Dental Stem Cells and Their Applications

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Stem cells are unspecialised cells that can divide, renew, and differentiate into more specialised cells. Due to their unique properties, stem cells are known for their use in therapies and treatments for missing tissues and damaged parts of the body. However, due to the invasive nature and other ethical issues with the retrieval process and usage of stem cells, stem cells are clinically being used in a limited manner. Furthermore, due to the invasive nature of the retrieval process elsewhere, dental tissues are one of the most preferred sources for stem cells. This review covers all of the characteristics of dental tissue-derived stem cells and their potential future uses.

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Introduction

Stem cells are undifferentiated cells that are capable of differentiating into more specialised cells with specific functions[1]. They mainly divide into two categories: pluripotent stem cells – cells that can differentiate into a variety of cells – and multipotent stem cells – cells that can differentiate into a limited variety of cells. Pluripotent stem cells further divide into embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSs), while multipotent stem cells consist of adult stem cells known as somatic or postnatal stem cells[2].

Embryonic stem cells

ESCs originate from the inner cell mass of a mammalian blastocyst, which is the early-stage of a preimplantation embryo[3]. To be considered an ESC, the cell must satisfy the following properties: 1) form from the inner cell mass of the blastocyst; 2) be capable of an unlimited number of divisions; 3) be capable of continuous proliferation; 4) be clonogenic; 5) have the transcription factor Oct4; 6) be able to differentiate into cells that are from all three primary germ layers of the embryo (endoderm, mesoderm and ectoderm); and 7) not require a signal or G1 stage in the cell cycle, to initiate DNA replication. The size of human ESCs are around 14 μm while mouse ESCs are closer to 8 μm[4]. ESCs are pluripotent and thought to have potential in cell replacement therapies, organ transplants and DNA restoration[5]. Despite its numerous beneficial characteristics and potential uses, today, ESCs are not being used for clinical use due to ethical issues that arise during its retrieval process. When isolating ESCs, the inner cell mass has to be isolated from the blastocyst and when the inner cell mass is isolated, the blastocyst or embryo destructs. In addition to the ethical issues, ESCs are not clinically being used due to the possibility of ESCs forming tumors[6].

Induced pluripotent stem cells

When c-Myc, KLF4, Oct4 and Sox2 are imposed into somatic cells, the cells become, like ESCs, pluripotent[7-9]. These cells are then, called iPSs. iPSs are obtained from adult tissues. The derivation period of iPSs from somatic cells is around 3 to 4 weeks for human cells and 1 to 2 weeks for mouse cells. On top of having a long derivation period, the derivation process of iPS is inefficient.

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The derivation efficiencies are around 0.01% to 0.10%[10]. Despite the fact that iPSs were made to become the alternative of ESCs and were studied due to the invasive nature of retrieving ESCs, iPSs are very limited compared to ESCs, in terms of clinical use, due to the reprogramming process. On top of this, iPSs have a long and inefficient derivation process and can be reprogrammed in a detrimental manner. For example, an iPS can end up inducing growth of a damaged body part. This can eventually lead to tumours and cancer[11]. Despite these issues, when fully understood, iPSs are believed to have potential in organ synthesis, tissue repair and formation of red blood cells[12].

Mesenchymal stem cells

Within adult stem cells, cells known as mesenchymal stem/stromal cells (MSCs), are considered to be special due to being immature and capable of a variety of differentiations. On top of being able to differentiate into more specialised cells with specific functions, MSCs have the ability to self-renew, grow fast, maintain their property after differentiation, and migrate to areas that need aid[13,14].

Positive markers that distinguish MSCs from other cells include CD13, CD73, CD105 and CD146. In addition to these positive markers, MSCs have negative markers: CD11b, CD14, CD19, CD34, CD45, CD79α and HLA-DR. However, to be officially recognised as a human MSC, on top of having both the positive and negative markers stated above, the cells must be adherent to plastic and be able to differentiate into at least osteoblasts, adipocytes and chrondroblasts[2,15].

MSCs can be retrieved from many locations: bone marrow, human endometrium, adipose tissue, amniotic fluid, the human amnion membrane, the chorion membrane, the placenta, cord blood, the umbilical cord, exfoliated deciduous teeth etc[16]. From these sources, dental tissues are considered a suitable source for future clinical stem cell usage, due to being a rich source and having less invasive stem cell-retrieval processes[2].

Dental stem cells

There are eight major populations of dental tissue-derived MSCs: dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), dental follicle progenitor cells (DFPCs), alveolar bone-derived mesenchymal stem cells (ABSMCs), stem cells from apical papilla (SCAPs), tooth germ progenitor cells (TGPCs) and gingival mesenchymal stem cells (GMSCs)[16,17].

Dental pulp stem cells (DPSCs)

DPSCs were the first adult human dental stem cells to be identified. They can be recognised through the following markers: CD9, CD10, CD13, CD14, CD19, CD24, CD29, CD31, CD34, CD44, CD45, CD59, CD73, CD90, CD105, CD106, CD117, CD133, CD146, CD166, CD271, dentin sialophosphoprotein during odontoblast differentiation, dentin matrix protein 1 during odontoblast differentiation, alkaline phosphatase during calcified and mineralized tissue differentiation, osteopontin during early stages of osteogenic differentiation and bone sialoprotein during late mineralized tissue differentiation[18]. Furthermore, STRO-1 and telomerase activities show the differentiation availability of each DPSC. Low telomerase activity indicates that the DPSC was differentiated. On the other hand, when the telomerase activity is high, the DPSC is undifferentiated. DPSCs are easily extractable and revivable[19]. Although DPSCs are inactive in their dental pulp form, when an injury occurs, DPSCs become active. Furthermore, similar to other dental tissue-derived MSCs, DPSCs do undergo multi-differentiation and self-renewal. DPSCs, along with SHEDs, are also known to take a long time in terms of initial colonisation periods[4]. However, unlike many other dental tissue-derived MSCs, DPSCs are known to be affected by their retrieval location. For example, MSCs from the inner dental pulp chamber have a higher growth rate compared to those from the outer pulp chamber[20]. DPSCs were also observed to react differently *in vitro* and in vivo. *In vitro*, DPSCs are able to differentiate into adipocytes, osteoblasts, odontoblasts, chondrocytes, myocytes, cardiomyocytes, active neurons, melanocytes and hepatocyte-like cells[21]. While in vivo, DPSCs can only differentiate into adipocytes, endotheliocytes and myofibers[22-25].

Stem cells from human exfoliated deciduous teeth (SHEDs)

SHEDS are tooth-derived MSCs that are commonly compared with DPSCs. They are more proliferative compared to DPSCs and bone marrow derived mesenchymal stem cells (BMSCs)[26,27]. In addition, they have a higher cell population doubling rate than DPSCs. The markers that distinguish SHEDs are CD11b, CD13, CD14, CD19, CD29, CD34, CD43, CD44, CD45, CD56, CD73, CD90, CD105, CD146 and CD166. SHEDs also contain embryonic stem cell markers, Oct4 and Nanog, neural stem cell markers, Nestin, and stage-specific embryonic antigens, SSEA-3 and SSEA-4[28]. In addition to being able to differentiate into osteogenic and adipogenic

cells, SHEDs encourage its host cells to form bone[29]. Although SHED's quality is not heavily affected from its extraction location, SHED was observed to differentiate differently *in vitro* and vivo. *In vitro*, SHED was observed to differente into osteocyte, odontocyte, adipocyte, chondrocyte, myocyte, neuronal cells, endothelia cells and other specific hepatic proteins. In vivo, SHEDs do not directly differentiate into osteogenic cells, instead they induce new bone formation, and when injected into an existing tooth, they are known to aid the creation of functional blood vessels[28]. Furthermore, in vivo, when injected along PEGylated fibrin, SHEDs can induce vascularised soft connective tissue[30,31].

Stem cells from apical papilla (SCAPs)

Like DFSCs, SCAPs also have a higher mineralization potential and proliferation rate compared to DPSCs. The positive and negative markers that distinguish SCAPs are the following: CD13, CD14, CD18, CD24, CD29, CD34, CD44, CD45, CD51, CD56, CD61, CD73, CD90, CD105, CD106, CD117, CD146, CD150 and CD166[18,32]. *In vitro*, SCAPs were observed to have additional neural markers such as neurofilament M, neuronal nuclear antigen, and neuron-specific enolase, and they differentiated into odontogenic, osteoblastic, neuronal cells, and hepatocyte-like cells. In vivo, SCAPs were observed to produce dentin-pulp like complexes and cement/woven bone-like tissue[33].

Periodontal ligament stem cells (PDLSCs)

Like SHEDs, PDLSCs are also more proliferative than BMSCs. On top of this, they are more clonogenic than BMSCs. PDLSCs contain the following markers: CD9, CD10, CD13, CD14, CD29, CD31, CD34, CD44, CD45, CD59, CD73, CD90, CD105, CD106, CD146 and CD166. The quality of PDLSCs, like DPSCs, depends on their harvest location. Compared to PDLSCs, which are harvested around the alveolar bone, those from the root surface are inferior. PDLSCs can restore periodontal tissues such as alveolar bone, cementum and periodontal ligaments[17]. *In vitro*, PDLSCs were observed to differentiate into cementoblasts, osteoblasts, adipocytes, neuronal cells and chondrogenic cells. While in vivo, they were observed to, as stated above, regenerate periodontal tissue[34].

Dental follicle progenitor stem cells (DFSCs)

From the dental tissue-derived MSCs, DFSCs have the best plasticity. DFSCs have a higher proliferation rate compared to DPSCs and SCAPs. They can produce cementum and bone, such as the root surface of teeth and are known to be used in periodontal and bone regeneration therapies[35]. DFSCs have CD9, CD10, CD13, CD29, CD31, CD34, CD44, CD45, CD53, CD59, CD73, CD90, CD105, CD106, CD133, CD166, CD271, Aggrecan, type 1 collagen, type 3 collagen, Notch-1 and Nestin as their markers[36]. DFSCs have heterogeneity: each DFSC harvested from different teeth have different mineralization and proliferation rates[2]. *In vitro*, DFSCs differentiate into calcified nodules, cementoblasts, chondrocytes, adipocytes and osteogenic cells. While in vivo, DFSCs were observed to produce woven bone-like tissue; however, DFSCs were not observed to form hard tissue[37].

Others

Unlike the other dental tissue-derived MSCs stated above, ABSMCs do not have all the positive and negative markers as stated in the human MSC criteria: ABSMC does not have CD14, CD34 and CD45 but additionally has CD90 and STRO-1. More specifically, ABSMCs have CD11b, CD13, CD14, CD19, CD29, CD31, CD34, CD44, CD45, CD71, CD73, CD90, CD105, CD146, CD166 and STRO-1[38-40]. *In vitro*, ABSMCs were observed to differentiate into osteoblastic, chrondrogenic and adipogenic cells. In addition to its own differentiation, ABSMCs can induce osteogenesis in its host. In vivo, ABSMCs were observed to form new bone[41].

Like SCAPs, TGPCs have a high proliferation rate. TGPCs have the following markers: CD14, CD29, CD34, CD44, CD45, CD73, CD90, CD105, CD106, CD133, CD166 and STRO-1[42-44]. *In vitro*, they differentiate into adipocytes, osteoblasts, odontoblasts, chondrocytes, endothelial cells and neuronal cells. While in vivo, they were observed to suppress liver inflammation[42].

Compared to BMSCs, GMSCs have a high proliferation and population doubling rate. The positive and negative markers GMSCs contain are CD14, CD29, CD34, CD44, CD45, CD73, CD90, CD105, CD106, CD117, CD146 and CD166. Furthermore, GMSCs have several addition positive markers on top of those included in the human MSC criteria: Oct4, Sox2, Nanog, Nestin, SSEA-4 and STRO-1[45-47]. *In vitro*, GMSCs differentiate into adipocytes, chondrocytes, osteoblasts, neuronal cells and endoderm-like cells. While in vivo, GMSCs were observed to produce tissues in fibrin gels and induce bone regeneration when injected into a osteogenic medium with collagen gel[2].

Applications of dental stem cells and their immunomodulatory properties

Today, dental tissues, salivary glands and orofacial bone marrow are known to be the safest and most accessible stem cell source. Applications of stem cells, which are an exciting prospect include bone regeneration, tooth regeneration, dentin-pulp formation, periodontal regeneration and neural tissue regeneration[21,48].

With regard to bone and tooth regeneration, DPSCs can form fibrous bone tissue, and those from human third molars were observed to restore human mandible defects[49]. SHEDs repair calvarial defects, while DFPCs induce new bone formation[29]. SCAPs can make bone-like mineralized tissue[50]. Not only do SCAPs make bone-like mineralized tissue but they also, along with PDLSCs, can form root periodontal complexes and support a dental crown on top[51]. GMSCs, like DPSCs and SHEDs, can repair mandibular and calvarial defects[2].

Not only can DPSCs form fibrous bone tissue but can also form dentin pulp-like structures along with odontoblast cells[22]. While SCAPs and SHEDs do not make dentin structures like DPSCs, SCAPs and SHEDs can make a layer of dentin-like tissue. Furthermore, SHEDs can differentiate into odontoblast cells during this process[52].

For periodontal regeneration, PDLSCs are known to differentiate into osteoblast and cementoblasts and induce new tissue formation around the surface of dental implants[28].

For neural tissue regeneration, SHEDs have shown the most promising results. When SHEDs were injected into Parkinsonian rats, the rats recovered from their behavioural disorder[17]. Along with SHEDs, dental pulp cells also demonstarted a positive effect on the tissue organisation of axons and neurons[53].

Although the most popular uses of dental tissue-derived stem cells are bone regeneration, tooth regeneration, dentin-pulp formation, periodontal regeneration and neural tissue regeneration, there are also other potential uses for these dental tissue-derived stem cells. PDLSCs can improve facial wrinkles, while SHEDs induce smooth skeletal muscle cells[54,55]. Furthermore, SHEDs are known to be capable of corneal reconstruction[56]. DPSCs improve ischemic diseases and could potentially offer a treatment alternative for strokes[57]. TGPCs can deter liver injuries from worsening[42]. GMSCs cannot only be used for skin injuries and inflammatory diseases but also reverse body weight loss in chemotherapy[58].

In general, dental tissue-derived MSCs play a unique role in the inhibition and rise of T cell proliferation. For example, DPSCs suppress T cell proliferation[59]. This infers that DPSCs can be used as an anti-inflammatory treatment. SHEDs help the growth of regulatory T cells (Tregs), while inhibiting the proliferation of T helper 17 (Th17). This specific type of inhibition and growth allows SHEDs to restore the balance between Th17 and Tregs, which induces the reversal of systemic lupus erythematosus-associated disorders[2]. PDLSCs, on the other hand, inhibit the induction of Tregs[60]. DFSC and ABSMC's relationship with T cell proliferation is not clear up until today. However, the pattern between DFSCs and the T cell proliferation infers that DFSCs can potentially become a treatment for tissue injuries. GMSCs, like DPSCs, inhibit T cell proliferation and show similar results to current anti-inflammatory therapies and medications[2]. In addition, by inducing M2 polarization, GMSCs accelerate wound healing, decrease oral mucositis caused by chemotherapy and treat allergic diseases[58].

Conclusion

With further research on the immunology of dental tissue-derived MSCs, it is believed that dental tissue-derived MSCs can treat parts of the body that currently have no therapies or treatments when damaged, such as the heart and brain. Furthermore, it is known that MSCs can potentially replace organ transplants and become a more personalised cure-all drug that does not cause side effects. Once there has been enough research on these MSCs, complete tooth regeneration will become possible and the supply of dental tissue-derived MSCs, whose retrieval process is not invasive, will become limitless and enough to be clinically used for numerous cases. Today, it is known that DPSCs, along with scaffold technology, can be used to solve the majority of dental-related problems. Numerous outcomes and research indicate that, once completed and mastered, dental tissue-derived MSC therapies will have a huge impact in the medical field.

References

1. Slack JM. Origin of stem cells in organogenesis. Science 2008;322:1498–1501.
2. Puri MC, Nagy A. Concise review: Embryonic stem cells versus induced pluripotent stem cells: the game is on. Stem Cells 2012;30:10–14.
3. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. Science 1998;282:1145–1147.
4. Zwaka TP, Thomson JA. Homologous recombination in human embryonic stem cells. Nat Biotechnol 2003;21:319–321.

5. Aladjem MI, Spike BT, Rodewald LW, et al. ES cells do not activate p53-dependent stress responses and undergo p53-independent apoptosis in response to DNA damage. Curr Biol 1998;8:145–155.
6. Knoepfler PS. Deconstructing stem cell tumorigenicity: a roadmap to safe regenerative medicine. Stem Cells 2009;27:1050–1056.
7. Kingham E, Oreffo RO. Embryonic and induced pluripotent stem cells: understanding, creating, and exploiting the nano-niche for regenerative medicine. ACS Nano 2013;7:1867–1881.
8. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006;126:663–676.
9. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. Nature 2007;448:313–317.
10. Zhou T, Benda C, Duzinger S, et al. Generation of induced pluripotent stem cells from urine. J Am Soc Nephrol 2011;22:1221–1228.
11. Zhao XY, Li W, Lv Z, et al. iPS cells produce viable mice through tetraploid complementation. Nature 2009;461:86–90.
12. Park TS, Bhutto I, Zimmerlin L, et al. Vascular progenitors from cord blood-derived induced pluripotent stem cells possess augmented capacity for regenerating ischemic retinal vasculature. Circulation 2014;129:359–372.
13. Egusa H, Iida K, Kobayashi M, et al. Downregulation of extracellular matrix-related gene clusters during osteogenic differentiation of human bone marrow- and adipose tissue-derived stromal cells. Tissue Eng 2007;13:2589–2600.
14. Ding DC, Shyu WC, Lin SZ. Mesenchymal stem cells. Cell Transplant 2011;20:5–14.
15. Potdar PD, Jethmalani YD. Human dental pulp stem cells: Applications in future regenerative medicine. World J Stem Cells 2015;7:839–851.
16. Hass R, Kasper C, Böhm S, Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal 2011;9:12.
17. Estrela C, Alencar AH, Kitten GT, et al. Mesenchymal stem cells in the dental tissues: perspectives for tissue regeneration. Braz Dent J 2011;22:91–98.
18. Gronthos S, Brahim J, Li W, et al. Stem cell properties of human dental pulp stem cells. J Dent Res 2002;81:531–535.
19. Odorico JS, Kaufman DS, Thomson JA. Multilineage differentiation from human embryonic stem cell lines. Stem Cells 2001;19:193–204.
20. Mauth C, Huwig A, Graf-Hausner U, Roulet JF. Restorative applications for dental pulp therapy topics in tissue engineering. Ashammakhi N, Reis R, Chiellini E (eds). In: Topics in Tissue Engineering, 2007:1–30.
21. Demarco FF, Conde MC, Cavalcanti BN, et al. Dental pulp tissue engineering. Braz Dent J 2011;22:3–13.
22. Gronthos S, Mankani M, Brahim J, et al. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 2000;97:13625–13630.
23. Stevens A, Zuliani T, Olejnik C, et al. Human dental pulp stem cells differentiate into neural crest-derived melanocytes and have label-retaining and sphere-forming abilities. Stem Cells Dev 2008;17:1175–1184.
24. Almushayt A, Narayanan K, Zaki AE, George A. Dentin matrix protein 1 induces cytodifferentiation of dental pulp stem cells into odontoblasts. Gene Ther 2006;13:611–620.
25. Waddington RJ, Youde SJ, Lee CP, Sloan AJ. Isolation of distinct progenitor stem cell populations from dental pulp. Cells Tissues Organs 2009;189:268–274.
26. Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 2009;88:792–806.
27. Pivoriuūnas A, Surovas A, Borutinskaite V, et al. Proteomic analysis of stromal cells derived from the dental pulp of human exfoliated deciduous teeth. Stem Cells Dev 2010;19:1081–1093.
28. Miura M, Gronthos S, Zhao M, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 2003;100:5807–5812.
29. Seo BM, Sonoyama W, Yamaza T, et al. SHED repair critical-size calvarial defects in mice. Oral Dis 2008;14:428–434.
30. Casagrande L, Demarco FF, Zhang Z, et al. Dentin-derived BMP-2 and odontoblast differentiation. J Dent Res 2010;89:603–608.
31. Galler KM, Cavender AC, Koeklue U, et al. Bioengineering of dental stem cells in a PEGylated fibrin gel. Regen Med 2011;6:191–200.
32. Ding G, Wang W, Liu Y, et al. Effect of cryopreservation on biological and immunological properties of stem cells from apical papilla. J Cell Physiol 2010;223:415–422.
33. Bakopoulou A, Leyhausen G, Volk J, et al. 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;56:709–721.
34. Seo BM, Miura M, Gronthos S, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004;364:149–155.
35. Shoi K, Aoki K, Ohya K, et al. Characterization of pulp and follicle stem cells from impacted supernumerary maxillary incisors. Pediatr Dent 2014;36:79–84.
36. Zhang W, Walboomers XF, Shi S, et al. Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. Tissue Eng 2006;12:2813–2823.
37. Morsczeck C, Petersen J, Völlner F, et al. Proteomic analysis of osteogenic differentiation of dental follicle precursor cells. Electrophoresis 2009;30:1175–1184.
38. Matsubara T, Suardita K, Ishii M, et al. Alveolar bone marrow as a cell source for regenerative medicine: differences between alveolar and iliac bone marrow stromal cells. J Bone Miner Res 2005;20:399–409.
39. Mason S, Tarle SA, Osibin W, et al. Standardization and safety of alveolar bone-derived stem cell isolation. J Dent Res 2014;93:55–61.
40. Park JC, Kim JC, Kim YT, et al. Acquisition of human alveolar bone-derived stromal cells using minimally irrigated implant osteotomy: in vitro and in vivo evaluations. J Clin Periodontol 2012;39:495–505.
41. Sonoyama W, Liu Y, Yamaza T, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 2008;34:166–171.
42. Ikeda E, Yagi K, Kojima M, et al. Multipotent cells from the human third molar: feasibility of cell-based therapy for liver disease. Differentiation 2008;76:495–505.
43. Yalvac ME, Ramazanoglu M, Rizvanov AA, et al. Isolation and characterization of stem cells derived from human third molar tooth germs of young adults: implications in neo-vascularization, osteo-, adipo- and neurogenesis. Pharmacogenomics J 2010;10:105–113.
44. Yalvaç ME, Ramazanoglu M, Tekguc M, et al. Human tooth germ stem cells preserve neuro-protective effects after long-term cryopreservation. Curr Neurovasc Res 2010;7:49–58.
45. Zhang Q, Shi S, Liu Y, et al. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 2009;183:7787–7798.
46. Tang L, Li N, Xie H, Jin Y. Characterization of mesenchymal stem cells from human normal and hyperplastic gingiva. J Cell Physiol 2011;226:832–842.
47. Wang F, Yu M, Yan X, et al. Gingiva-derived mesenchymal stem cell-mediated therapeutic approach for bone tissue regeneration. Stem Cells Dev 2011;20:2093–2102.
48. Nör JE. Tooth regeneration in operative dentistry. Oper Dent 2006;31:633–642.
49. d'Aquino R, De Rosa A, Lanza V, et al. 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.

50. Huang GT, Yamaza T, Shea LD, et al. Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng Part A 2010;16:605–615.
51. Trubiani O, Orsini G, Zini N, et al. Regenerative potential of human periodontal ligament derived stem cells on three-dimensional biomaterials: a morphological report. J Biomed Mater Res A 2008;87:986–993.
52. Shi S, Bartold PM, Miura M, et al. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofac Res 2005;8:191–199.
53. de Almeida FM, Marques SA, Ramalho Bdos S, et al. Human dental pulp cells: a new source of cell therapy in a mouse model of compressive spinal cord injury. J Neurotrauma 2011;28:1939–1949.
54. Fang D, Seo BM, Liu Y, et al. Transplantation of mesenchymal stem cells is an optimal approach for plastic surgery. Stem Cells 2007;25:1021–1028.
55. Kerkis I, Ambrosio CE, Kerkis A, et al. Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic J Transl Med 2008;6:35.
56. Monteiro BG, Serafim RC, Melo GB, et al. Human immature dental pulp stem cells share key characteristic features with limbal stem cells. Cell Prolif 2009;42:587–594.
57. Gandia C, Armiñan A, García-Verdugo JM, et al. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells 2008;26:638–645.
58. Zhang Q, Nguyen AL, Shi S, et al. Three-dimensional spheroid culture of human gingiva-derived mesenchymal stem cells enhances mitigation of chemotherapy-induced oral mucositis. Stem Cells Dev 2012;21:937–947.
59. Pierdomenico L, Bonsi L, Calvitti M, et al. Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. Transplantation 2005;80:836–842.
60. Liu D, Xu J, Liu O, et al. Mesenchymal stem cells derived from inflamed periodontal ligaments exhibit impaired immunomodulation. J Clin Periodontol 2012;39:1174–1182.