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Potential for Stem Cell-Based Periodontal Therapy

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Abstract

Periodontal diseases are highly prevalent and are linked to several systemic diseases. The goal of periodontal treatment is to halt the progression of the disease and regenerate the damaged tissue. However, achieving complete and functional periodontal regeneration is challenging because the periodontium is a complex apparatus composed of different tissues, including bone, cementum, and periodontal ligament. Stem cell-based regenerative therapy may represent an effective therapeutic tool for periodontal regeneration due to their plasticity and ability to differentiate into different cell lineages. This review presents and critically analyzes the available information on stem cell-based therapy for the regeneration of periodontal tissues and suggests new avenues for the development of more effective therapeutic protocols.

Keywords

Periodontal Regeneration; Cell-based Therapy; Stem Cells; Periodontal Diseases; Induced Pluripotent Stem Cells; Periodontal Ligament Stem Cells; Skeletal Stem Cells

INTRODUCTION

Periodontal diseases are characterized by bacterial-induced chronic inflammation that cause destruction of tooth supporting structures, including periodontal ligament (PDL), cementum, and alveolar bone (Pihlstrom et al., 2005). Periodontal diseases are highly prevalent, and in fact severe periodontitis is the sixth-most prevalent health condition worldwide (Kassebaum et al., 2014). Current scientific evidence highlights the association and possible cause-effect correlation between periodontitis and other high prevalence diseases, such as diabetes, cardiovascular diseases, chronic kidney diseases, and pulmonary infections (Borgnakke et al., 2013; Fisher et al., 2008; Friedewald et al., 2009; Scannapieco et al., 2003). Thus,

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periodontal disease is an important public health issue and the development of effective therapies to treat periodontal disease should be a major goal of the scientific community.

The goal of periodontal treatment is to stop the progression of the disease and regenerate the structure and function of the damaged tissues. Conventional non-surgical or surgical treatments, such as scaling and root planing, open flap debridement, and osseous surgery can control the progression of periodontal disease (Salvi et al., 2014). However, achieving complete and functional periodontal regeneration is still challenging (Giannobile, 2014). The challenge in periodontal regeneration lies in the ability to induce the regeneration of a complex apparatus composed of different tissues, including bone, cementum, and periodontal ligament.

Periodontal regeneration has been defined as the regeneration of alveolar bone, PDL, and cementum over a previously diseased root surface (1996). Several treatment modalities have been developed to achieve periodontal regeneration, including guided tissue regeneration, use of bone grafts, application of growth factors and host modulating factors, and the combination of the above methodologies (Reynolds et al., 2015). Although there is some evidence showing that periodontal regeneration can be achieved by employing these techniques, all regenerative treatment modalities have shown limited success, especially in challenging clinical situations (Reynolds et al., 2015). Thus, alternative treatment approaches to achieve predictable periodontal regeneration are still highly desirable.

Recent efforts have focused on cell-based regenerative approaches using stem cells. Stem cells appear to have a promising therapeutic potential in regenerative medicine due to their plasticity and ability to differentiate into different cell lineages, thus providing a cellular source for the regeneration of the different missing periodontal tissues (PDL, cement, and bone). In this review we explore the current and potential applications of stem cell-based therapeutic approaches in periodontal regeneration.

STEM CELL BIOLOGY

A brief introduction to the biology of stem cells is required in order to fully understand the potentials and the efficacy of stem cell-based therapeutic approaches to periodontal regeneration.

Stem cells have two important characteristics: self-renewal and differentiation potential. Self-renewal refers to their ability to renew themselves through mitosis, even after long periods of inactivity (Bianco et al., 2010). The differentiation potential entails stem cells to differentiate into a different phenotype. These two qualities, together, allow stem cells to proliferate and regenerate missing or compromised tissues.

Based on their differentiation potential, stem cells can be categorized in totipotent cells (able to differentiate into cells of all three germ lines as well as cells of the extraembryonic tissue), pluripotent cells (able to differentiate into cells of all three germ lines but not in cells of the extraembryonic tissue), multipotent (able to differentiate into cells of only one or two germ lines), and unipotent (able to differentiate into only one cell type).

Based on their derivation or methods of generation, stem cells are denominated as: 1) embryonic stem cells, 2) post-natal stem cells, and 3) reprogrammed stem cells. Embryonic stem cells are pluripotent cells derived from the inner cell mass of the blastocyst (Thomson et al., 1998). These cells have the ability to form derivatives of all three embryonic germ layers (Thomson et al., 1998). Therefore, embryonic stem cells have great potential for cell-based regenerative therapy. However, the therapeutic use of embryonic stem cells has raised major ethical concerns and other safety concerns such as those related to their immunogenicity and tumorigenicity. (Jung, 2009; Lu et al., 2009). The same ethical concerns are not present with the use of post-natal stem cells. Post-natal stem cells have been isolated from a variety of tissue sources, including bone marrow, epithelium, adipose tissue, liver, nervous system, teeth, and periodontal ligament (Barker, 2014; Caplan, 2007; Codega et al., 2014; Gronthos et al., 2000; Seo et al., 2004). It is generally believed that tissue-resident post-natal stem cells play a role in maintaining tissue homeostasis, physiological tissue renewal, and regeneration after tissue damage (Li and Clevers, 2010). In contrast to embryonic stem cells, post-natal stem cells are multipotent, and therefore are more limited in their differential potential. However, post-natal stem cells may represent a safer approach to stem cell–based tissue regeneration (Jing et al., 2008). Consequently, numerous pre-clinical and clinical studies have investigated the application of these stem cells for cell-based regenerative therapy in a variety of conditions.

Among the large variety of post-natal stem cells that can be found within the human body, skeletal stem cells (SSCs) (Bianco and Robey, 2015) are of interest to periodontists because of their potential to regenerate periodontal tissues (Hynes et al., 2012; Seo et al., 2004). SSCs are multipotent adult stem cells able to form mesenchymal and connective tissues (Pittenger et al., 1999). They can differentiate into at least three mesenchymal cell lineages, including osteoblasts, chondrocytes, and adipocytes (Huang et al., 2009; Pittenger et al., 1999). SSCs were initially isolated from bone marrow more than 50 years ago (Becker et al., 1963). Since then, SSC-like cells have been isolated from several tissues using various methods of isolation and expansion, and therefore, in order to standardize the isolation and preparation methods, the International Society for Cellular Therapy proposed the following criteria to identify human SSCs (defined by the Society as adult mesenchymal stem cells): 1) Adherence to plastic when maintained in standard culture conditions; 2) Expression of markers including CD105, CD73 and CD90, and lack expression of hematopoietic cell markers such as CD45, CD34, CD14 or CD11b, CD79alpha or CD19 and HLA-DR; 3) Capacity to differentiate into osteoblasts, adipocytes and chondroblasts in vitro (Dominici et al., 2006).

Reprogrammed stem cells are cells whose genetic program is modified to induce a switch from one cell phenotype to another (Gurdon and Melton, 2008). Cell reprogramming can be achieved using the following four different methodologies (discussed in more details elsewhere (Gurdon and Melton, 2008; Intini, 2010)): a) nuclear transfer from somatic cells to oocytes, b) overexpression of certain genes or modulation of certain signaling pathways, c) lineage switching, and d) direct conversion. Takahashi and Yamanaka were the first to induce plasticity in mouse fibroblasts by viral transduction of four genes named *Oct 3/4*, *Sox2*, *c-Myc*, and *KLF4* (Takahashi and Yamanaka, 2006). The transduced cells, named

induced pluripotent stem (iPS) cells, exhibited plasticity similar to that observable in embryonic stem cells, thus, by all means, iPS cells may be considered as pluripotent stem cells. Since their inception, extensive efforts have been made to improve the iPS technology and to develop iPS cell-based therapeutic approaches for regeneration of a wide variety of tissues (Duan et al., 2011; Revilla et al., 2015). Further improvement of iPS-related technologies could lead to development of effective, safe, and ethically acceptable stem cell–based therapeutic approaches for regeneration of desired tissues.

This review intends to introduce the readers to the present available information on the use of stem cell-based therapy for periodontal regeneration. We present and critically analyze the current scientific evidences on the use of non dental- derived post-natal stem cells, dental-derived adult stem cells, and iPS-derived cells for the regeneration of periodontal tissues.

NON DENTAL-DERIVED POST-NATAL STEM CELLS

In this section we review the periodontal tissue regenerative potential of non dental- derived adult stem cells, including bone marrow-derived skeletal stem cells (BMSSCs) and adipose tissue-derived stem cells (ATSCs).

Bone Marrow Skeletal Stem Cells for Periodontal Regeneration

Bone marrow-derived skeletal stem cells (BMSSCs) are adult multipotent cells that can differentiate into cells identified as components of the periodontal tissues (Huang et al., 2009; Pittenger et al., 1999). The potential of BMSSCs for periodontal regeneration has been widely investigated, and multiple periodontal defects have been treated by autologous or allogeneic skeletal stem cells derived from bone marrow (Table 1) (Chen et al., 2008; Du et al., 2014; Hasegawa et al., 2006; Kawaguchi et al., 2004; Li et al., 2009; Tan et al., 2009; Wei et al., 2010; Yang et al., 2010; Yu et al., 2013; Zhou et al., 2011; Zhou and Mei, 2012).

Autologous BMSSCs are usually isolated from iliac crest bone marrow (Pittenger et al., 1999). The capacity of these cells to regenerate periodontal tissue has been demonstrated in several studies (Hasegawa et al., 2006; Kawaguchi et al., 2004; Li et al., 2009; Wei et al., 2010). For instance, Kawaguchi and colleagues evaluated the potential of autologous BMSSCs isolated from iliac crest to regeneration furcation periodontal defects in a beagle dog model of periodontal defect (Kawaguchi et al., 2004). They created class III furcation defects surgically, and treated the so created defects with BMSSCs delivered by means of a collagen gel carrier. Although a complete regeneration of defects was not achieved, tissue regeneration was marked by formation of new bone, cementum, and Sharpey’s fibers inserted into the cementum (Kawaguchi et al., 2004). It should be noted, however, that, with the exception for the adherence to plastic when maintained in standard culture conditions, cells used in the Kawaguchi’s study do not meet the definition criteria of skeletal stem cells, as in fact they were not selected for the expression of the markers proposed by the International Society for Cellular Therapy (Dominici et al., 2006). In addition, the ability of these cells to effectively differentiate into periodontal tissues was not properly reported in this study. Therefore, results of this study should be interpreted with caution.

To understand the behavior and fate differentiation of autologous bone marrow skeletal stem cells into periodontal tissue after transplantation into periodontal defects, labeling experiments have been performed (Hasegawa et al., 2006; Wei et al., 2010). For instance, using a canine model, Wei and colleagues labeled BMSSCs isolated from tibias with bromodeoxyuridine (BrdU) (Wei et al., 2010). The labeled autologous BMSSCs were transplanted into surgically created class III furcation defects using alginate gel as a delivery vehicle. Although not contributing to the actual formation of the regenerated tissues, this study indicates that the transplanted cells migrated into PDL, cementum, alveolar bone, and blood vessels. It was only demonstrated that some of the labeled cells expressed markers of mature osteoblasts and fibroblasts, suggesting differentiation of BMSSCs into fibroblasts and osteoblasts after transplantation (Wei et al., 2010).

A limitation of using autologous BMSSCs is due to the fact that the number of autologous stem cells declines by aging (Stenderup et al., 2003). Furthermore, proliferative potential of stem cells is reduced over an extended culture period (Bonab et al., 2006). To overcome these limitations, scientists propose cryopreservation of bone marrow skeletal stem cells as a viable option. The capacity of cryopreserved autologous BMSSCs for periodontal regeneration has been reported by Li and colleagues in a canine model (Li et al., 2009). Li et al compared the regenerative potential of cryopreserved autologous BMSSCs with that of freshly isolated BMSSCs when transplanted into periodontal fenestration defects using collagen sponge scaffolds as a carrier (Li et al., 2009). Regeneration of periodontal defects was observed in both groups. Histomorphometric analysis revealed that amounts of formation of new bone, cementum, and PDL fibers were significantly higher in both groups compared to the control group, which was treated with scaffold alone, and no significant differences were found in the regeneration of periodontal tissue between the two test groups, indicating that cryopreservation of BMSSCs may represent a valid alternative to other stem cell-based treatment modalities (Li et al., 2009).

Allogeneic bone marrow-derived skeletal stem cells can be another alternative to autologous BMSSCs. Several studies have tested the potential of allogeneic BMSSCs for regeneration of periodontal defects (Du et al., 2014; Yang et al., 2010; Yu et al., 2013; Zhou et al., 2011). Using a rat model, Yang and colleagues studied the fate and the regeneration potential of allogeneic BMSSCs labeled with green fluorescent protein (GFP) expanded on gelatin beads (Yang et al., 2010). This study demonstrated that transplantation of the GFP-labeled cells resulted in regeneration of the defect with significantly more new bone formation and greater number of functionally orientated periodontal ligament fibers compared to defects treated with gelatin beads alone; however, no significant difference was found regarding formation of new cementum between the groups (Yang et al., 2010). They also found that GFP-labeled cells were integrated into newly formed periodontal tissue (Yang et al., 2010), indicating the direct contribution of transplanted allogeneic BMSSCs to the regeneration of the periodontal defects. Interestingly, tracking of GFP labeled BMSSCs after intravenous administration of the cells in a mouse model also demonstrated that these cells contributed to periodontal regeneration and differentiated into tissue specific cells including PDL fibroblasts and osteoblasts (Zhou et al., 2011). In this respect, it is, however, important to mention that isolation and enrichment of bone marrow-derived skeletal stem cells result in a heterogeneous cell population (Ho et al., 2008; Kassem and Abdallah, 2008). Therefore, it is

not clear whether new bone, cementum, and periodontal ligament were formed by multipotent cells or by lineage-specific progenitor cells within the heterogeneous cell population.

Signaling molecules such as growth factors, cytokines, and chemokines, are important factors implicated in tissue regeneration and can therefore be employed to modulate cellular functions including cell proliferation and differentiation (Yoshida and Fujii, 1999). One of the approaches in cell-based therapy is based on the use of genetically-manipulated cells as carriers for the delivery of signaling molecules (Lin et al., 2015). Hence, the delivery of regenerative signaling molecules using BMSSCs as vehicle for periodontal cell-based regenerative therapy has also been investigated (Chen et al., 2008; Tan et al., 2009; Zhou and Mei, 2012). Chen and colleagues, using a rabbit model, demonstrated that transplantation of BMSSCs engineered to over-express bone morphogenetic protein-2 (BMP-2) enhanced periodontal regeneration by forming more new bone than the un-manipulated BMSSCs (Chen et al., 2008). Another study by Tan and colleagues evaluated the potential of BMSSCs engineered to over-express basic fibroblast growth factor (bFGF) for treatment of class III furcation defects in a canine model of periodontal defect (Tan et al., 2009). These authors reported that periodontal regeneration was evident in sites treated with bFGF-overexpressing BMSSCs or BMSSCs alone, while more new bone formation was observed in sites treated with the bFGF-overexpressing BMSSCs (Tan et al., 2009). Despite the promising results, the current available data on the efficacy of delivery of signaling molecules using post-natal stem cells in the treatment of periodontal defects are still very limited, and more studies are needed to confirm the efficacy and the safety of this treatment approach.

It is worth to mention that the surgically created periodontal defects in preclinical animal models do not accurately resemble the defects induced by periodontal disease. Human periodontal lesions are associated with inflammation, and the defects are populated with pathogenic biofilm, granulation tissue, calculus, and plaque (Pihlstrom et al., 2005; Socransky and Haffajee, 1992). To recreate this extremely contaminated environment while developing a periodontal defect with reproducible defect morphology is extremely difficult. Thus, the above-mentioned pre-clinical studies may still have limited efficacy when translated in human clinical trials. Clearly, the promising results of the mentioned preclinical studies must be further confirmed by clinical studies.

One of the few clinical studies reporting on the use of BMSSCs for the treatment of periodontal defects is a case report published by Yamada and colleagues (Yamada et al., 2006). These authors reported successful treatment of intrabony periodontal defects in one patient using the local application of a combination of expanded autologous BMSSCs harvested from iliac crest and platelet-rich plasma (PRP) (Yamada et al., 2006). The same cell transplantation approach was utilized by Yamada et al in a larger scale clinical study with 104 subjects treated for alveolar bone regeneration, sinus floor elevation, ridge preservation, and regeneration of periodontal defects (Yamada et al., 2013). 17 patients in this study received the stem-cell transplantation for the treatment of periodontal defects. The clinical outcomes revealed a significant improvement in clinical periodontal parameters compared to the baseline: an average 5.12 ± 2.45 mm reduction of probing depth, an average

4.29 ± 1.32 mm gain of clinical attachment, and an average 3.12 ± 1.23 mm gain of bone as measured by radiographic evaluations were achieved by using the combination of BMMSCs and PRP (Yamada et al., 2013). It is important to note that no side effects were evident in all 104 participants of this study. Although impressive clinical outcomes were observed, there was no control group in this study, which limits the interpretation of the outcomes. Therefore, further well-designed clinical studies are still needed to establish feasible and safe BMSSCs-based therapeutic approaches for the treatment of periodontal defects.

Adipose Tissue Stem Cells for Periodontal Regeneration

Adipose tissue-derived stem cells (ATSCs) have recently been widely studied as a viable cell source for cell-based regenerative medicine (Locke and Feisst, 2015). These cells have shown to have properties similar to BMSSCs. Several studies have demonstrated the ability of ATSCs to differentiate into adipogenic, chondrogenic, and osteogenic cells as well as myogenic and neurogenic cells (Gimble and Guilak, 2003; Lee et al., 2004; Planat-Benard et al., 2004). ATSCs express mesenchymal cell surface markers such as CD29, CD44, CD71, CD90, CD105, and STRO-1 and lack expression of the hematopoietic markers such as CD31, CD34, and CD45 (Zuk et al., 2002). The use of these cells offers several advantages over the use of BMSSCs, including the easy harvesting process and the minimal donor site morbidity (Huang et al., 2009; Zuk et al., 2002). Therefore, ATSCs represent a highly attractive cell source alternative for stem cell-based therapeutic approaches in periodontology.

Indeed, the potential of ATSCs for regeneration of periodontal defects has been demonstrated in some preclinical studies (Table 2) (Akita et al., 2014; Tobita et al., 2008; Tobita et al., 2013). For instance, Tobita and colleagues isolated ATSCs from inguinal fat pads of Wistar rats, mixed them with PRP, and transplanted them into surgically created fenestration periodontal defects. (Tobita et al., 2008). The results showed that new bone, cementum, and perpendicular periodontal ligament-like fibers were formed 8 weeks after transplantation. No cementum or PDL formation was evident in the defects that did not received the treatment or that were treated with PRP alone (Tobita et al., 2008). Furthermore, labeling of ATSCs with GFP demonstrated that GFP-positive cells were present on the surface of the regenerated alveolar bone and within the PDL structures, suggesting that transplanted ATSCs underwent differentiation, becoming osteocytes or periodontal ligament cells (Tobita et al., 2008). Another study in a murine model published by Akita and colleagues reported similar findings (Akita et al., 2014). Akita and his collaborators isolated the ATSCs using the protocol detailed in Tobita et al (Tobita et al., 2008), and transplanted the isolated ATSCs using poly-lactic-glycolic acid (PLGA) scaffold into surgically created periodontal defects. Histomorphometric analysis revealed that the amount of newly formed cementum and width of regenerated PDL were significantly greater when ATSCs and PLGA were transplanted into the defect then when PLGA alone was used. Histomorphometric analysis and quantitative micro-CT analysis confirmed the existence of significantly higher bone formation in the defects treated with the combination of ATSCs and PLGA (Akita et al., 2014).

In addition to murine models, the efficacy of ATSCs-mediated regeneration of periodontal defects was also reported in a larger animal model (Tobita et al., 2013). Tobita and colleagues, using a canine model, investigated the potential of the combination of autologous ATCSs and PRP for the regeneration of class III periodontal furcation defects. By means of histologic analysis they demonstrated that newly formed PDL ligament were evident only when defects were treated with the combination of ATSCs and PRP, while no newly formed PLD structures were found in PRP-only treated group and in the non-implantation group. However, no significant differences in the amount newly formed bone and cementum were found between groups (Tobita et al., 2013). Thus, despite promising results, data obtained from many pre-clinical studies appear to be still inconsistent. We conclude that the available data in support of their clinical use is still scarce and further preclinical and clinical investigations are needed to fully exploit the potential of ATSCs for periodontal regeneration. Dental-derived multipotent stem cells may provide a suitable and worth to be explored alternative to ATSCs.

DENTAL-DERIVED POST-NATAL STEM CELLS

Dental-derived post-natal stem cells have recently gained a great deal of attention since they can be isolated from tissues that are often discarded in dental clinics and since their isolation presents with lower morbidity compared to non dental-derived post-natal stem cells.

There are many research groups that have investigated the potential of dental-derived post-natal stem cells for periodontal regeneration. In this section we summarize the available information on the potential of these cells for regeneration of periodontal tissue. We analyze results obtained using stem cells isolated from periodontal ligament, dental pulp, exfoliated deciduous teeth, dental follicle, dental apical papilla, and extraction sockets.

Periodontal Ligament Stem Cells for Periodontal Regeneration

It has been proposed that periodontal regeneration is mediated by a heterogeneous cell population present in the PDL that can differentiate into fibroblasts, osteoblasts, and cementoblasts (Melcher, 1976). However, the presence of multipotent stem cells in PDL remained elusive until they were isolated from PDL of extracted human third molars by Seo and colleagues in 2004 (Seo et al., 2004). Seo and colleagues found that human PDL contains a group of cells that express mesenchymal stem cell surface markers such as STRO-1 and CD146. These cells, which were defined as periodontal ligament stem cells (PDLSCs), present with self-renewal ability and have multipotent capacity, being able to differentiate into cementoblasts/osteoblasts, adipocytes, and collagen-forming cells. Furthermore, these cells formed cementum-like and PDL-like structures after ectopic transplantation into the dorsal surface of immunocompromised mice (Seo et al., 2004). This research represents the milestone of all studies investigating the potential of periodontal-derived stem cells for cell-based regenerative periodontal therapy. Subsequent studies focused on the characterization of PDLSCs; for instance several studies compared the properties of these cells with the properties of mesenchymal stem cells or skeletal stem cells. These studies showed that PDLSCs present with self-renewal and multilineage differentiation capabilities, expression of mesenchymal stem cell surface markers such as CD44, CD73, CD 90, CD105, CD106 (VCAM-1), CD146 (MUC-18), and Stro-1, and lack

of expression of hematopoietic markers such as CD31, CD34, and. CD45 (Fujii et al., 2008; Huang et al., 2009; Wada et al., 2009). In addition, PDLSCs possess unique characteristics that make them distinct from other mesenchymal or skeletal stem cells. In fact, it has been shown that these cells have higher proliferation rate than skeletal stem cells derived from bone marrow (Seo et al., 2004). Furthermore, PDLSCs express scleraxis, a tendon/ligament-specific transcription factor, at higher level compared to bone marrow or dental pulp derived stem cells (Seo et al., 2004). It has also been demonstrated that PDLSCs are able to generate PDL attachment in vivo by forming Sharpey's fiber-like collagen bundles that are connected to cementum-like structure (Seo et al., 2004). These unique features of PDLSCs make them a promising cell source for cell-based regenerative periodontal therapy. Hence, several studies have evaluated the potential of these cells for regeneration of periodontal defects (Table 3).

A study by Liu et al used autologous PDLSCs extracted from teeth of miniature pigs for periodontal regeneration in a swine periodontitis model (Liu et al., 2008). The periodontal defect was created by surgically removing alveolar bone around teeth and by subsequently inserting a ligature around them. In this study, the so formed periodontal defect was treated with a combination of alloplasts (hydroxyapatite and tricalcium phosphate) and cultured autologous PDLSCs; results showed newly formed bone, cementum, and periodontal ligament in the treated defect, supporting the feasibility of periodontal regeneration therapies based on the use of ex vivo expanded PDLSCs (Liu et al., 2008). The application of autologous PDLSCs for the treatment of periodontal defects is also reported in one clinical case series (Feng et al., 2010). Feng and colleagues treated intrabony periodontal defects in a limited number of patients with autologous PDLSCs from extracted third molars using hydroxyapatite as a carrier. They reported that periodontal parameters were significantly improved in all shown cases, without any adverse event during 32–72 months of follow-up (Feng et al., 2010). Obviously, further well-designed clinical trials with larger patient population and appropriate controls are needed before drawing a conclusion regarding the clinical efficacy and safety of autologous PDLSCs.

Although autologous PDLSCs have shown promising results in preclinical studies, sources of autologous PDLSCs are limited for clinical applications as isolation of PDLSCs involves extraction of teeth. Furthermore, availability and function of PDLSCs is influenced by the age and disease status of donors (Mrozik et al., 2013). Indeed, it appears that the proliferative capacity, migratory potential, and multi-lineage differentiation ability of PDLSCs diminishes in older compared with younger individuals (Zhang et al., 2012). Hence, additional studies started to investigate the potential of cryopreserved or allogeneic PDLSCs as a feasible alternative cell source for PDLSCs-based regenerative periodontal therapy.

Two studies have shown that human PDLSCs can be recovered from cryopreserved PDLSCs and that cryopreservation does not affect the growth capacity of these cells (Seo et al., 2005; Vasconcelos et al., 2012). The cryopreserved PDLSCs maintained their stem cell characteristics such as expression of STRO-1, multipotent differentiation capacity, and ability to form cementum/periodontal-ligament-like tissues (Seo et al., 2005; Vasconcelos et

al., 2012). These data suggest that utilization of cryopreserved human PDLSCs for cell-based therapy may be a valid clinical approach.

In lack of autologous PDLSCs, allogeneic PDLSCs may represent a valid alternative. A key feature that allows for the utilization of allogeneic PDLSCs is that, similar to BMSSCs, these cells appear to be immunoprivileged (Mrozik et al., 2013). It has also been shown that PDLSCs have the ability to suppress immune responses and inflammatory reactions (Wada et al., 2009). A study by Ding and colleagues compared the autologous and allogeneic PDLSCs for the treatment of induced periodontitis in a swine model of periodontal disease. They demonstrated successful periodontal regeneration of the defects with both autologous and allogeneic PDLSCs. Importantly, they reported that there were no significant differences in percentage of T cell-related immunological markers such as CD3, CD4, and CD8 between the autologous or the allogeneic PDLSCs, suggesting that transplanted allogeneic PDLSCs cause no immunological rejection (Ding et al., 2010a). Similar observation was reported by Mrozik and colleagues using allogeneic PDLSCs to reconstruct surgically created periodontal dehiscence in an ovine model of periodontal defects. Although no immunological evaluations were performed in this study, they reported that allogeneic PDLSC implants were in general well tolerated, as no inflammation, infection, or root exposure was observed in any of the animals (Mrozik et al., 2013). Thus, it seems that allogeneic PDLSCs have the ability to evade or suppress the immune system response.

The putative immunoprivilege status of stem cells has formed the basis for the development of allogeneic stem cell-based therapies in various medical fields. There are several clinical trials that have reported that SSCs may be administered to humans without inducing clinically relevant immune reactions (Ascheim et al., 2014; Hare et al., 2012; Hare et al., 2009). However, it should be noted that there are some studies that have raised concerns over the clinical application of allogeneic stem cells. An animal study by Huang and colleagues reported that there is the possibility that allogeneic bone marrow-derived stem cells lose their immnoprivileged status during differentiation (Huang et al., 2010). They demonstrated that levels of expression of the major histocompatibility complex-Ia and –II are very low in undifferentiated cells, while their level of expression increases significantly after differentiation into endothelial or smooth muscle cells. (Huang et al., 2010). Thus, it becomes crucial to determine, by means of additional studies, if the phenomenon translates into the use of dental-derived stem cells, such as allogeneic PDLSCs.

Dental Pulp Stem Cells for Periodontal Regeneration

Dental pulp-derived stem cells (DPSCs) were the first identified human dental stem cells (Gronthos et al., 2000). Dental pulp contains a heterogeneous population of cells, including fibroblasts, nerve cells, vascular cells, and undifferentiated stem cells or dental pulp stem cells. DPSCs are often found in highly vascularized regions of the pulp and can be collected from the pulp tissue of clinically extracted human teeth of both young and aged individuals by various isolation methods (Huang et al., 2006; Laino et al., 2005). It has been shown that DPSCs are able to differentiate into odontoblast-like cells, osteoblasts, adipocytes, and smooth and skeletal muscle cells (d'Aquino et al., 2007; Karaoz et al., 2010). Additionally, several studies have reported that DPSCs express mesenchymal stem cell surface markers

such as CD10, CD13, CD29, CD44, CD59, CD73, CD90 and CD105, and do not express CD14, CD34, CD45, HLA-DR (Huang et al., 2009; Tran and Doan, 2015).

In vivo ectopic transplantation experiments demonstrated that DPSCs can form a dentin-pulp-like complex associated with vascularized pulp-like tissue (Gronthos et al., 2000), as well as a bone-like tissue (Graziano et al., 2008). However, the potential of DPSCs for periodontal regeneration may be questionable because of their limited capacity to form cementum. In fact, Xu and colleagues have shown that these cells are not able to form distinct cementum-like structure after ectopic transplantation in a rat model (Xu et al., 2009). Another study by Park et al, using a canine model, compared the regenerative potential of autologous DPSCs with that of autologous PDLSCs for the treatment of surgically created periodontal defects (Park et al., 2011). In a 3-mm-wide circumferential periodontal defect, they reported only 0.35 mm attachment gain for sites treated with DPSCs group compared to 3.02 mm gain for sites treated with PDLSCs. Furthermore, histological evaluations revealed that periodontal regeneration was not achieved in DPSCs group, while the defects in PDLSCs groups were regenerated by means of new cementum, bone, and Sharpey's fibers connecting the tooth to the alveolar bone (Park et al., 2011). Therefore, the current evidence, although somehow limited, indicates that DPSCs may not be the most ideal multipotent stem cells for periodontal regeneration.

Stem Cells from Human Exfoliated Deciduous Teeth for Periodontal Regeneration

A multipotent stem cell population can be isolated from the remnant pulp of human exfoliated deciduous teeth. Similar to DPSCs, stem cells from exfoliated deciduous teeth (SHED) have the capacity to differentiate into osteogenic, adipogenic, chondrogenic, and myogenic cell lineages (Miura et al., 2003). These cells also express Oct4, CD13, CD29, CD44, CD73, CD90, CD105, CD146 and CD166, but do not express hematopoietic markers such as CD14, CD34 or CD45 (Liu et al., 2015; Pivoriuunas et al., 2010). Furthermore, it has been shown that SHED have higher proliferation rate compared to BMSSCs and DPSCs (Nakamura et al., 2009). SHED are able to form bone and dentin structures after ectopic transplantation into brain of mice. Unlike DPSCs, these cells were not able to form complete dentin-pulp complex (Miura et al., 2003). However, it has been shown that these cells might be promising for bone regeneration as Ma and colleagues reported that both fresh and cryopreserved SHED were able to repair calvarial critical size bone defects in immunocompromised mice (Ma et al., 2012). Despite promising outcome in bone regeneration, the evidence supporting the use of SHED in periodontal regeneration is still elusive.

Dental Follicle Stem Cells for Periodontal Regeneration

Dental follicle is a loose connective tissue derived from ectomesenchymal cells. It surrounds the enamel organ and the developing tooth germ before eruption and plays different roles during tooth development (Honda et al., 2010). It has been shown that dental follicle-derived stem cells (DFSCs) can undergo osteogenic, chondrogenic, and adipogenic differentiation in vitro (Kemoun et al., 2007). DFSCs express CD9, CD10, CD13, CD29, CD44, CD53, CD59, CD73, CD90, CD105, CD106, CD166 and CD271, but not hematopoietic markers such as Cd31, CD34, CD45 and CD133 (Liu et al., 2015). DFSCs have shown to possess

potential for regeneration of periodontal tissue. Yokoi et al. transplanted immortalized mouse DFSCs subcutaneously into immunocompromised mice (Yokoi et al., 2007). Their study showed that, after ectopic transplantation, these cells can form PDL-like structures with collagen type I fibril assemblies. Furthermore, the PDL-like structures showed expression of periostin, scleraxis, and type XII collagen, indicating the ability of DFSCs to regenerate PDL in vivo (Yokoi et al., 2007). A canine study by Park and colleagues demonstrated that transplantation of autologous DFSCs into surgically created periodontal defects results in regeneration of the defects (Park et al., 2011). Histological analysis revealed the regeneration of the defects with newly formed alveolar bone, cementum, and PDL. The net volume of regenerated bone in defects treated with DFSCs, however, was lower compared with defects treated with autologous PDLSCs (Park et al., 2011). The potential of DFSCs for formation of PDL is further supported by a study by Guo et al. (2012), where DFSCs isolated from human impacted third molars (Guo et al., 2012) and implanted into nude mice subcutaneously induced the formation of cementum-PDL-like complex. In this study, DFSCs were found to express high level of CD146 and STRO1 and were positive for other markers, such as Notch-1, alkaline phosphatase, COL-I, COL-III, osteocalcin, bone sialoprotein (BSP) and Runx2 (Guo et al., 2012). Similar findings were reported by Han et al., who detected formation of a cementum-PDL complex upon ectopic transplantation of DFSCs in mice (Han et al., 2010). It appears, therefore, that DFSCs cells may represent an alternative to PDLSCs, although not an equally effective one.

Dental Apical Papilla Stem Cells for Periodontal Regeneration

The apical papilla is the soft tissue found at the apices of developing teeth. This tissue is only present during root development, before the eruption of teeth into the oral cavity (Sonoyama et al., 2006; Sonoyama et al., 2008). The third molars are commonly extracted while undergoing root formation and therefore they may represent an excellent source of dental apical papilla stem cells (DAPSCs) **(**other than DPSCs and PDLSCs). DAPSCs have the potential to differentiate into odontoblast-like cells, osteoblasts, adipocytes, and neuronal cells. (Huang et al., 2009) In addition, they have capacity to induce root formation (Huang et al., 2009; Liu et al., 2015). They express mesenchymal markers, including STRO-1, CD13, CD24, CD29, CD44, CD73, CD90, CD105, CD106 and CD146, and not hematopoietic markers such as CD18, CD34, CD45 or CD150 (Ding et al., 2010b; Huang et al., 2009; Liu et al., 2015).

The potential use of DAPSCs for periodontal regeneration is supported by the study of Xu et al (Xu et al., 2009). In this study, Xu and colleagues isolated DAPSCs from the apical region of developing mandibular first molars of Sprague–Dawley rats. Using a model of ectopic transplantation, they demonstrated that the mineralization potential of these cells is superior to that of DPSCs and that DAPSCs are able to form PDL-like, dentin-like, cementum-like, and bone-like tissues (Xu et al., 2009).

Not enough studies evaluating the potential use of DAPSCs in periodontal regeneration exist and therefore we feel that a recommendation for the use of DAPSCs in periodontal regeneration cannot be made at the present time.

Dental Socket Stem Cells for Periodontal Regeneration

Evian and colleagues in 1982 studied the osteogenic activity of healing extraction sockets in humans by analyzing the bone cores removed from post-extraction sockets (Evian et al., 1982). Histological evaluations of the retrieved tissue samples revealed the presence of osteoblasts and osteoid starting from the 4^{th} up to the 8^{th} week post-extraction, suggesting a high osteogenic activity during this healing phase. This study, however, did not specifically evaluated the presence of skeletal stem cells within the healing extraction sockets (Evian et al., 1982). Nevertheless, dental sockets have been recently shown to be a potential source for stem/progenitor cells. A study performed in a canine model of extraction sockets showed that the granulation tissue collected 3 days after extraction contains dental socket-derived stem cells (DSSCs) expressing CD44, CD90 and cD271 and not expressing hematopoietic markers such as CD34 and CD45. They showed that these cells are able to differentiate into osteogenic, adipogenic and chondrogenic cells in vitro (Nakajima et al., 2014). In addition, autologous transplantation of these cells into one-wall periodontal defects resulted in regeneration of the defects by formation of new bone, periodontal ligament-like fibers, and cementum-like tissue (Nakajima et al., 2014). Therefore, although limited to an animal model, data in support of the use of DSSCs for periodontal regeneration exist and appear promising. Besides the use of extraction sockets as source of DSSCs, intraoral small bone defects in remote areas of the mandible (i.e. the ascending part of the ramus) may be generated ad hoc prior to the regenerative procedure and serve as source for multipotent stem cells during the periodontal regenerative procedure.

IPS-DERIVED CELLS IN PERIODONTAL REGENERATION

Reprogramming somatic cells to become pluripotent cells (induced pluripotent stem cells, iPS cells) may represent an alternative to multipotent adult stem cells in periodontal regenerative therapy.

Initial approaches to nuclear reprogramming initiated in 1960 (Yamanaka and Blau, 2010) and generation of iPS cells was successfully achieved in 2006 by inducing overexpression of four key transcription factors, Oct3/4, c-Myc, Klf4, and Sox2, into fibroblasts (Takahashi and Yamanaka, 2006). Since then, iPS cells have been tested for regeneration of diverse tissues such as heart, pancreatic islets, liver, bone, cartilage, and brain (Duan et al., 2011; Revilla et al., 2015). Few studies have used iPS cells for periodontal regeneration as well (Table 4) (Duan et al., 2011; Hynes et al., 2013; Yang et al., 2014). In a study by Duan et al, iPS cells from mice, delivered by means of a silk scaffold in a combination with enamel derived factors, were used to induce regeneration of periodontal tissues in a murine model of periodontal defect (Duan et al., 2011). After treatment, histomorphometric analysis revealed that significantly greater amounts of new bone and cementum formation were evident when defects were treated with the combination of cells, scaffold, and enamel derived factors rather than scaffold alone or a combination of scaffold and growth factors, suggesting that transplantation of iPS cells can enhance periodontal regeneration (Duan et al., 2011).

Prevention of periodontal bone resorption was observed when iPS cells were transplanted into circumferential defects in a rat model of periodontal disease (Yang et al., 2014). In this model, periodontal defects were developed by means of wire ligature and inoculation of *P.*

gingivalis into the oral cavity. Application of iPS cells showed decreased inflammation and inhibition of alveolar bone resorption. Although the mechanism by which iPS cells controlled bone resorption was not investigated, one possibility is that these pluripotent cells can control inflammation, therefore indirectly preventing bone destruction. In another study, surgically created periodontal fenestration defects in immunodeficient rats treated with human iPS cells clotted with fibrinogen and thrombin and labeled with BrdU exhibited significantly greater area of mineralized tissue formation compared to non-treated defects and defects treated with clotting factors only (Hynes et al., 2013). The used iPS cells were shown to express mesemchymal stem cell markers such as CD73, CD90, CD105, CD146, CD106, HSP90 and pluripotency markers such as TRA160, TRA180, and ALKPOS. The BrdU –labeled cells were found to be integrated into the newly formed tissues, suggesting that iPC cells can directly contribute to the regeneration of the defects (Hynes et al., 2013).

Unfortunately, these studies do not fully address the safety and the efficacy of iPS for periodontal regeneration. It is well described that iPS cells may not exhibit phenotypic stability once transplanted in vivo (Hynes et al., 2013) and might become immunogenic due to abnormal gene expression upon differentiation (Zhao et al., 2011). Defining the number of cells that will suffice the threshold for tissue regeneration will also need to be accomplished in future investigations in order to avoid uncontrollable regeneration of tissues (Lin et al., 2015). Thus, future studies are needed to identify the exact environmental, chemical, and biomechanical cues to modulate the therapeutic use of iPS. Until then, these cells remain just a promising tool for periodontal tissue regeneration.

CLINICAL APPLICABILITY AND FUTURE DIRECTIONS

The use of pluripotent stem cells, such as iPS cells, for regeneration of periodontal tissue appears far from being feasible at the moment. On the contrary, several of the mentioned animal studies seem to indicate that multipotent stem cells can be effectively used for regeneration of the periodontium. However, apart from a few case reports (Table 5), there is no available information on the safety and efficacy of multipotent stem cells for periodontal regeneration in the clinical setting. If long-term clinical trials confirm the safety and efficacy of multipotent stem cells, standard clinical protocols may be developed for the effective use of these cells in periodontal regenerative therapy.

Allogeneic multipotent stem cells have been proposed as an alternative to autologous multipotent stem cells for periodontal regenerative therapies. However, a major challenge of non-autologous stem cell therapy includes graft-versus-host disease (Li et al., 2009). Thus, at least for now, it appears that the safest approach to multipotent stem cell-based therapy is the autologous transplantation. Based on the studies evaluated in this review, it appears that PDLSCs are the most promising phenotype although SSCs may also be effectively used for periodontal regeneration. Other dental derived stem cells, such as DPSCs, SHEDs, DFSCs, and DAPSCs do not appear to be as effective in regenerating periodontal tissues and therefore it may not be worth to test them in future clinical trials.

If effective clinical therapeutic protocols are established, a challenge remains in terms of tissue culturing and ex-vivo expansion of the autologous multipotent stem cells. Culturing

SSCs or PDLSC is challenging, time-consuming, time-sensitive and, as per today, very costly. Additionally, the FDA has implemented strict regulations for stem cell tissue culturing (Halme and Kessler, 2006). This is because xenogenic products, such as fetal bovine serum, are often used in stem cell-culture medium. Furthermore, the culture conditions that would maintain the cells in an optimal environment without cross-contaminations or infections have neither been fully studied nor fully developed (Karring et al., 1993; Lin et al., 2009). Another technical challenge arises from cell manipulation as instability and gene mutations have been observed after prolonged culturing of stem cells, thus suggesting that freshly prepared stem cell cultures should be used (Jo et al., 2007). Furthermore, cryopreservation has been routinely utilized for hematopoietic stem cells and other stem cells (Hunt, 2011); however, questions remain on whether or not it transmission of infections may occur during direct immersion of cells into liquid nitrogen (Hawkins et al., 1996; Mazzilli et al., 2006).

One effective way to by-pass the problems identified above is to develop safer, more efficient, and cost effective “disposable” bioreactors for the ad hoc preparation of autologous multipotent stem cells (Martin et al., 2009). Until then the only alternative could be based on the utilization of bone competent cells isolated from extraction sockets or from previously created intraoral bone defects that may serve as in vivo autologous bioreactors during procedures of periodontal regeneration.

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Table 1

Animal studies of periodontal regeneration using bone marrow-derived skeletal stem cells

Reference	Cell type	Defect type	Animal model	Cell delivery method	Significance
(Kawaguchi et al., 2004)	Autologous BMSSCs	Surgically created class III furcation defects	Beagle dogs	Collagen gel carrier	Formation of new bone, cementum, and PDL was observed in the test group, but complete regeneration of defects was not achieved. Significantly higher percentages of new cementum length and bone area were observed in defects treated with cells + carrier compared to carrier alone.
(Hasegawa et al., 2006)	Autologous BMSSCs labeled with GFP	Surgically created class III furcation defects	Beagle dogs	Collagen gel carrier	Formation of new bone, cementum, and PDL was observed in the test group, but complete regeneration of defects was not achieved. Differentiation of labeled BMSSCs into cementoblasts, osteoblasts, osteocytes, and fibroblasts was observed.
(Wei et al., 2010)	Autologous BMSSCs labeled with BrdU	Surgically created class III furcation defects	Beagle dogs	Alginate hydrogel carrier	New periodontal tissue formation with new bone, PDL and cementum was observed. Labeled cells were distributed into PDL, cementum, alveolar bone, and blood vessels. These cells expressed markers of osteoblasts and fibroblasts.
(Li et al., 2009)	Autologous Cryopr served BMSSCs	Surgically created fenestration defects	Beagle dogs	Collagen spongec rrier	No significant differences were found in the regeneration of periodontal tissue between defects treated with cryopreserved and the non-cryopreserved BMSSCs. Amounts of formation of new bone, cementum, and PDL fibers were significantly higher in both groups compared to the carrier alone group.
(Du et al., 2014)	Allogeneic BMSSCs	Periodontal defects caused by periodontitis by binding wire around teeth and inoculation of *P. gingivalis*	Sprage Dawleyrats	Cells were delivered by local injection into defects using 0.9% NaCl solution	Inhibition of pro-inflammation factors and enhanced periodontal tissue regeneration were observed in the animals treated with BMSSCs.
(Yang et al., 2010)	Allogeneic BMSSCs labeled with GFP	Surgically created periodontal window defects on the buccal surface of the mandibular 1st molar	Sprage Dawleyrats	Gelatin beads carrier	Significantly more new bone formation and greater number of functionally orientated PDL fibers were found in the test defects. GFP-labeled cells were integrated in newly formed periodontal tissue.
(Zhou et al., 2011)	Allogeneic BMSSCs labeled with GFP	Surgically created intradentinal periodontal defects on mesial root of maxillary 1st molar	Irradiated wild-type mice	Cells were delivered by IV injection; Defects filled with ceramic bovine bone	New periodontium formation was observed. Labeled BMSSCs contributed to periodontal regeneration and differentiated into fibroblasts and osteoblasts.
(Yu et al., 2013)	Allogeneic BMSSCs labeled with enhanced-GFP	Surgically created periodontal window defects on the buccal	Irradiated Sprague Dawley rats	Cells were delivered by intra–bone marrow transplantati on 4 wks before creation of defects	GFP-labeled cells were detected in the defects one week after surgery. Labeled cells were observed in the newly formed bone, PDL, and cementum 4 weeks after creation of the defects.

Reference	Cell type	Defect type	Animal model	Cell delivery method	Significance
		surface of the mandibular 1 st and 2 nd molars			
(Chen et al., 2008)	Autologous BMSSCs engineered to overexpres s BMP-2 gene	Surgically created transgingival Periodontal defects over the palate of maxillary incisors	New Zealand white rabbits	Pluronic F-127 gel carrier	More new bone formation was found in the defects treated with engineered-BMSSCs compared to the defect treated with the unmodified BMSSCs.
(Tan et al., 2009)	Autologous BMSSCs transfected with human bFGF	Surgically created class III furcation defects	Beagle dogs	Calcium alginate gel carrier	More new bone formation was observed in sites that treated with bFGF-modified BMSSCs compared to unmodified BMSSCs.
(Zhou and Mei, 2012)	Autologous BMSSCs modified to overexpres s OPG	Surgically created periodontal window defects on buccal surface of mandibular premolars	Beagle dogs	PLGA carrier	The amounts of formation of new bone, cementum, and connective tissue were significantly higher in defects treated with modified or non-modified BMSSCs compared to the control defects.

bFGF- basic Fibroblast Growth Factor; BMP-2- Bone Morphogenetic Protein 2; BMSSCs- Bone Marrow- derived Skeletal Stem Cells; BrdU- Bromodeoxyuridine; GFP- Green Fluorescent Protein; OPG- Osteoprotegerin; PDL-Periodontal Ligament; PLGA- Poly Lactic-co-Glycolic Acid.

Table 2

Animal studies of periodontal regeneration using adipose tissue-derived stem cells

Reference	Cell type	Defect type	Animal model	Cell delivery method	Significance
(Tobita et al., 2013)	Autologous ATSCs	Surgically created class III furcation defects	Beagle dogs	PRP carrier	Newly formed PDL fibers were observed in defects treated with ATSCs + PRP, but not in defects treated with PRP alone. No significant differences in the amount new bone and cementum formation were found between the groups.
(Akita et al., 2014)	Allogeneic ATSCs	Surgical created fenestration defects	F344 and Sprague Dawley rats	PLGA carrier	Significantly greater amount of newly formed cementum and width of newly formed PDL were observed in ATSCs + PLGA group compared to PLGA alone group. Higher bone formation was found in defects treated with ATSCs + PLGA.
(Tobita et al., 2008)	Allogeneic ATSCs labeled with GFP	Surgically created fenestration defects	Wistar rats	PRP carrier	New bone, cementum, and PDL fibers were formed in the defects treated with the mixture of ATSCs and PRP. GFP-positive cells were observed on the surface of the regenerated alveolar bone and PDL structures.

ATSCs- Adipose Tissue- derived Stem Cells; PDL- Periodontal Ligament; PLGA- Poly Lactic-co-Glycolic Acid; PRP-Platelet-Rich Plasma.

Table 3

Animal studies of periodontal regeneration using periodontal ligament-derived stem cells

Reference	Cell type	Defect type	Animal model	Cell delivery method	Significance
(Liu et al., 2008)	Autologous PDLSCs	Surgical created periodontal defects in the mesial of 1st molars and induction of inflammation by insertion of ligature	Miniature pigs	HA/TCP carrier	Formation of new bone, cementum, and PDL was reported. Significant improvements in clinical parameters, and height of regenerated alveolar bone were observed in the cells + HA/TCP group compared to HA/TCP alone and no treatment groups.
(Park et al., 2011)	Autologous PDLSCs; DFSCs; and DPSCs	Surgically created circumferential apical involvement defects	Beagle dogs	No carrier	PDLSCs had the best regenerative potential. Defects in PDLSCs groups were regenerated by means of new cementum, bone, and PDL fibers. Periodontal regeneration was not achieved in DPSCs group.
(Wei et al., 2012)	Autologous vitamin C-induced PDLSC sheets; UpCell dish PDLSC sheets; Autologous dissociated PDLSCs	Same as Liu et al	Miniature pigs	No carrier for cell sheets; Gelfoam for autologous dissociated PDLSCs	Regeneration of defects up to normal levels was observed in Vitamin C- induced PDLSCs sheet group. Formation of Sharpey's fibers was seen in all groups, but fibers were irregular in dissociated PDLSCs group. Significantly higher percentage of newly formed alveolar bone was evident in Vitamin C- induced PDLSCs sheet group compared to the other groups
(Ding et al., 2010a)	Autologous PDLSC sheets; Allogeneic PDLSC sheets; Autologous heterogenic PDL cells (PDLC)	Same as Liu et al	Miniature pigs	HA/TCP carrier	Significantly improved clinical and histological outcomes were observed for allogeneic and autologous PDLSCs sheets compared to PDLCs and control groups. Similar regenerative outcomes were obtained using allogeneic and autologous PDLSCs sheets. No evidence of immunological rejections of allogeneic PDLSCs was found.
(Mrozik et al., 2013)	Allogeneic PDLSCs	Surgically created zero-wall dehiscene defects	Merino sheep	Gelfom sponge carrier	No significant differences were found in mean area of new bone formation, length of new cementum formation, and Sharpey's fiber thickness between PLDSCs + Gelfoam group compared to Gelfoam alone group. All regenerative parameters were significantly improved in both group compared to untreated defects.
(Guo et al., 2014)	Monolayered PDLSC cell sheets (MCSs); Monolayer PDLSC pellets (MCPs); Multilayered PDLSC pellets (MUCPs)	Surgically created periodontal defects in the mesial region of the maxillary 1st molars.	Sprague–Dawley rats	None	Perpendicular insertion of fibers into defects was evident in MUCPs and MCPs groups. Higher formation of mineralized tissue was found in MUCPs group compared to MCPs group.
(Seo et al., 2004)	Xenogeneic (human) PDLSCs	Surgically created periodontal defects on buccal surface of the mandibular molars	Immunodeficient rats	HA/TCP carrier	Human PDLSCs integrated into the periodontal tissue in two of six samples
(Iwasaki et al., 2014)	Xenogeneic (human) PDLSCs	Surgically created class II furcation defects	Immunodeficient rats	Amniotic membrane	Enhanced regeneration of periodontium and a higher percentage of bone fill in were observed in defects treated with PDLSCs-amnion compared to the amnion-alone.

DFSCs- Dental Follicle- derived Stem Cells; DPSCs- Dental Pulp- derived Stem Cells; HA/TCP: Hydroxyapatite/Tricalcium Phosphate; PDL- Periodontal Ligament; PDLSCs- Periodontal Ligament-derived Stem Cells.

Table 4

Animal studies of periodontal regeneration using induced pluripotent stem (iPS) cells.

Reference	Cell type	Defect type	Animal model	Cell delivery method	Significance
(Yang et al., 2014)	Allogeneic iPS cells; Allogeneic iPS engineered to overexpress TSG-6	Experimental periodontitis was established by ligature and inoculation of *P. gingivalis* around the maxillae 1st molars	Sprague-Dawley rats	Systemic and local injections	Inflammation mediators, osteoclasts and bone loss decreased in the animal treated with modified and unmodified iPS cells compared to no treatment group. The amounts of reduction in pro-inflammatory mediators were significantly higher in TSG-6 iPS cells.
(Duan et al., 2011)	Allogeneic iPS cells; Xenogeneic iPS cells	Surgically created fenestration defects	Immunod eficient mice	Apatite-coated silk fibroin scaffolds +EMD	Significantly greater amounts of new bone and cementum formation were seen when defects were treated with the cells+ scaffold + EMD versus treating the defects with scaffold alone or scaffold + EMD
(Hynes et al., 2013)	Xenogeneic iPS cells labeled with BrdU	Surgically created fenestration defects	Immunod eficient Rats	Cells were clotted with 5 μL fibrinogen and 5 μL thrombin	Significantly higher area of mineralized tissue formation was observed in the test group. The labeled cells were integrated into the newly formed tissues.

BrdU- Bromodeoxyuridine; EMD- Enamel Matrix Derivatives; iPS cells- induced pluripotent stem cells; TSG-6- tumor necrosis factor alpha-stimulated gene-6

Table 5

Clinical studies of periodontal regeneration using stem cell-based regenerative approaches.

Reference	Type of Study	Cell type (Cell Source)	Defect type	Carrier	Significance
(Yamada et al., 2006)	Case Report (n=1)	Autologous BMSSCs *(from iliac crest)*	Angular interproximal defect	PRP	Highest PPD reduction and CAL gain were both 4mm after 1 year.
(Yamada et al., 2013)	Case Series (n=17)	Autologous BMSSCs *(from iliac crest)*	Angular interproximal defect	PRP	The treatment resulted in mean PPD reduction of 5.12± 2.45 mm, CAL gain of 4.29 ± 1.32 mm, and radiographic bone height gain of 3.12 ± 1.23. No side effects were observed.
(Feng et al., 2010)	Case series (n=3)	Autologous PDLPs and PDLSCs *(from extracted 3rd Molars)*	Intrabony		