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# Stem Cells as Therapeutic Option in Periodontal Regeneration: A Myth or a Reality?

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**Review Article**

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**Abstract:** Periodontitis are multifactorial infections that begin with a period of inflammation of the supporting tissues of the teeth and then progress destroying the tissues until loss of the teeth. The goal of periodontal treatment is to stop the progression of the disease, restore functions and regenerate the damaged tissue. However, regeneration of different tissues, by conventional treatments, still remains great challenge. To address this, there are several approaches to tissue engineering for regenerative dentistry, among them, the use of stem cells by including dental pulp stem cells (DPSCS), periodontal ligament stem cells (PDLSCS), stem cells from the dental apical papilla (SCAPS), stem cells from human exfoliated deciduous teeth (SHEDS), dental follicle stem cells (DFSCS), dental epithelial stem cells (DESCS), bone marrow mesenchymal stem cells (BMMSCS), adipose-derived stem cells (ADSCS), embryonicstem cells (ESCS) and induced pluripotent stem cells (IPSCS). This novel approach may represent an effective therapeutic tool for periodontal regeneration. However, there are still obscurities regarding the mechanisms underlying in periodontal regeneration and challenges in applications of dental stem cell. Further efforts are necessary before moving to clinical trials for future applications.
**Keywords**: stem cells, periodontal treatment, Mesenchymal stem cells, periodontal regeneration.

## INTRODUCTION

Periodontitis is a chronic inflammatory disease of the supportive tissues of the teeth.

This disease is caused by specific microorganisms or groups of specific microorganisms, which result in a pathological disinsertion of the collagen fibres of the cementum; progressive destruction of the periodontal ligament and alveolar bone with increased probing depth formation, recession, or both, and apical migration of the union epithelium [1]. Periodontal diseases are highly prevalent as 15-50% of adults in developed countries are concerned with them [2] and, generaly, severe periodontitis is the sixth-most prevalent health condition worldwide [3]. This affects the chewing, phonation, aesthetics and may also affect quality of life of the patient [4]. 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 [5-7]. Conventional periodontal treatments include basic treatment such as scaling and root planing, open flap debridement, guided tissue regeneration (GTR) and guided bone regeneration(GBR). That fill defects and replace lost dental tissue, but these approaches are not substitutes for a real regeneration of tissue with a physiological architecture and function. The outcomes of these methods are limited and associated with poor clinical predictability [8,9]. To address this, new therapeutic options using stem cells have been proposed. 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) (Figure 1) [7,10]. This altrnative encompasses numerous elements, including biomaterials, stem cells, tissue-inducing substances and biomimetic regenerative environments [9].

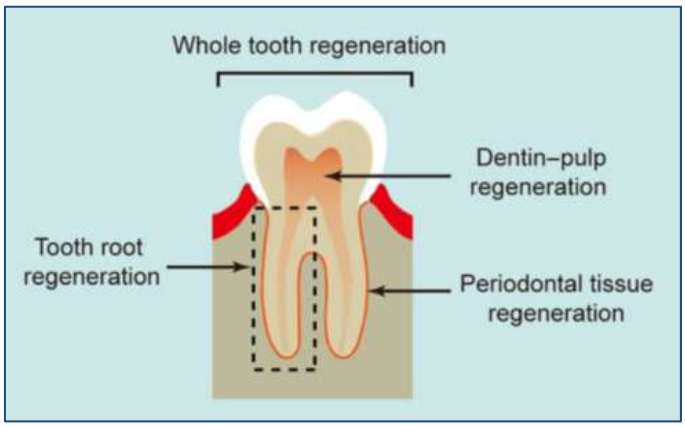


**Fig-1: stem cell approache to regenerate dentin–pulp, tooth root, whole tooth, and periodontal tissues [9]**

This work presents the current and potential applications of stem cells and its efficacy to periodontal regeneration and suggests new avenues for the development of more effective therapeutic protocols.

**Biology of stem cells**

Stem cells are undifferentiated cells, that 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 [10]. They can be isolated in the early stages of embryogenesis (embryonic stem cells) or in various postnatal tissues (adult stem cells). Although embryonic stem cells have a long lifespan and potential for high differentiation, the bioethical aspects involved in their production have oriented the research on adult stem cells, which are considered pluripotent and found in most tissues, with the potential to repair damaged tissues or renew cell populations that are constantly being replaced [4,11]. Ideally a stem cell should be easily accessible and highly available.

Based on their differentiation potential, stem cells can be categorized as [7]:

- 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:

- embryonic stem cells: these are pluripotent cells derived from the inner cell mass of the blastocyst. They have the ability to form derivatives of all three embryonic germ layers. 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 [7,12]
- post-natal stem cells: they have been isolated from a variety of sources tissues, including bone marrow, epithelium, adipose tissue, liver, nervous system, teeth and periodontal ligament [13] (Figure 2). 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 [14]. As the ethical concerns are not present with its use, they can be a safer approach to tissue regeneration. Consequently, different pre-clinical and clinical investigations have been conducted for the application of these stem cells.
- reprogrammed: they are cells whose genetic program is modified to induce a switch from one cell phenotype to another [15]. Cell reprogramming can be achieved using the following four methodologies: 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 [16].

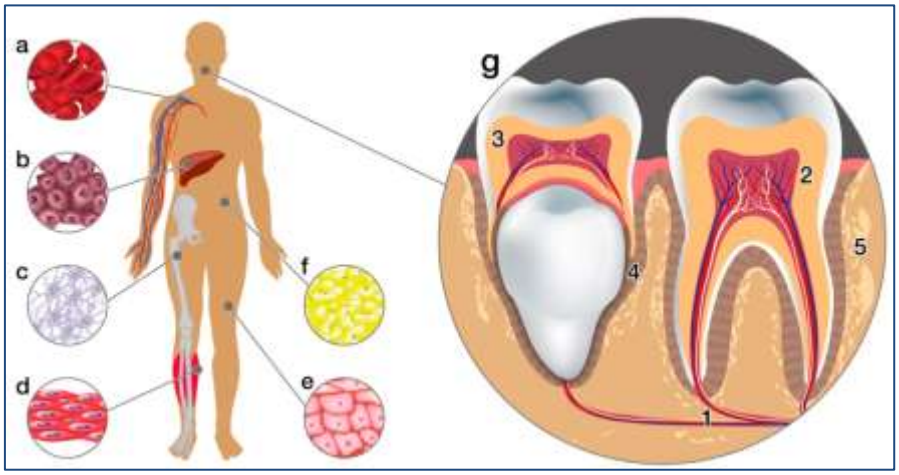


**Fig-2: differents sources of adult stem cells ls in the human organism : (a) Peripheral blood; (b) Liver; (c) Bone marrow; (d) Muscles; (e) Skin; (f) Adipose tissue; (g) Dental tissues: (1. Apical dental papilla, 2. Adult pulp, 3. Pulp of deciduous teeth, 4. Periodontal ligament and 5. Alveolar. [4])**

**Potential of stem cells in periodontal regeneration**

The stem cells used for tooth and periodontal regeneration are both dental and non-dental mesenchymal stem cells (MSCs). Previous studies mainly combined the stem cells with a scaffold for periodontal regeneration, for example, collagen, fibrin, hydrogel and gelatin. However, major concerns are the complicated transplantation process and the potential for host rejection. Recently, non-scaffold tissue engineering has been of increasing interest to researchers. There are primarily two non-scaffold strategies : local cell injection and cell sheets [9]. Theremore stem cells can be banked and cryopreserved in nitrogen. This cryopreservation does not affect their biological and immunological properties [9].

**The non-dental adult stem cells**

The non dental adult stem cells used for tooth and periodontal regeneration can be from differnts sources, but we will be interested in bone marrow-derived skeletal stem cells (BMSSCs) and adipose tissue-derived stem cells (ADCs).

**Bone Marrow-Derived Skeletal Stem Cells (BMSSCs)**

BMSSCs were the first discovered MSCs. They are adult multipotent cells that have shown a capacity for osteogenic, adipogenic, chondrogenic and myogenic differentiation. For tooth and periodontal regeneration, they can differentiate into cells identified as components of the periodontal tissues that can upregulate the expression of odontogenic genes and contribute to tooth regeneration after being recombined with embryonic oral epithelium [15,16]. Moreover, the capacity of these cells to regenerate periodontal tissue has been demonstrated in differrent studies [17] in which multiple periodontal defects have been treated by BMSSC [18-21]. These authors reported that periodontal regeneration was evident in sites treated with BMSSCs while more new bone formation was observed in sites treated. *Kawaguchi and colleagues* evaluated the potential of autologous BMSSCs isolated from iliac crest to regenerate furcation periodontal defects in a beagle dog model. They created class III furcation defects surgically and treated them with BMSSCs delivered by means of a collagen gelcarrier. 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 [17]. in addition, *wei* were transplanted BMSSC into surgically created class III furcation defects using alginate gel as a delivery vehicle although their actual contribution to the periodontal regeneration was not proven. Thus, despite the promising results, pre-clinical studies may still have limited efficacy, and human clinical trials are necessary to confirm the efficacy of BMSSC . 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*. They reported successful treatment of intrabony periodontal defects for one patient using the local application of a combination of expanded autologous BMSSCs harvested from iliac crest and platelet-rich plasma (PRP) [22]. 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 [23]. The clinical outcomes revealed a significant improvement in clinical periodontal parameters. However, clinical studies are still needed to establish feasible and safe BMSSCs therapeutic for the treatment of periodontal defects.

**Adipose Tissue-Derived Stem Cells (ATSCs)**

Adipose tissue derived stem cells (ADSCs) have been widely invistigated as a viable cell source for regenerative medicine [24]. These cells have shown regenerative potential similar to BMMSC, they can promote cementum and organize periodontal ligament

fibers and periodontal vessel regeneration [25]. The use of these cells offers several advantages over the use of BMSSCs, including the ease of harvesting and the minimal donor site morbidity. Therefore, ATSCs represent a highly attractive cell source alternative for stem cell-based therapeutic approaches in periodontology [26]. *Hung et al*. transplanted ADSCs into adult rabbit extraction sockets. Regeneration of dentin, periodontal ligament and alveolar bone structure were obtained [26]. Moreover, *Tobita* and colleagues isolated ATSCs from inguinal fat pads of rats, mixed them with PRP and transplanted them into surgically created fenestration periodontal defects. The results showed that new bone, cementum and perpendicular periodontal ligament-like fibers were formed 8 weeks after transplantation. No new formations was proven in the defects that were treated with PRP alone [27]. Another pre-clinical study by *Akita* and his collaborators, in which they isolated the ATSCs and transplanted them into surgically created periodontal defects. Histologic analysis revealed new formations of cementum and regeneration of PDL. These results were confirmed by micro-CT analysis [28]. In 2013, *Tobita etal.*, transplanted conbination of ATCSs and PRP into class III periodontal furcation defects, on dogs. Histologic results demonstrated that newly formed PDL ligaments were evident only when defects were treated with the combination of ATSCs and PRP [29]. Thus, more pre-clinical and clinical investigations are needed to exploit the potential of ATSCs for periodontal regeneration.

**Dental-derived adult stem cells**

There are many studies that have investigated the potential of dental-derived adult stem cells for periodontal regeneration. In this section we review the available data on the potential of these cells, including stem cells isolated from periodontal ligament, dental pulp, exfoliated deciduous teeth, dental follicle and dental apical papilla [30,31].

One of the advantages of adult stem cells of dental origin is that they can be safely used in allogeneic transplants, because they have immunosuppressive properties such as those found in bone marrow-derived cells [9]. Good immune tolerance and tissue repair ability have both been observed in different models.

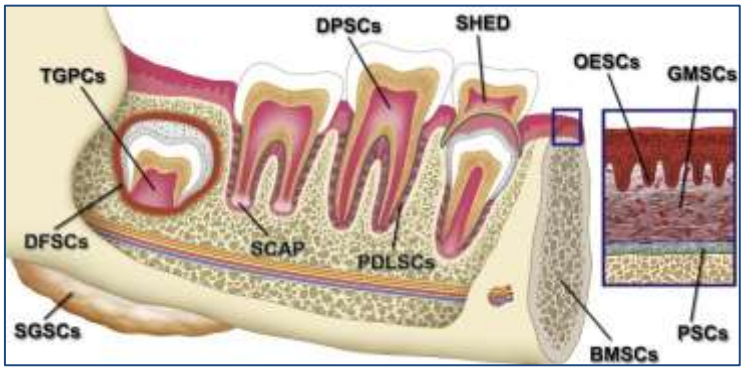


**Fig-3: different sources of dental-derived adult stem cells [31]**

**Periodontal Ligament Stem Cells (PDLSCs)**

Periodontal ligament is an extensively investigated dental stem cells source along with the dental pulp. First isolated from human third molars, PDLSCs could differentiate into cementoblast-like cells, adipocytes and collagen forming cells in vitro and showed the capacity to contribute periodontal tissue repair by generating a cementum/PDL structure in vivo [30,31]. In fact, it has been shown that these cells have higher proliferation rates than skeletal stem cells derived from bone marrow[30]. Moreover, *Seo* et al. 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 structures[30]. These unique features of PDLSCs make them a promising cell source for cell-based regenerative periodontal therapy. *Liu* et al. used PDLSCs extracted from teeth of pigs for periodontal regeneration in a swine periodontitis model. The periodontal defect was created surgically and was treated with a combination of alloplasts (hydroxyapatite and tricalcium phosphate) and cultured autologous PDLSCs. The histologic analysis showed new formation of bone, cementum and periodontal ligament in the treated defect [32]. *Feng* et al., in clinical case series, tranplanted autologous PDLSCs from extracted third molars using hydroxyapatite as a carrier, in intrabony periodontal defects for a limited number of patients. They showed that periodontal parameters were significantly improved in all cases, without any adverse events during 32–72 months of follow-up [33]. However *Morzik et al.* showed that the efficacy potential of PDLSCs is influenced by the age and disease status of donors [34].

**Dental Pulp Stem Cells (DPSCs)**

They were the first identified human dental stem cells[35]. The use of DPSCs seeded scaffolds has yielded successful outcomes for in vivo bone regeneration when transplanted subcutaneously or in bone defects. Although woven bone samples obtained by in vitro conditions without scaffolding can lead to in vivo bone formation [36]. Aquino and Karaoz has

demonstreated that DPSCs are able to differentiate into odontoblastlike cells, osteoblasts, adipocytes and smooth and skeletal muscle cells [37,38]. In vivo, Gronthos showed that DPSCs can form a dentin-pulp-like complex associated with vascularized pulp-like tissue [35]. However, the potential of these cells, for periodontal regeneration may be subject to discussion 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 transplantation in a rat model [39]. Also, Park et al., using a canine model, compared the regenerative potential of autologous DPSCs with that of PDLSCs for the treatment of periodontal defects[40]. Histological results 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 [40]. 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**

A distinct population of stem cells can be isolated from the remnant crown pulp tissue of exfoliated human deciduous teeth, SHED, which is highly, proliferative and can induce bone formation in vivo [41]. In 2017, Ching et al. demonstrated, in comparison for osteogenic capacity of SHED and DPSCs, that SHED exhibit more enhanced osteogenic properties than DPSCs [42]. Furthermore, Nakamura et al. has shown that SHED have higher proliferation rates compared to BMSSCs and DPSCs[43].Miura and colleagues revealed that SHED are able to form bone and dentin structures after ectopic transplantation into brain of mice [44]. Furthermoe, 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 mice[45]. Additional studies are needed to confirm potential of SHED in periodontal regeneration.

**Dental Follicle-derived Stem Cells (DFSCs)**

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 [44]. It has been shown that dental follicle-derived stem cells (DFSCs) can undergo osteogenic, chondrogenic and adipogenic differentiation in vitro[44]. During tooth development, dental follicle has key functions in bone resorption and development of the periodontium. Stem cells can be isolated from teeth follicles of impacted human third molars. When transplanted in critical size defects in calvaria, DFSCs harvested at the early stage of crown formation showed newly formed bone and vascularization after 4 weeks similar to that of BMMSCs [47]. Furthermore, *Park* and Colleagues demonstrated, on a canine model, that transplantation of autologous DFSCs into surgically created periodontal defects results in regeneration of the defects. 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 to those of defects treated with autologous PDLSCs [48]. The potential of DFSCs for formation of PDL is further supported by a study by *Guo et al*. [49], where DFSCs isolated from human impacted third molars and implanted into nude mice subcutaneously induced the formation of cementum-PDL-like complex.

**Dental Apical Papilla Stem Cells DAPSCs**

Dental papilla is the soft tissue at the apex of a developing permanent tooth, which contributes to tooth formation and evolves into the dental pulp. The apical papilla contains fewer blood vessels and less cellular components compared to pulp tissue and it is separated from the pulp by an apical cell rich zone [50]. 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 [51]. Also, they have capacity to induce root formation [51,52]. For periodontal regeneration, *Xu* and colleagues isolated DAPSCs from the apical region of developing mandibular first molars of 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, dentin, cementum, and bone tissues [39]. These results were supported by *Nakajima*, who showed that these cells are able to differentiate into osteogenic, adipogenic and chondrogenic cells in vitro [53]. 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-liketissue [53]. More studies are needed to clear potential of DAPSCs in periodontal regeneration.

**Induced Pluripotent Stem Cells (iPSCs)**

Induced pluripotent stem cells (iPSCs) were firstly discovered in 2006 and then raised substantial interest in regenerative medicine [54]. *Yamanka and Blau* initiated reprogramming somatic cells to become pluripotent cells in 1960 and generation of iPS cells was successfully achieved in 2006 by inducing overexpress of four key transcription factors: Oct3/4, c-Myc, Klf4 and Sox2, into fibroblasts [55]. Since then, iPS cells have been tested for regeneration of diverse tissues such as heart, pancreatic islets, liver, bone, cartilage, and brain [9]. Few studies have used iPS cells for periodontal regeneration. *Duan et al*. demonstrated that the use of iPS cells from mice in a combination

with enamel derived factors induced regeneration of periodontal tissues defect [56]. After treatment, histological analysis revealed that significantly greater amounts of new bone and cementum formation were evident, suggesting that transplantation of iPS cells can enhance periodontal regeneration [56].

Recently, *Hynes et al.* exhibited significantly greater area of mineralized tissue formation of periodontal defect treated by IPSs compared to non-treated defects [57]. *Yang et al.* revealed that prevention of periodontal bone resorption was observed when iPS cells were transplanted into circumferential defects in the case if a rat model of periodontal disease[58]. 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. Nevertheless, the mechanism by which iPS cells controlled bone resorption was not investigated [58]. In addition, in 2015, *Hynes et al.* showed that iPSCs can differentiate into mesenchymal stem cells and osteoprogenitor cells, which have potential for dental tissue regeneration [59].

**Challenges and perspectives**

Studies of non dental stem cells is a widely explored that demonstreted that the future for stem cell-based periodontal regeneration is very promising. However, as with all new technologies, those of dental origin are in an incipient stage. In addition , it could be argued that we still lack the required biological understanding of these cells to apply them to a human patient [60].

However, these experimental animal data provide only an approximation of the anticipated behaviour in a human being. Clinical trials have not been widely reported and the available human studies suggest that there are no adverse effects or ethical implications, but there are still very few of them that are needed to reach definitive conclusions and to confirm the safety and efficiency of stem cells. However, there are critical steps in moving the field towards human clinical utility. Issues such as appropriate delivery devices, immunogenicity, autologous cells vs. allogeneic cells, which tissues provide the most appropriate donor source, control of the whole process and cost-effectiveness are all important considerations that should not be overlooked [7]. Furthermore, before we can confidently move forward, the next critical phase is the systematic validation of specific stem cells as reliable sources for cytotherapeutic use. Finally, the establishment of large-scale preparation facilities incorporating the stringent protocols of good manufacturing procedures will be an absolute necessity [9,60].

**CONCLUSION**

Pre-clinical evidence supports the potential use of stem cells as an optionnal therapy in diverse pathologies. In this sense, the use of these cells of dental origin to repair and regenerate periodontal defects can offer advantages over other more exploited cellular sources. Because of its similarity to target tissue and remarkable accessibility, it seems reasonable to conclude that using stem cells of dental origin, especially DPSCs and PDLSCs to treat periodontitis, is the most logical option. In addition, this assumption needs to be verified in future experimental and clinical studies about the therapeutic efficacy of dental stem cells in periodontal regeneration. Thus, it is likely that in near future periodontist will be able to isolate and store stem cells using the ready to use dental stem cell kits as part of their routine practice.

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