noting that while many of today's conflicts take place in low- and middle-income countries, Kerecis® Omega3 Wound Matrix is still associated with a high cost, possibly rendering the product prohibitively expensive for many regions in war. However, Kerecis® claims to donate some of their product to victims of conflict and natural disasters (https://www.kerecis.com/charitable-use/).

Yoon *et al.* presented a case of a 52-year-old female with full-thickness contact burn wounds and exposed adipose tissue on her left thigh[6]. Five days after surgery, Kerecis® Omega3 Wound Matrix was applied to the wound in combination with NPWT. By the 13th and 14th postoperative day, plentiful granulation tissue was observed. In further consequence, the wound was suitable for STSG[6].

FSGs accelerate healing in acute full-thickness wounds

When it comes to acute wounds, a distinction needs to be made between simple wounds, which are limited to the superficial dermis, and complex wounds, which also affect deeper tissue structures such as fascia, muscle, tendon or bone and create significant deficits in contour and/or function[40]. Complex soft tissue wounds often demand extensive reconstructive procedures involving pedicled or free flaps[41]. Skin substitutes promoting regeneration and new tissue formation could potentially allow for a step-down in the reconstructive ladder in certain cases. One clinical study investigated the regenerative potential of Kerecis® Omega3 Wound Matrix in acute full-thickness wounds.

Kirsner *et al.* reported on a prospective, double-blinded RCT comparing the usage of Kerecis® Omega3 Wound Matrix and dehydrated human amnion/chorion membrane allograft (dHACM) (EpiFix® MiMedx Group Inc.) on acute full-thickness defects measuring 4 mm in diameter in otherwise healthy individuals[11]. They defined the primary endpoint as time to full epithelialization at set fixed time points and the secondary

endpoints as epithelialization at the 28th day, erythema, pain, infection and price comparison of both treatment groups[11]. Wounds treated with Kerecis® Omega3 Wound Matrix showed significantly shorter healing times ($P = 0.0014$) compared with dHACM-treated defects, with an expected healing time of 22 days for 50% of defects treated with Kerecis® Omega3 Wound Matrix versus 24 days for dHACM[11]. No significant difference was observed between both groups regarding adverse reactions. While Kerecis® Omega3 Wound Matrix required on average 1.6 applications per participant and dHACM 1.4 applications, a significant overall increase in the cost to treat was observed in the dHACM group compared to Kerecis® Omega3 Wound Matrix ($P < 0.001$), with 76% higher costs[11].

Adverse events and safety of FSG

No adverse events were observed across the included studies. No patient showed any reaction associated with the two different types of FSGs that were discussed here. This indicates a high safety level for patients. Moreover, there were no incidences of infection in wounds that were devoid of signs of infection prior to the application of the product.

Conclusion

FSGs seem to be a promising new tool in the field of burn care and complex trauma wounds, which have been shown to be beneficial for easy- and hard-to-heal wounds likewise[20,24,25]. Most studies on SPTB have been conducted with NTG, whereas Kerecis® Omega3 Wound Matrix was used in all studies on donor sites[15,19,23,24]. Despite the lack of comparability, both types of wounds can be considered easy-to-heal wounds, in which similar beneficial effects on pain control, re-epithelialization time, and the required number of dressing changes were achieved through both Kerecis® Omega3 Wound Matrix and NTG[15,18,24]. The reduced number of dressing changes, especially in large-scale burns, seems to be one of the main advantages of both types of acellular FSGs[14]. However, in donor sites, careful cost-benefit considerations should be made when using a costly product such as Kerecis® Omega3 Wound Matrix. Unfortunately, no direct comparison between Kerecis® Omega3 Wound Matrix and NTG can be made due to the different indications, study designs, and different control products.

In the context of deep dermal burns and complex trauma wounds, FSGs might allow for a step-down of the reconstructive ladder. Some authors were able to use Kerecis® Omega3 Wound Matrix on priorly debrided deep partial-thickness burns as an alternative to autografting with superior long-term results to autografts[16]. Lima Júnior *et al.* showed in their RCT that in small-size deep partial-thickness burns, conservative treatment with NTG performed well in terms of re-epithelialization time, pain control, and the frequency of dressing changes[14]. Kerecis® Omega3 Wound Matrix might also have beneficial impacts on the healing outcomes in full-thickness burns[25]. As the product seems to strongly promote new tissue formation[11], it can be used after deep excision and before grafting in order to minimize contour deficits[25]. The same applies to complex trauma wounds, where wound conditioning and new tissue formation to reliably cover and protect deeper structures could allow for less invasive reconstructive surgery[20]. Most studies on deep dermal burns and complex wounds were conducted with Kerecis® Omega3 Wound Matrix. Nevertheless, the question of which fish skin product to use is irrelevant in most cases, as only Kerecis® Omega3 Wound Matrix is FDA-approved and available worldwide. NTG seems to be a cost-effective alternative for countries that have direct access to the fish.

However, again, among the available studies, there is a limited number of high-quality studies, such as RCTs. In order to generate a meaningful treatment algorithm and to make use of the full potential of the product, more high-quality studies are urgently needed.

Limitations

Within this systematic review, we only searched two common literature databases: PubMed and Web of Science. This leaves the risk of studies being missed. Three out of four RCTs included in this study were all performed by the same research group, and only NTG was used here. Only 7 out of the 11 included studies investigated FSG in a controlled clinical setting, but lacked big cohorts and longtime follow-ups. The other included studies were case reports or series. Moreover, there is a paucity of recent publications; most included studies (9/11) are over two years old. Therefore, the results presented in this systematic review should be interpreted with caution.

DECLARATIONS

Authors' contributions

Conceptualization, review and editing: Pignet AL, Kamolz LP
Methodology: Pignet AL, Hecker A, Kamolz LP
Writing: Pignet AL, Voljc T, Carnieletto M, Watzinger N
All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

All relevant information for this systematic review is either part of the manuscript, figures, tables, or digital supplemental content. Any additional information can be found on the PROSPERO protocol for this paper. If any further information is required or unclear, the reader is more than welcome to contact the corresponding author for clarification.

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Consent for publication

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