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Review

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Stem cells and periodontal regeneration: present and future

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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-

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[1,2], may cause edentulism, and has been listed as a risk factor for major systemic diseases, such as atherosclerosis, cardiovascular diseases[3], diabetes[4], and rheumatoid arthritis[5,6]. This may directly influence the general health, social life, and nutritional state of affected individuals, jeopardizing their overall quality of life[7-10]. 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[11].

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 ≤ 6 mm[12,13]. 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[14]) throughout a life-long supportive care program, which is generally enough to prevent masticatory dysfunction. However, patients who still present multiple PDs ≥ 5 mm, or even one PD ≥ 6 mm after non-surgical therapy, take significant risk to experience disease progression and require additional surgical treatment[15,16] 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[17]. 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[18,19]. 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[20].

Periodontal regeneration has been shown to be effective in the treatment of intrabony and furcation defects with varying degrees of efficacy[21,22]; 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[23]. 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[24]. To overcome these limitations, new access flaps[25-28] and biological agents[29,30] 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[31], 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[32]. In periodontal regeneration, mesenchymal stem cells (MSCs) have been tested *in vitro* and in humans with promising results[33]. MSCs from dental and non-dental tissues have been harvested and used[34]. Among MSCs from dental tissue, we may list dental pulp stem cells (DPSCs)[35], human exfoliated deciduous teeth cells (SHEDs)[36], periodontal ligament stem

cells (PDLSCs)[37], dental follicle precursor cells (DFPCs)[38], and stem cells from apical papilla (SCAPs)[39]. 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[40]. 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[41] and still show potential to differentiate into osteoblast-like cells. Second, DPSCs share origin, antigenic pattern, and differentiation lineages with periodontal stem cells[35,40,41]. 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[42]. Third, they easily interact with biomaterials[43,44].

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[45] and could express an immuno-regulatory potential on T cells, macrophages, and dendritic cells[46]. Nakamura *et al.*[47] 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*[48]. 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[49], 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[50].

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[51,52] and periodontal tissue cells and can enhance periodontal regeneration[53,54]. 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[55].

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[56]. 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[57,58]. 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[59]. 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[60,61], and iPCSs 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)[62,63]. 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[64]; the isolation and expansion

Table 1. Summary of available evidence for periodontal regeneration with mesenchymal stem cells

	Bone regeneration	PL	Cementum
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 reservable 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 reservable 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[65]; the way of delivery and implantation in combination with membranes[66]; and biomaterials[67,68] or bioactive molecules[69,70].

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 (≤ 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[64].

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[64].

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[71]; 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 rational 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[30]. Similarly, these effects have been found in cell-based regenerative therapies, with promising results from beta-TCP[46,72-74], hyaluronic acid[75,76], and nano designed materials[77-79].

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 *et al.*[72] 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 *et al.*[53] 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)	Defects treated with MSC showed newly formed of cementum, PL and alveolar bone
Simsek *et al.*[70] 2012	BMSC	PRP autologues	DOG Mongrel	6 (a, b, c, d, e)	Class II furcation defects 5 mm × 2 mm	(a) BMSC+PRP (b) autogenous cortical bone + PRP (c) PRP (d) autogenous cortical bone (e) no treatment	2 months	-Histological: HES -Histomorphometric	All the groups with PRP showed regeneration of bone and cementum. BMSC showed the best potential for periodontal regeneration.
Paknejad *et al.*[68] 2015	BMSC	Demineralized bovine bone matrix	DOG Mongrel male 14-22 kg	9 (a, b)	Intrabony defects 4 mm × 4 mm	(a) BMSC + Demineralized bovine bone matrix (b) Demineralized bovine bone matrix	2 months	-Histological: HES -Histomorphometric	Test group showed higher percentage of cementum and PL regeneration. Bone formation was equal in test and control group
Hung *et al.*[85] 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	Genetic expression of ASCs and DPSCs are similar. Transplanted ASCs regenerate PL, and alveolar bone
Ding *et al.*[86] 2010	PDLSC	HA/β-TCP + gelatin membrane	MINIATURE PIG female Wuzhishan, male Guizhou 6-8 months old	6 (a, b, c, d, e)	Intrabony defects 3 mm × 7 mm × 5 mm	(a) PDLSC autologous + HA-β-TCP (b) PDLSC allogenic + HA-β-TCP (c) PDLC autologous and allogenic + HA-β-TCP (d) HA + β-TCP (e) empty defect	3 months	-Clinical: CAL, PD, GR, blood and biochemical tests -Histological: HES -Histomorphometric -Radiological: CT	Bone, cementum and ligament resulted regenerated in both allogenic and autogenous PDLSC
Fu *et al.*[87] 2014	PDLSC SHED	HA/β-TCP	MINIATURE PIG female 9-12 months old	6 (a, b, c)	Intrabony defects 5 mm × 7 mm × 7 mm	(a) PDLSC + HA-β-TCP (b) SHED + HA-β-TCP (c) HA-β-TCP	3 months	-Clinical: CAL, PD, GR -Histological: HES -Radiological: CT	Both cell types improved regeneration
Gao ZH *et al.*[91] 2016	PLSC DPSC	HA/TCP	MINIATURE PIG 18 months old	46 (a) 9 (b)	Root-shaped implant socket 4.1 mm × 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	PL and bone tissue had been generated in both groups

Tsumanuma *et al*.[88] 2016	PDLSC	PGA/β-TCP/ collagen and absorbable membrane (GTR)	DOG Beagle	8 (a, b, c)	Intrabony defects 5 mm × 5 mm	(a) PDLSC autologous/PGA + β-TCP + collagen (b) PDLSC allogenic/PGA + β-TCP + collagen (c) β-TCP + collagen	2 months	-Histological: Azan staining -Enzyme-linked immunosorbent assay: CRP, IL-10, IFN-c, CD30 -Histomorphometric -Radiological: micro-CT	No differences were found between autologous and allogenic groups. a) and c) showed collagen fibers oblique and parallel to the root. b) had perpendicularly inserted fibers in new cementum
Zhu *et al*.[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 × 5 mm	(a) jBMSC + PDLSC + TDM + CA (b) iBMSC + PDLSC + TDM + CA	3 months	-Histological: HES, Masson's trichrome -Immunohistochemical: Col1	PL regeneration was found in both groups
Nuñez *et al*.[90] 2012	CDC PDLSC	Collagen	DOG Beagle male 1 year old	8 (a, b, c)	Intrabony defects 3 mm × 4 mm	(a) CDC + collagen (b) PDLSC + collage (c) collagen	3 months	-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 *et al*.[95] 2005	PDLC	Hyaluronic acid sheet	DOG Beagle female 3 years-old	5 (a, b)	Dehiscence defects 5 mm × 5 mm	(a) PDLC + hyaluronic acid sheet (b) hyaluronic acid sheet	2 months	-Histological: HES, Masson trichrome staining -Histomorphometric	Group with PDLC showed more cementum, bone and ligament formation
Nakahara *et al*.[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 × 4 mm	(a) PDLSC + atecollagen (b) empty	1 month	-Histological: HES, Masson's trichrome staining -Histomorphometric	No differences in bone formation between the groups, but PDLSC showed more cementum formation
Iwata *et al*.[97] 2009	PDLC	PGA/β-TCP	DOG Beagle male	4 (a, b)	Intrabony defects 5 mm × 5 mm × 4 mm	(a) PDLC + PGA + β-TCP (b) PGA + β-TCP	1, 5 months	-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 *et al*.[140] 2008	PDLSC	HA/β-TCP + gelatin membranes	MINIATURE PIG 12 months old	24 (a) 12 (b, c)	Intrabony defects 7 mm × 3 mm × 5 mm	(a) PDLSC + HA-β-TCP (b) HA-β-TCP (c) no treatment	3 months	-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 *et al*.[98] 2013	PDLC	AC	DOG 18-36 months old	5 (unclear)	Intrabony defects 4 mm × 5 mm	(a) PDLC + AC (b) AC + PBS (c) no treatment	1 month	-Histological: HES -Histomorphometric -Radiological: dental X-ray	Cementum, bone heights and surface were more significant in group a
Khorsand *et al*.[67] 2013	DPSC	3-4 Demineralized bovine bone matrix granules	DOG Mongrel male 1-2 years old	10 (a, b)	Intrabony defects 3 mm × 5 mm × 8 mm	(a) DPSC + Demineralized bovine bone matrix (b) Demineralized bovine bone matrix	2 months	-Histologic: HES -Histomorphometric	Bone formation was similar between the groups. Cementum and ligament were regenerated more in the test group

Fu *et al.*[87] 2014	PDLSC SHED	HA/β-TCP	MINIATURE PIG female 9-12 months old	6 (a, b, c)	Intrabony defects 5 mm × 7 mm × 7 mm	(a) PDLSC + HA-β-TCP (b) SHED + HA-β-TCP (c) HA-β-TCP	3 months	-Clinical: CAL, PD, GR -Histological: HES -Radiological: CT	PDLSC and SHED significantly improved periodontal regeneration
Fawzy El-Sayed *et al.*[104] 2012	GMSC	Demineralized bovine bone matrix + collagen membrane	MINIATURE PIG 1 female and 7 males 1841 months old	8 (a, b, c, d, e, f)	Intrabony defects 3 mm × 7 mm × 5 mm	(a) GMSC + Demineralized bovine bone matrix (b) GMSC + collagen scaffold (c) Demineralized 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	Better results were found for groups with GMSC
Ozasa M *et al.*[139] 2014	ASC	Fibrin gel	DOG Beagle female 50-56 months old	unknown	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 *et al.*[103] 2013	ASC	PRP gel	DOG Beagle 9 or 10 months old	8 (a, b, c)	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; PDLSC: periodontal ligament stem cells; PDLC: 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

BMMSCs 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[82]. 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[68]. 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[83,84]. Finally, ASCs in extraction sockets in a rabbit model showed potential for the regeneration of the alveolar bone structure[85].

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[86-91], as reported in a recent systematic review of pre-clinical studies[92]. Importantly, PDLSCs have unique properties to form a cementum/PL complex-like structure when ectopically transplanted in animals[93,94].

In fenestration defects (applied with hyaluronic acid sheet), they showed significantly greater formation of cementum, bone, and periodontal ligament than in control group[95]. 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)[96]. 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[90]. 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[86,97]. The benefits were less pronounced but still maintained when used with non-supportive biomaterials[90,98]. PDLSCs' protocols without manipulation before direct implantation have been tested and proven effective, thus making the use of this cell type more sustainable[95].

Dental pulp stem cells

DPSCs are another well studied lineage in the animal model. These cells were isolated almost 20 years ago[35] and found to be capable of forming lamellar bone after grafting[43,99]. Their use in different ways (injected, organized in sheet, and on different carriers) showed potential in regenerative procedure[67]. 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[67], 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[100].

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[46]. 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[89].

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[101].

Other cells

Other stem cell types are less studied, yet have positive effects, especially APCs[102], ASCs[103], and GMSCs[104]. 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 form 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[105,106] and three case series[107-109]. One of the case reports provided positive results for allogenic transplantation of DPSCs[106]. In parallel, some initial reports in humans demonstrated the clinical and radiographic efficacy of dental pulp micrografts in post-extraction alveolar defects[110,111]. 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[112]. 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[113]. Later on, in a case report[114], 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[115-118] and Grade III furcation defects[119].

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[120-123] have been published on assessing the efficacy of stem cells in periodontal regeneration, compared to open flap debridement[121,123] or compared to the use of a scaffold alone without

stem cells[120,122] 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[121] 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[120] 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[122] 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[123] 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[113], 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[121,122]; 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[124].

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[125]. 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 linage 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 *et al*.[112] 2005	RCT with a split mouth design	PDLSC	Direct application of PDL tissue collected from an extracted molar, into the defect	Class II mandibular furcation defects	10 patients 20 defects: 10test/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 *et al*.[120] 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 *et al*.[123] 2015	Parallel designed RCT	UC-MSC	Collection from the hospital in a sterile tube Followed by isolation and culture on β-TCP scaffold	Intrabony defects	14 patients 24 defects: 12 test/ 12 control	Test: ODF applying allogeneic UC-MSCs on a β-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 *et al*.[122] 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 ≥ 4 mm were significantly more frequent in the test group. No adverse effects were reported

Shalini *et al*.[121] 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/114 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[69]. 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[126,127]. Furthermore, iPSCs can differentiate into cells that promote tooth regeneration[128,129].

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[130]. 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-1α (SDF-1α). 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[131]. SDF-1α-loaded gelatin sponges have been proven to enhance bone and PL regeneration[132]. 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[133,134].

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[135]. Vertical layering of PDLSCs sheets, woven polyglycolic acid, and porous beta-TCP demonstrated the recreation of bone and cementum with inserted collagen fibers[97] 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[136].

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[137].

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)[138]. 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

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All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

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