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REVIEW

Dental stem cell and dental tissue regeneration

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Abstract **The teeth are highly differentiated chewing organs formed by the development of tooth germ tissue located in the jaw and consist of the enamel, dentin, cementum, pulp, and periodontal tissue. Moreover, the teeth have a complicated regulatory mechanism, special histologic origin, diverse structure, and important function in mastication, articulation, and aesthetics. These characteristics, to a certain extent, greatly complicate the research in tooth regeneration. Recently, new ideas for tooth and tissue regeneration have begun to appear with rapid developments in the theories and technologies in tissue engineering. Numerous types of stem cells have been isolated from dental tissue, such as dental pulp stem cells (DPSCs), stem cells isolated from human pulp of exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAPs), and dental follicle cells (DFCs). All these cells can regenerate the tissue of tooth. This review outlines the cell types and strategies of stem cell therapy applied in tooth regeneration, in order to provide theoretical basis for clinical treatments.**

Keywords stem cells; pulp regeneration; periodontal regeneration

Introduction

Stem cells are primitive cells with self-replicating and multi-directional differentiation potentials. Moreover, they can be differentiated into various functional cells or tissues and organs under certain conditions, and are thus known as "universal cells." Stem cell therapy is the use of the multi-directional differentiation characteristics of stem cells in order to repair diseased cells or reconstruct normal functioning of cells and tissues [1]. Therefore, stem cell therapy has provided new hope for tissue and organ regeneration.

In recent years, new ideas for tooth and tissue regeneration have been proposed secondary to the rapid developments in theories and technologies in tissue engineering. Various types of stem cells and new biological methods such as those that use bioactive factors, have been widely applied in tooth regeneration research [2]. The teeth are highly differentiated chewing organs formed by the development of tooth germ tissue located in the jaw and consist of the enamel, dentin, cementum, pulp, and periodontal tissue. Moreover, the teeth have a complicated regulatory mechanism, special histologic origin, diverse structure, and important function. These characteristics, to a certain extent, greatly complicate the research in tooth regeneration.

Feasible availability is one of the superior properties of dental stem cells. Dental stem cells can be easily obtained from premolar and wisdom teeth and are thus extracted for orthodontics use; moreover, dental stem cells are increasingly becoming the source for regenerative medicine research. Healthy dental tissues contain large amounts of normal stem cells that are needed to maintain normal function, whereas inflamed or traumatized tissues have a low amount of robust stem cells, which leads to failure in tissue repair [3,4]. Thus, *ex vitro* expansion/manipulation of stem cells is seen to be an important source when it comes to supplementing host cells and promoting tissue

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regeneration [5]. The transplanted cells not only participate in the regeneration directly, but also produce building blocks and secrete trophic factors to regulate regeneration procedures [6]. These developments and findings in stem cells will broaden the regeneration medicine research fields, as well as become the basis for translation research. In this paper, the research background, research status, and several common dental stem cells are reviewed. Moreover, the prospect of future applications is discussed.

Stem cells of dental origin

Stem cells from pulp tissue

Dental pulp stem cells (DPSCs), located in pulp tissue, were the first stem cells isolated from adult human dental pulp [7]. DPSCs, which show strong ability to proliferate and self-renew and eventually differentiate into odontoblast-like cells and osteoblasts to form dentin and bone, are best sourced from the third molar [7]. DPSCs composited with hydroxyapatite/tricalcium phosphate (HA/TCP) produced dentin-like structures with pulp tissue inside and an odontoblastic cell lining after being transplanted into immunocompromised mice [7]. Moreover, stem cells isolated from the human pulp of exfoliated deciduous teeth (SHED) not only generated bone and dentin from the odontoblasts, but also differentiated into other mesenchymal and non-mesenchymal derivative stem cells *in vitro*, such as adipocytes and neural cells [8]. In addition to forming mineralized tissues, DPSCs and SHED expressed neural markers and had the capacity to differentiate into adipocytes. SHED displayed a faster rate of proliferation than DPSCs, but showed noticeable defects in forming a complete dentin/pulp-like complex *in vivo*.

Moreover, DPSCs and SHED can generate bone-like tissues *in vivo* [9]. A comparison on the genetic profiles between DPSCs and normal osteoblasts with microarrays was done by Carinci *et al.*, and they identified several up- and downregulated genes. Therefore, several functional activities were differentially regulated between DPSCs and normal osteoblasts, including cell differentiation, adhesion, developmental maturation, and production of cytoskeletal elements [10]. Furthermore, SHED have not only the ability to form bone-like tissue directly, but also the unique potential of osteo-inductive ability. When SHED and DPSCs were respectively mixed with HA/TCP, odontoblast-like cells can differentiate from recipient murine cells only with SHED but not DPSCs transplanted into immunocompromised mice [11,12].

Because of the above characteristics, DPSCs and SHED were considered potential dental stem cells for pulp, dentin, and periodontal regeneration. As a result, when transplanted subcutaneously into the immunodeficient mice, functional dental pulp was developed by the SHED injected into the roots of human premolars with either PuramatrixTM (BD Biosciences, Bedford, MA, USA) or rhCollagen type I (human recombinant BDTM Fibrogen Collagen Type I; BD Biosciences) [13]. Additionally, under certain environments, DPSCs and SHED can be induced into neural cells, and thus may become useful for treating neurological deficits and mortality [14,15]. DPSCs have already been initially and successfully applied for clinical alveolar bone reconstruction in a clinical study [16]. This study showed that DPSC/collagen sponge biocomposites can restore human mandibular bone defects completely, indicating that DPSCs can be used for tissue and organ reparation/or regeneration [16]. Thus, stem cells derived from pulpal tissue are an alternative and available source for regenerating pulp and periodontal tissue.

Periodontal ligament stem cells

The periodontal ligament is located between the teeth and the inner wall of the alveolar fossa and is surrounded by fibrous connective tissue. The presence of multiple types of cells indicates that the periodontal ligament contains stem cells, which are responsible for maintaining homeostasis and building periodontal tissue. Periodontal ligament stem cells (PDLSCs) were first discovered in 2004 by Professor Songtao Shi using mesenchymal stem cell-related markers. PDLSCs can differentiate along the mesenchymal cell lineage to produce osteoblast-like cells, adipocytes, and collagen-forming cells *in vitro*. Moreover, PDLSCs express an increased level of tendon-specific transcription factors, suggesting that PDLSCs belong to the unique postnatal mesenchymal stem cell population [17]. Transplantation of PDLSCs into immunocompromised mice can regenerate the cementum/PDL tissue, which contains dense collagen I-positive tissue [17]. These collagen-positive tissues mimicked the physiologic attachment of Sharpey's fibers. Therefore, PDLSCs can form osteoblast-like cells, cementum/PDL tissue, and Sharpey's fibers. Moreover, human PDLSCs restored defects and migrated into the PDL compartment after being transplanted into defects created surgically at the periodontal area of mandibular molars, thus suggesting that PDLSCs can be used in periodontal tissue regeneration. PDLSCs also successfully regenerated periodontal tissue in surgically periodontal defect areas in swine or canine [18,19]. PDLSCs exist not only in the root surface, but also in the alveolar bone surface with stronger ability of differentiation and proliferation [20]. Furthermore, the root of human deciduous teeth will gradually become a new source for PDLSCs that are highly purified by magnetic cell sorting [21]. To promote periodontal regenerative treatment technology, various immortalized clonal human PDLSC cell lines have been established [22,23]. This series of studies have confirmed that PDLSCs

are crucial cells for maintaining dynamic balance and defect restoration in periodontal tissue, and can form new periodontal structures in the animal body, thereby providing basis for PDLSC applications in periodontal regeneration, with further focus on safety and stability of PDLSC cell lines.

PDLSCs have been used in clinical studies to observe periodontal regeneration. A clinical trial showed the regeneration potential of PDLSCs in three patients with severe periodontal disease: two of these patients regained healthy periodontal tissue with reasonable clinical regeneration; and the loosening and probing depth degree of another patient was significantly reduced and the periodontal attachment was stabilized. This study summarizes the significant effects of autologous PDLSCs in periodontal disease [24]. Host immune rejection can be avoided with the application of autologous PDLSCs. However, the shortage of PDLSCs collected from the same donor limits the use of PDLSCs as a treatment for periodontal disease. Recently, PDLSCs have been shown to have the same low immunogenicity as BMMSCs and have important immunomodulatory functions [25]. When transplanted into periodontal bone defects in a pig model, allogeneic PDLSCs reversed periodontitis without immunological rejection [18]. Therefore, PDLSCs has become the first choice for periodontal regeneration [26], with a successful protocol proposed for clinical trials. Recent clinical studies have reported the use of autologous PDLSCs to repair periodontal defects, suggesting that autologous PDLSCs therapy is safe and efficient to cure periodontal defects [27].

Microenvironment around the stem cells has a profound impact on their function. PDLSCs differentiate along the cementoblastic lineage to form cementum/PDL tissue when cultured with conditioned medium of apical tooth germ cells to provide a highly cementogenic microenvironment [28]. Moreover, recent data has shown that inflamed microenvironments regulate PDLSC osteogenesis via epigenetic modification and endoplasmic reticulum stress [29,30]. Further elaboration of molecular mechanisms can further increase our understanding of chronic inflammatory diseases and provide theoretical basis for PDLSC-based stem cell therapy of periodontitis [2].

Root apical papilla stem cells

The apical papilla is located only in growing teeth before completion of root development. The dental papilla contributes to the formation of teeth, and stem cells from apical papilla (SCAPs) are mesenchymal stem cell-like cells isolated from human immature permanent apical papilla [31]. Like DPSCs and SHED, SCAPs are positive for STRO-1 and CD146, and negative for CD34 and CD45. However, CD24 is only present in SCAP and not in DPSCs and SHED. The expression level of CD24 is downregulated when SHED undergoes odontogenic differentiation *in vitro*. The clonal cell population could be obtained via culturing by using type I collagenase and neutral protease to digest the apical papilla tissue and prepare a single cell suspension. The cells are induced *in vitro* to be transformed into odontoblast-like cells, adipocytes cells, and neuron-like cells. Moreover, cultured SCAPs show positive staining even in the absence of neurogenic stimuli.

SCAP, which has high proliferative potential, is suitable for cell-based tooth regeneration. Moreover, SCAP appears to be more suitable for tissue regeneration than DPSCs because SCAP has higher proliferative capacity and mineralization potential [32]. SCAP appears to be the source of primary odontoblasts when forming root dentin, whereas DPSCs are the likely source of replacement odontoblasts to form reparative dentin. Compared with PDLSCs, SCAPs show a higher rate of proliferation than PDLSCs at 3rd passages [33]. To construct the bio-root with SCAPs, PDLSCs and SCAPs were co-implanted around the root into the alveolus of minipigs. The results showed that the combined use of these stem cells can produce a root/periodontal complex to repair root function [31]. Furthermore, recent data have reported that stem cells derived from the apical end of developing roots displayed unique "embryonic" characteristics [34] and formed a typical cementum/PDL-like complex *in vivo*.

Dental follicle stem cells

The dental follicle is a loose connective tissue capsule derived from ecto-mesenchyme, and surrounds the developing tooth germ. The dental follicle contains progenitor cells that can differentiate into periodontal ligament cells and osteoblasts [35,36]. Human dental follicle cells (DFCs) are the most common cells extracted from the sac of the third molar. Their ability to adhere on plastic surface makes DFCs easy to be isolated from human dental follicles during culturing [37]. Moreover, DFCs have putative markers of mesenchymal stem cells, such as Nestin and Notch-1, and other markers of Stro-1, namely, CD105 and CD90. Furthermore, DFCs displays multipotency similar to BMMSCs and PDLSCs. Characteristics of the different dental stem cells are concluded in Table 1.

The DFC can differentiate into PDL cells, osteoblasts, and cementoblasts because of its heterogeneity. After being transplanted into nude mice, DFCs differentiated into PDL fibroblasts that secrete collagen and interact with fibers on the surface of adjacent bone and cementum to further generate cementum/PDL-like tissue [38]. In addition, the morphology, proliferation, immune profile, and mineralization characteristics of DFCs can still be observed after more than 30 passages, and finally result in cementum/PDL-like tissue formation [39]. Furthermore, DFCs combined with treated dentin matrix (TDM) regenerated

Table 1 Characteristics of different types of dental stem cells

Types	Tissue sources	Markers	Differentiation potency
DPSC	Adult human dental pulp	STRO-1, CD146	Odontoblast-like cells, osteoblasts, adipocytes, neural cells
SHED	Pulp of exfoliated deciduous teeth	STRO-1, CD146/MUC18, CD90, CD29, CD44, CD166, CD105, CD13	Odontoblasts, osteoblasts, adipocytes, neural cells
PDLSC	Periodontal ligament	STRO-1, CD146, CD73, CD90, CD105	Osteoblast-like cells, adipocytes, collagen-forming cells
SCAP	Apical papilla	STRO-1, CD146, CD24	Odontoblasts
DFC	Dental follicle	STRO-1, CD105, CD90, nestin, notch-1	Periodontal ligament cells, osteoblasts, cementoblasts

root-like tissue with pulp-dentin complex and a PDL-connecting cementum-like layer after being transplanted into rat alveolar fossa, and this phenomenon suggested that DFCs can form tooth roots [39]. The heterogeneity of DFCs and the potential of multidirectional differentiation suggested that targeting their differentiation into the desired cell lineage may be a strategy for root regeneration. Moreover, bone morphogenetic protein (BMP) and enamel matrix derivatives (EMD) may be important in cementoblast and osteoblast differentiation of DFCs [40,41]. Recent studies have reported that DFC sheets induced by Hertwig's epithelia root sheath cells can produce periodontal tissue through epithelial-mesenchymal interaction [42]. The critical factors that induce DFC differentiation remain unclear; however, DFCs are still worth considering for tooth regeneration.

Stem cells and dentin/pulp regeneration

Conventional endodontic treatments use biocompatible materials to fill in roots without any regeneration of dentin and pulp tissue. Given the impact of the loss of pulp vitality on the prognosis of the tooth [43], scientists are devoted to regenerating dentin pulp complex in endodontic treatments [44]. Recent advances in stem cell therapy have paved the way for dentin and pulp regeneration. Moreover, only implantable scaffoldings cannot guide the functional regeneration of the pulp-dentin complex harmonically and consistently. Appropriate microenvironment for dentin-pulp complex regeneration is produced by the interaction between scaffolds and cells.

The dentin slices were prepared *in vitro*, seeded with stem cells, and then implanted into the immunocompromised mice subcutaneously [45]. Our group developed a treated dentin matrix (TDM), which contained a rich extracellular matrix that exists in dentin [46]. Combination of TDM and DFCs were capable of regenerating complete and prefabricated shaped dentin tissues [46]. Moreover, the pulp-like tissue in full-length human roots was regenerated by injectable scaffolds mixed with SHED [13].

Pulp-like tissue can be formed after implantation of dental stem cells onto the matrix scaffold; however, present scaffolds do not fully possesses the characteristics of the ideal material. Many currently available scaffolds fail to mimic essential functions of natural ECM. Compared with the traditional scaffold-mediated method, self-assembly of monodisperse cells into 3D structures produce more extracellular matrix and promote intercellular communication. The use of scaffold-free microtissue DPSC spheroids that are pre-vascularized by suspending with human umbilical vein endothelial cells showed regeneration with good vascularization and pulp-like tissue [47]. Cell aggregates mimic the cell condensation and thus provide appropriate stimulation for organ development and regeneration. We used autogenous stem cells from pig deciduous teeth (SPED) to form cell aggregates, which contained cells and extracellular cell matrix, and regenerated pulp-like tissue in the root canal of miniature pig (Fig. 1). The pulp tissue was removed before implantation with calcium hydroxide (control) and cell aggregates of SPED (regenerated). Cell aggregates have succeeded in avoiding the major drawbacks of scaffolding methods, such as the inability to mimic natural extracellular matrix, lack of intercellular cross-talk, selective degradation, and remodeling [48,49].

Stem cells and periodontal regeneration

The effectiveness of periodontal therapy based on stem cells has been strongly supported in recent years. Numerous animal studies have reported that implantation of stem cells into periodontal wounds contributes to the periodontal regeneration process. Moreover, cell sheet engineering has recently been developed as a scaffold-free strategy for cell delivery [50]. To generate cell sheets, cells are grown to confluence in a cell culture dish and develop cell-to-cell interactions and ECM over time. The monolayer is then removed without the use of proteolytic enzymes to preserve the sheet structure. To facilitate cell metabolism, motivation, and communication in native

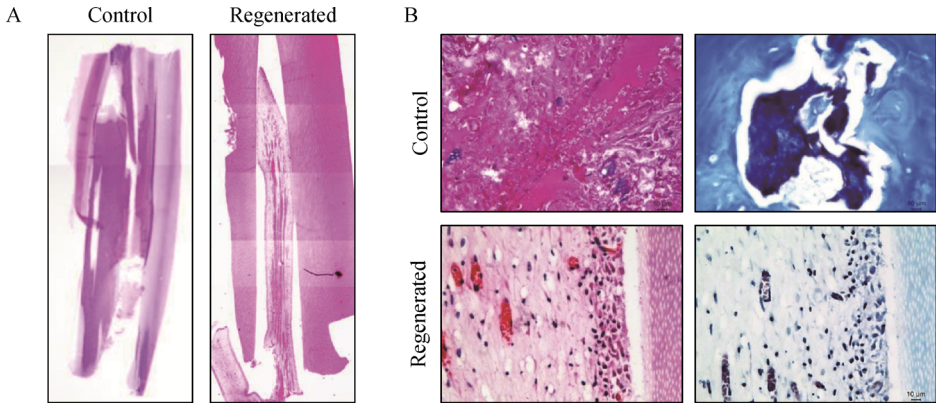

Fig. 1 Cell aggregates of SPED regenerated pulp-like tissue in root canal of miniature pig. (A) Full-length root canal of pigs inserted with calcium hydroxide or cell aggregates. (B) H&E and Masson staining showed that pulp tissue was regenerated by SPED implantation after 3 months. In the control group, calcium hydroxide was inserted into young permanent incisors in miniature pigs (n = 3). After 3 months, no pulp tissue was regenerated and only calcium hydroxide was observed.

tissues, establishing a microenvironment similar to that in the real regeneration process is important. Cell sheet engineering aims to prevent ECM degradation using enzymes that are usually used for isolating cells, so that the ECM can be retained completely and finally ensure normal cell function. Numerous types of scaffold have been reported, and these types have prospects in view of functional layers of cells produced in cell sheet engineering [51,52]. In addition to providing support for cells and tissues, ECM contributes a 3D sub-structure for cell adhesion and movement. Furthermore, ECM contains growth factors and facilitates signal delivery, which are necessary for morphogenesis and differentiation.

PDLSCs may be the first choice for periodontal regeneration in regenerating typical cementum/PDL structures with HA/TCP as a carrier. Moreover, cell sheets of PDLSCs have been developed successfully. The cement/periodontal ligament complex has been observed by grafting monolayered or layered PDLSC *in vivo* [53,54]. Several pilot studies have shown that PDLSC sheet transplants can regenerate periodontal tissues in experimental deficiencies in rats, dogs, and pigs [16,52,53]. In a mesial dehiscence model in a immunodeficient rat, the dentin surface showed a thin layer of cementum-like tissue formation and new fibrous structure insertion after being restored by PDLSC sheets, indicating that cell sheet transplantation can be used for periodontal regeneration [55]. In addition, PDLSCs sheets promoted the potential of periodontal tissue regeneration in a beagle dog in an experimental group, wherein the specimens showed newly generated cementum and periodontal ligament, as well as new blood supply of collagen fibers that were inserted vertically into the new bone and cement [52]. In the mini pig periodontitis model, obvious periodontal regeneration was seen in the autologous and allogeneic PDLSC transplantation groups with no significant differences [18].

In addition to animal models, autologous periodontal ligament progenitor cells (PDLPs) with bone grafting material have shown their therapeutic benefit in humans when implanted into bone defects, as demonstrated in a retrospective pilot study [24]. Periodontal defects were reconstructed, and clinical trials and basic studies proved the security and efficiency of autologous PDL cells in periodontitis therapy [24]. Moreover, our recent clinical trial showed that autologous PDLSC therapy is safe and effective for treating periodontal intrabony defects [27]. PDLSCs have a low chance of immune rejection, and have become a good resource for periodontal tissue therapy; furthermore, PDLSCs have greater potential for periodontal regeneration than other mesenchymal stem cells.

Challenges and prospects

Research and clinical application of dental stem cells have reached several breakthroughs; however, they are still in infancy. Moreover, many theoretical mechanisms still need to be studied for successful clinical applications and real tissue regeneration. Many technical problems have not yet been overcome. However, its incomparable advantages in self-renew ability, potential for multidirectional differentiation, availability, and low autologous transplant rejection result in a broad prospect of its application in dental tissue engineering. With rapid developments in science and technology, people will be able to induce dental stem cells and genetically modify them, thus opening up a new way for tissue regeneration and replacement therapy.

Conclusions

The purpose of this review was to illustrate the types and

characteristics of the commonly applied dental stem cells, namely, DPSCs, SHED, PDLSCs, SCAPs, and DFCs. Stem cells isolated from teeth are readily available and can be applied to stem cell therapy. Moreover, cell aggregates and cell sheets continue to be practical biotechnological materials for scaffold-free tissue reconstruction. However, given the clinical application of dental stem cells, a comprehensive global strategy is needed to be developed to address the multiple issues involving safety, harmonization, privacy, and transparency. Stem cell-based tooth regeneration has shown potential clinical use in the near future. With the rapid developments in science and technology, the research in dental stem cells will open up broader application prospects for tissue engineering and regenerative medicine.

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Compliance with ethics guidelines

Qiming Zhai, Zhiwei Dong, Wei Wang, Bei Li, and Yan Jin declare that they have no conflict of interest or financial conflicts to disclose. This manuscript is a review article and did not involve a research protocol requiring approval by the relevant institutional review board or ethics committee.

References

1. Catón J, Bostanci N, Remboutsika E, De Bari C, Mitsiadis TA. Future dentistry: cell therapy meets tooth and periodontal repair and regeneration. J Cell Mol Med 2011; 15(5): 1054–1065
2. Yoshida T, Washio K, Iwata T, Okano T, Ishikawa I. Current status and future development of cell transplantation therapy for periodontal tissue regeneration. Int J Dent 2012;2012:307024
3. Liu N, Shi S, Deng M, Tang L, Zhang G, Liu N, Ding B, Liu W, Liu Y, Shi H, Liu L, Jin Y. High levels of β-catenin signaling reduce osteogenic differentiation of stem cells in inflammatory microenvironments through inhibition of the noncanonical Wnt pathway. J Bone Miner Res 2011; 26(9): 2082–2095
4. Liu Y, Liu W, Hu C, Xue Z, Wang G, Ding B, Luo H, Tang L, Kong X, Chen X, Liu N, Ding Y, Jin Y. miR-17 modulates osteogenic differentiation through a coherent feed-forward loop in mesenchymal stem cells isolated from periodontal ligaments of patients with periodontitis. Stem Cells 2011; 29(11): 1804–1816
5. Chen FM, Jin Y. Periodontal tissue engineering and regeneration: current approaches and expanding opportunities. Tissue Eng Part B Rev 2010; 16(2): 219–255
6. Mooney DJ, Vandenburgh H. Cell delivery mechanisms for tissue repair. Cell Stem Cell 2008; 2(3): 205–213
7. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. Proc Natl Acad Sci USA 2000; 97(25): 13625–13630
8. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA 2003; 100(10): 5807–5812
9. Yasui T, Mabuchi Y, Toriumi H, Ebine T, Niibe K, Houlihan DD, Morikawa S, Onizawa K, Kawana H, Akazawa C, Suzuki N, Nakagawa T, Okano H, Matsuzaki Y. Purified human dental pulp stem cells promote osteogenic regeneration. J Dent Res 2016; 95(2): 206–214
10. Carinci F, Papaccio G, Laino G, Palmieri A, Brunelli G, D'Aquino R, Graziano A, Lanza V, Scapoli L, Martinelli M, Pezzetti F. Comparison between genetic portraits of osteoblasts derived from primary cultures and osteoblasts obtained from human pulpar stem cells. J Craniofac Surg 2008;19(3):616–625; discussion 626–627
11. Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, Smith AJ, Nör JE. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 2008; 34(8): 962–969
12. Telles PD, Machado MA, Sakai VT, Nör JE. Pulp tissue from primary teeth: new source of stem cells. J Appl Oral Sci 2011; 19(3): 189–194
13. Rosa V, Zhang Z, Grande RH, Nör JE. Dental pulp tissue engineering in full-length human root canals. J Dent Res 2013; 92 (11): 970–975
14. Yamamoto A, Sakai K, Matsubara K, Kano F, Ueda M. Multifaceted neuro-regenerative activities of human dental pulp stem cells for functional recovery after spinal cord injury. Neurosci Res 2014; 78: 16–20
15. Wang J, Wang X, Sun Z, Wang X, Yang H, Shi S, Wang S. Stem cells from human-exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. Stem Cells Dev 2010; 19(9): 1375–1383
16. d'Aquino R, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A, Desiderio V, Laino G, Papaccio G. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. Eur Cell Mater 2009; 18: 75–83
17. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004; 364(9429): 149–155
18. Ding G, Liu Y, Wang W, Wei F, Liu D, Fan Z, An Y, Zhang C, Wang S. Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. Stem Cells 2010; 28(10): 1829–1838
19. Park CH, Rios HF, Jin Q, Sugai JV, Padial-Molina M, Taut AD, Flanagan CL, Hollister SJ, Giannobile WV. Tissue engineering bone-ligament complexes using fiber-guiding scaffolds. Biomaterials 2012; 33(1): 137–145
20. Wang L, Shen H, Zheng W, Tang L, Yang Z, Gao Y, Yang Q, Wang C, Duan Y, Jin Y. Characterization of stem cells from alveolar periodontal ligament. Tissue Eng Part A 2011; 17(7-8): 1015–1026
21. Silvério KG, Rodrigues TL, Coletta RD, Benevides L, Da Silva JS, Casati MZ, Sallum EA, Nociti FH Jr. Mesenchymal stem cell properties of periodontal ligament cells from deciduous and permanent teeth. J Periodontol 2010; 81(8): 1207–1215
22. Tomokiyo A, Maeda H, Fujii S, Wada N, Shima K, Akamine A. Development of a multipotent clonal human periodontal ligament

cell line. Differentiation 2008; 76(4): 337–347
23. Singhatanadgit W, Donos N, Olsen I. Isolation and characterization of stem cell clones from adult human ligament. Tissue Eng Part A 2009; 15(9): 2625–2636
24. Feng F, Akiyama K, Liu Y, Yamaza T, Wang TM, Chen JH, Wang BB, Huang GT, Wang S, Shi S. Utility of PDL progenitors for *in vivo* tissue regeneration: a report of 3 cases. Oral Dis 2010; 16(1): 20–28
25. Kim HS, Kim KH, Kim SH, Kim YS, Koo KT, Kim TI, Seol YJ, Ku Y, Rhyu IC, Chung CP, Lee YM. Immunomodulatory effect of canine periodontal ligament stem cells on allogenic and xenogenic peripheral blood mononuclear cells. J Periodontal Implant Sci 2010; 40(6): 265–270
26. Iwata T, Yamato M, Zhang Z, Mukobata S, Washio K, Ando T, Feijen J, Okano T, Ishikawa I. Validation of human periodontal ligament-derived cells as a reliable source for cytotherapeutic use. J Clin Periodontol 2010; 37(12): 1088–1099
27. Chen FM, Gao LN, Tian BM, Zhang XY, Zhang YJ, Dong GY, Lu H, Chu Q, Xu J, Yu Y, Wu RX, Yin Y, Shi S, Jin Y. Treatment of periodontal intrabony defects using autologous periodontal ligament stem cells: a randomized clinical trial. Stem Cell Res Ther 2016; 7 (1): 33
28. Yang ZH, Zhang XJ, Dang NN, Ma ZF, Xu L, Wu JJ, Sun YJ, Duan YZ, Lin Z, Jin Y. Apical tooth germ cell-conditioned medium enhances the differentiation of periodontal ligament stem cells into cementum/periodontal ligament-like tissues. J Periodontal Res 2009; 44(2): 199–210
29. Li B, Sun J, Dong Z, Xue P, He X, Liao L, Yuan L, Jin Y. GCN5 modulates osteogenic differentiation of periodontal ligament stem cells through DKK1 acetylation in inflammatory microenvironment. Sci Rep 2016; 6(1): 26542
30. Xue P, Li B, An Y, Sun J, He X, Hou R, Dong G, Fei D, Jin F, Wang Q, Jin Y. Decreased MORF leads to prolonged endoplasmic reticulum stress in periodontitis-associated chronic inflammation. Cell Death Differ 2016; 23(11): 1862–1872
31. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Wang S, Shi S. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One 2006; 1 (1): e79
32. Bakopoulou A, Leyhausen G, Volk J, Tsiftsoglou A, Garefis P, Koidis P, Geurtsen W. Comparative analysis of *in vitro* osteo/odontogenic differentiation potential of human dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP). Arch Oral Biol 2011; 56(7): 709–721
33. Han C, Yang Z, Zhou W, Jin F, Song Y, Wang Y, Huo N, Chen L, Qian H, Hou R, Duan Y, Jin Y. Periapical follicle stem cell: a promising candidate for cementum/periodontal ligament regeneration and bio-root engineering. Stem Cells Dev 2010; 19(9): 1405–1415
34. Jo YY, Lee HJ, Kook SY, Choung HW, Park JY, Chung JH, Choung YH, Kim ES, Yang HC, Choung PH. Isolation and characterization of postnatal stem cells from human dental tissues. Tissue Eng 2007; 13(4): 767–773
35. Handa K, Saito M, Tsunoda A, Yamauchi M, Hattori S, Sato S, Toyoda M, Teranaka T, Narayanan AS. Progenitor cells from dental follicle are able to form cementum matrix *in vivo*. Connect Tissue Res 2002; 43(2-3): 406–408
36. Luan X, Ito Y, Dangaria S, Diekwisch TG. Dental follicle progenitor cell heterogeneity in the developing mouse periodontium. Stem Cells Dev 2006; 15(4): 595–608
37. Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, Sippel C, Hoffmann KH. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 2005; 24(2): 155–165
38. Handa K, Saito M, Yamauchi M, Kiyono T, Sato S, Teranaka T, Sampath Narayanan A. Cementum matrix formation *in vivo* by cultured dental follicle cells. Bone 2002; 31(5): 606–611
39. Guo W, Chen L, Gong K, Ding B, Duan Y, Jin Y. Heterogeneous dental follicle cells and the regeneration of complex periodontal tissues. Tissue Eng Part A 2012; 18(5-6): 459–470
40. Wu J, Jin F, Tang L, Yu J, Xu L, Yang Z, Wu G, Duan Y, Jin Y. Dentin non-collagenous proteins (dNCPs) can stimulate dental follicle cells to differentiate into cementoblast lineages. Biol Cell 2008; 100(5): 291–302
41. Kémoun P, Laurencin-Dalicieux S, Rue J, Farges JC, Gennero I, Conte-Auriol F, Briand-Mesange F, Gadelorge M, Arzate H, Narayanan AS, Brunel G, Salles JP. Human dental follicle cells acquire cementoblast features under stimulation by BMP-2/-7 and enamel matrix derivatives (EMD) *in vitro*. Cell Tissue Res 2007; 329(2): 283–294
42. Bai Y, Bai Y, Matsuzaka K, Hashimoto S, Fukuyama T, Wu L, Miwa T, Liu X, Wang X, Inoue T. Cementum- and periodontal ligament-like tissue formation by dental follicle cell sheets co-cultured with Hertwig's epithelial root sheath cells. Bone 2011; 48 (6): 1417–1426
43. Setzer FC, Kim S. Comparison of long-term survival of implants and endodontically treated teeth. J Dent Res 2014; 93(1): 19–26
44. Nakashima M, Akamine A. The application of tissue engineering to regeneration of pulp and dentin in endodontics. J Endod 2005; 31 (10): 711–718
45. Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, Smith AJ, Nör JE. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 2008; 34(8): 962–969
46. Guo W, He Y, Zhang X, Lu W, Wang C, Yu H, Liu Y, Li Y, Zhou Y, Zhou J, Zhang M, Deng Z, Jin Y. The use of dentin matrix scaffold and dental follicle cells for dentin regeneration. Biomaterials 2009; 30(35): 6708–6723
47. Dissanayaka WL, Zhu L, Hargreaves KM, Jin L, Zhang C. Scaffold-free prevascularized microtissue spheroids for pulp regeneration. J Dent Res 2014; 93(12): 1296–1303
48. Kelm JM, Fussenegger M. Microscale tissue engineering using gravity-enforced cell assembly. Trends Biotechnol 2004; 22(4): 195–202
49. Kelm JM, Djonov V, Ittner LM, Fluri D, Born W, Hoerstrup SP, Fussenegger M. Design of custom-shaped vascularized tissues using microtissue spheroids as minimal building units. Tissue Eng 2006; 12(8): 2151–2160
50. Chen FM, Sun HH, Lu H, Yu Q. Stem cell-delivery therapeutics for periodontal tissue regeneration. Biomaterials 2012; 33(27): 6320–6344
51. Nishida K, Yamato M, Hayashida Y, Watanabe K, Yamamoto K, Adachi E, Nagai S, Kikuchi A, Maeda N, Watanabe H, Okano T, Tano Y. Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. N Engl J Med

2004; 351(12): 1187–1196

52. Hoashi T, Matsumiya G, Miyagawa S, Ichikawa H, Ueno T, Ono M, Saito A, Shimizu T, Okano T, Kawaguchi N, Matsuura N, Sawa Y. Skeletal myoblast sheet transplantation improves the diastolic function of a pressure-overloaded right heart. J Thorac Cardiovasc Surg 2009; 138(2): 460–467
53. Flores MG, Hasegawa M, Yamato M, Takagi R, Okano T, Ishikawa I. Cementum-periodontal ligament complex regeneration using the cell sheet technique. J Periodontal Res 2008; 43(3): 364–371
54. Iwata T, Yamato M, Tsuchioka H, Takagi R, Mukobata S, Washio K, Okano T, Ishikawa I. Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. Biomaterials 2009; 30(14): 2716–2723
55. Flores MG, Yashiro R, Washio K, Yamato M, Okano T, Ishikawa I. Periodontal ligament cell sheet promotes periodontal regeneration in athymic rats. J Clin Periodontol 2008; 35(12): 1066–1072