REVIEW

Mesenchymal Stem Cells and Tooth Engineering

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Abstract

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Tooth loss compromises human oral health. Although several prosthetic methods, such as artificial denture and dental implants, are clinical therapies to tooth loss problems, they are thought to have safety and usage time issues. Recently, tooth tissue engineering has attracted more and more attention. Stem cell based tissue engineering is thought to be a promising way to replace the missing tooth. Mesenchymal stem cells (MSCs) are multipotent stem cells which can differentiate into a variety of cell types. The potential MSCs for tooth regeneration mainly include stem cells from human exfoliated deciduous teeth (SHEDs), adult dental pulp stem cells (DPSCs), stem cells from the apical part of the papilla (SCAPs), stem cells from the dental follicle (DFSCs), periodontal ligament stem cells (PDLSCs) and bone marrow derived mesenchymal stem cells (BMSCs). This review outlines the recent progress in the mesenchymal stem cells used in tooth regeneration.

Keywords mesenchymal stem cell, tooth engineering, dental pulp stem cell

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Introduction

A commonly applied definition of tissue engineering, as stated by Langer and Vacanti, is “an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function or a whole organ” (Langer and Vacanti, 1993). Tissue engineering has also been defined as “understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use” (MacArthur and Oreffo, 2005). Tissue engineering aims to stimulate the body either to regenerate tissue on its own or to grow tissue outside the body which can then be implanted as natural tissue.

Stem cells are characterized by the ability to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types. According to developmental stages, stem cells can be divided into embryonic stem cells and adult stem cells. Differentiation and proliferation of embryonic stem cells constitute the basis of animal development. The further differentiation of adult stem cells is the prerequisite of tissues and organs’ repair and regeneration. Embryonic stem cells are the progenitors of undifferentiated cells, which are “totipotent” (totipotency is the ability of a single cell to divide and produce all the differentiated cells in an organism, including extraembryonic tissues) and can differentiate into a variety of cells to form various organs, also known as the “all-competent cells”. In the process of cell differentiation, they can gradually differentiate into a stable form of “pluripotent stem cells”. With the features of highly proliferative capacity and plasticity, stem cells are regarded as a new source of seed cells in tissue engineering in a wide range of applications.

There is no doubt that the description of tissue engineer offers a new hope to both patients who

suffer with tooth loss and the dentists as well. The exploration of tooth tissue engineering mainly focuses on three parts: seeding cells, scaffolds and growth factors. Here we summarized the recent studies on tooth engineering using mesenchymal stem cells. Numerous attempts have been made to "create" tooth and very promising results been made. Single cell suspensions obtained from rat, pig or mice tooth germs or bone marrow were seeded onto the surface of biodegradable polymer scaffolds (e.g. collagen-coated polyglycolic acid, calcium phosphate material, collagen sponges, PGA/PLLA scaffolds) and the cell/polymer constructs were successfully re-implanted into a suitable immunocompromised host, so a sufficient blood supply could support the growth of higher ordered structures (Duailibi *et al*., 2004; Honda *et al*., 2006, 2007a, 2007b; Hu *et al*., 2006). All these reports described the formation of dentin or enamel or even both of them. Besides dissociated cells, the dissociated tooth tissues were also used to bioengineer complex tooth crowns resembling those of naturally developing teeth successfully (Young *et al*., 2002). This indicated that the seed cells or tissues could differentiate properly into odontoblast-like and ameloblast-like cells. However, these bioengineered teeth were produced in ectopic sites and lacked of some essential elements such as the complete root and periodontal tissues which allow their correct anchoring into the alveolar bone (Bluteau *et al*., 2008). Recently, a three-dimensional organ-germ culture method has been proposed for growing teeth in the mouse mandible (Nakao *et al*., 2007). In this study, epithelial and mesenchymal cells were sequentially seeded into a collagen gel drop and then implanted into the tooth cavity of adult mice. With this technique the bioengineered tooth germ generated a structurally correct tooth, showing all dental structures such as odontoblasts, ameloblasts, dental pulp, blood vessels, crown, periodontal ligament, root and alveolar bone (Nakao *et al*., 2007). Thus, the development of bioengineered organ replacement strategies and the appropriate seeding cells, plus biodegradable polymer scaffolds and proper microenvironment ensure a substantial advance in tooth engineering science.

In the field of tooth engineering, efforts have been made to explore mesenchymal stem cells (MSCs) such as stem cells from human exfoliated deciduous teeth (SHEDs), adult dental pulp stem cells (DPSCs), stem cells from the apical part of the papilla (SCAPs), stem cells from the dental follicle (DFSCs), periodontal ligament stem cells (PDLSCs), bone marrow derived mesenchymal stem cells (BMSCs) and epithelium-originated dental stem cells (Bluteau *et al*., 2008). The recent advances of MSCs in tooth engineering were reviewed as followings.

Stem cells from human exfoliated deciduous teeth

The discovery of stem cell in deciduous teeth (Miura *et al*., 2003) sheds a light on the intriguing possibility of using dental pulp stem cells for tissue engineering (Murray and Garcia-Godoy, 2004; Sloan and Smith, 2007). The obvious advantages of SHEDs (stem cells from human exfoliated deciduous teeth) are: higher proliferation rate compared with stem cells from permanent teeth (Miura *et al*., 2003), easy to be expanded *in vitro*, high plasticity since they can differentiate into neurons, adipocytes, osteoblasts and odontoblasts, readily accessible in young patient (Miura *et al*., 2003), especially suitable for young patients with mix dentition (Nör, 2006). Miura demonstrated that SHEDs could not differentiate directly into osteoblasts but did induce new bone formation by forming a template to recruit murine host osteogenic cells (Miura *et al*., 2003). SHEDs are distinctive with the osteoinductive ability and high plasticity. Cordeiro seeded SHEDs in PLLA (porous poly L-lactic acid) prepared within human tooth slice scaffolds and transplanted them into the subcutaneous tissue of immunodeficient mice. They observed that SHEDs differentiated into odontoblast-like cells and showed morphologic characteristics resembled those of odontoblast cells. Moreover, an increase in microvessel density was found in the co-implantation. They also verified that the transplanted SHEDs were capable of differentiating into blood vessels that anastomosed with the host vasculature (Cordeiro *et al*., 2008). These studies proved that SHEDs might be an ideal resource of stem cells to repair damaged tooth structures and induce bone regeneration.

Adult dental pulp stem cells

The regenerative capacity of the human dentin/pulp complex enlightens scientists that dental pulp may contain the progenitors that are responsible for dentin repair. Gronthos first identified adult dental pulp stem cells (DPSCs) in human dental pulp in 2000 and found DPSCs could regenerate a dentin-pulp-like complex, which is composed of mineralized matrix with tubules lined with odontoblasts, and fibrous tissue containing blood vessels in an arrangement similar to the dentin-pulp complex found in normal human teeth (Gronthos *et al*., 2000). The same group further verified DPSCs possessed striking features of self-renewal capability and multi-lineage differentiation by finding that DPSCs were capable of forming ectopic dentin and associated pulp tissue *in vivo* and differentiating into adipocytes and neural-like cells (Gronthos *et al*., 2002). An *in vivo* study showed that DPSCs produced bone when implanted into subcutaneous sites in immunocompromised mice with HA/TCP powder as carrier. In addition, the potential of DPSCs for long-term storage was analyzed. They found that even after storage for 2 years, DPSCs were still capable of differentiating into pre-osteoblasts and produced woven bone tissues. In addition, DPSCs still expressed certain surface antigens, confirming cellular integrity (Papaccio *et al*., 2006, Otaki *et al*., 2007). Scientists have been working to find an efficient scaffold that can be loaded with DPSCs and an appropriate microenvironment to promote the differentiation of DPSCs. In a recent study DPSCs were seeded onto different 3-dimensional (3-D) scaffold materials (a spongeous collagen, a porous ceramic, and a fibrous titanium mesh) and implanted in nude mice for 6 or 12 weeks, the formed tissue was not dentin-pulp-like complex but something resembled connective tissue (Zhang *et al*., 2006). These studies indicate the potential of DPSCs in tooth tissue engineering.

Stem cells from dental follicle

The dental follicle is a mesenchymal tissue that surrounds the developing tooth germ. During tooth root formation, periodontal components, such as cementum, periodontal ligament (PDL), and alveolar bone, are created by dental follicle progenitors (Yokoi *et al*., 2007). Stem cells from dental follicle (DFSCs) have been isolated from follicle of human third molars and express the stem cell markers: Notch1, STRO-1 and nestin (Morsczeck *et al*., 2005). DFSCs were found to be able to differentiate into osteoblasts/cementoblasts, adipocytes, and neurons (Kémoun *et al*., 2007, Yao *et al*., 2008, Coura *et al*., 2008). In addition, immortalized dental follicle cells were transplanted into immunodeficient mice and were able to recreate a new periodontal ligament (PDL)-like tissue after 4 weeks (Yokoi *et al*., 2007). These cells may be a useful research tool for studying PDL formation and for developing regeneration therapies. Wu showed that dNCPs (dentin non-collagenous proteins) extracted from dentin could stimulate DFSCs to differentiate into cementoblast lineages (Wu *et al*., 2008). Tsuchiya reported that Col-Ⅰ facilitated the differentiation of DFSCs along the mineralization process (Tsuchiya *et al*., 2008). Kémoun proved that enamel matrix derivatives (EMD) activated human dental follicle stem cells (hDFSCs) toward the cementoblastic phenoltype. hDFSCs acquired cementoblast features under stimulation of BMP-2/-7 and EMD *in vitro* (Kémoun *et al*., 2007). These studies provide new insights into the mechanism of cementogenesis. In addition, Luan indicated that DFSCs lines were heterogeneous. The three main lineages were highly undifferentiated state of periodontal ligament-type lineage and cementoblastic or alveolar bone osteoblastic lineage. The profound cellular heterogeneity of DFSCs suggests that heterogeneous cellular constituents might play a role in tissue regeneration as much as the individual lineages might do (Luan, 2007).

Bone marrow derived mesenchymal stem cells

Human bone marrow-derived stem cells (hBMSCs) originate from cell populations in the bone marrow and are capable of differentiating along multiple mesenchymal lineages. Bone regeneration has long been the critical point of BMSCs' research. In the

aspect of tooth engineering, current research focused on the fields of tooth-like structures formation and periodontal regeneration. The bone marrow derived (BMD) cells are a mixed population which consist of fibroblasts, osteoblast, adipocyte progenitors and up to 0.01% stem cells (Pereira *et al*., 1998; Pittenger *et al.*, 1999). Most tooth engineering researches were carried out with cell populations of purified stem cells, however, Ohazama challenged them by proving that bone and soft tissues could also be formed from a heterogeneous population such as BMD cells. They reported that tooth structures could also be formed when intact explants which formed from BMD cells were transferred into renal capsules. Meanwhile, they recombined embryonic oral epithelium with three non-dental mesenchymal cells such as embryonic stem cells, neural stem cells and adult BMD cells, and transferred the recombinations into adult renal capsules and adult jaw. The results showed tooth structures and associated bone were developed in the adult environment (Ohazama *et al*., 2004). Li came to the coincident conclusion with that of Ohazama group. They verified that the recombination of BMSCs with oral epithelial cells derived from rat embryos expressed odontogenic genes such as *Pax*9, *DMP*1, and *DSPP* and demonstrated the presence of tooth-like structures (Li *et al*., 2007). BMSCs have been tested for their ability to recreate periodontal tissue and restore periodontal defects. It was proved that auto-transplantation of BMSCs are able to form *in vivo* cementum, periodontal ligament, and alveolar bone after implantation into defective periodontal sites. Thus, bone marrow provides an alternative source of MSC for the treatment of periodontal diseases (Kawaguchi *et al*., 2004). Interestingly, when BMSCs were regarded as a source of mesenchymal seed cells, Hu and his colleagues investigated the possibility that BMSCs give rise to different types of epithelial cells and their potential to serve as a source for ameloblasts. Their results showed, for the first time, that BMSCs can be reprogrammed to give rise to ameloblast-like cells (Hu *et al*., 2006). They offered BMSCs a novel possibility for tooth-tissue engineering and could be induced into both mesenchymal and epithelium cells in tooth tissue engineering. Not all the scientists identify BMSCs with the ideal seeding cells for tooth engineering. Jing pointed out that the differentiation abilities of BMSCs decrease significantly with the increasing age of donors. They hold the opinion that adipose derived stem cells could be induced into odontogenic lineage and might be used as suitable seeding cells for tooth regeneration to replace the lost tooth of elderly patients (Jing *et al*., 2008).

Periodontal ligament stem cells

The periodontal ligament is a specialized connective tissue, derived from dental follicle and originated from neural crest cells. Recent studies have shown that mesenchymal stem cells obtained from periodontal ligament (PDLSCs) are multipotent cells with similar features of the BMSCs and DPSCs, capable of developing different types of tissues such as bone and tooth associated-tissues. It was reported that PDLSCs could differentiate into cells that can colonize and grow on biocompatible scaffold, suggesting an easy and efficient autologous source of stem cells for bone tissue engineering in regenerative dentistry (Trubiani *et al*., 2008). Orciani verified the osteogenic ability of PDLSCs and pointed out that differentiating cells were also characterized by an increase of Ca^{2+} and nitric oxide production. The authors demonstrated that local reimplantation of expanded cells in conjugation with a nitric oxide donor could represent a promising method for treatment of periodontal defects (Orciani *et al*., 2008). Besides osteogenic ability, differentiation of PDLSCs to the cementoblastic lineage was also emphasized. The conditioned medium from developing apical tooth germ cells (APTG-CM) was shown to be able to provide a cementogenic microenvironment and induce differentiation of PDLSCs along the cementoblastic lineage. When transplanted into immunocompromised mice, the induced PDLSCs showed tissue-regenerative capacity to produce cementum/periodontal ligament-like structures, characterized by a layer of cementum-like mineralized tissues connected with periodontal ligament-like collagen fibers. This has important implications for periodontal engineering (Yang *et al*., 2008). Dentin noncollagenous proteins (DNCPs) were also proved to increase proliferation and adhesion ability of HPDLSCs. Induced HPDLSCs

presented several features of cementoblast differentiation (Ma *et al*., 2008).

Moreover, some studies paid more attention to the identification and character of cells produced from human periodontal ligament. There is evidence that human periodontal ligament, with its mesodermal derivatives, produced neural crest-like cells. Such features suggested a recapitulation of their embryonic state. The human periodontal ligament revealed itself as a viable alternative source for possible primitive precursors to be used in stem-cell therapies (Coura *et al*., 2008).

Adipose-derived stromal cells

Adipose-derived stromal cells (ADSCs) are considered to contain a group of pluripotent mesenchymal stem cells and manifest multilineage differentiation capacity, including osteogenesis, chondrogenesis and adipogenesis (Liu *et al*., 2008). ADSCs exhibit stable growth and proliferation kinetics *in vitro*. Adipose tissue can be obtained by less invasive methods and in larger quantities than bone marrow cells, making the use of hADSCs as a source of stem cells very appealing (Zuk *et al*., 2002). In 2005, a research team first proposed the hypothesis that adipose derived stem cells could be induced into odontogenic lineage and might be used as suitable seeding cells for tooth regeneration to replace the lost tooth of elderly patients (Jing *et al*., 2008). The team holds the opinion that the seeding cells for tooth regeneration such as odontoblasts from dental germ, stem cells from dental pulp and deciduous teeth, and ectomesenchymal cells from the first branchial arch are difficult, even impossible to harvest in clinic. Bone marrow mesenchymal stem cells have odontogenic capacity, but their differentiation abilities significantly decrease with the increasing age of the donors. Therefore, the cells mentioned above are not practical in the clinical application of tooth regeneration in the old. They tried to find ideal alternative seeding cells and an appropriate inducing method to overcome the problems mentioned above. The team reported that overexpression of DSPP enhanced expression of genes related to mineralization, such as *Cbfa*1, *Osx*, *BSP*, *OCN* and *DMP*1 in ADSCs and early odontogenic marker genes, such as *Msx*1, *Msx*2, *Lhx*7 and *Pax*9, which implied that these cells may differentiate into functional odontoblast-like cells (Wu *et al*., 2008). In addition, the osteogenesis potential of ADSCs has prompted wide attention. It was reported that ADSCs expressed bone marker proteins including alkaline phosphatase, type Ⅰ collagen, osteopontin, and osteocalcin and produce mineralized matrix. In the current study, the ADSCs ability to form osteoid matrix *in vivo* was determined, proved them a novel therapeutic for bone repair and regeneration (Hicok *et al*., 2004). Kakudo investigated the possibility of using honeycomb collagen scaffold to culture ADSCs in bone tissue engineering. It showed that the scaffold was filled with the grown ADSCs and calcification materials. When the ADSCs-loaded honeycomb collagen scaffolds were subcutaneously transplanted into nude mice, bone formation *in vivo* was identified after 8 weeks (Kakudo *et al*., 2008).

From this review, we can conclude that tooth engineer seeding cells may come from both dental stem cells and non-dental stem cells, which share the similar features such as high proliferation rate, multi-differentiation ability, easy accessibility, high viability and easy to be induced. There has been great interest in mesenchymal stem cells and their roles in maintaining the physiological structure of tissues. The progress of tooth engineering can not be made without the support of other research of organ regeneration. There is a comment interpretating the relationship: “the acessibility of teeth, together with the fact that they are not essential organs, mean that teeth provide an attractive organ with which to test the practicalities and feasibility of tissue-engineered organ replacement (Ohazama *et al*., 2004).

Since these cells are considered as candidates for regenerative medicine, the knowledge of the cell differentiation mechanisms is imperative for the development of tooth engineering. Further studies will be carried out to elucidate the molecular mechanisms involved in their maintenance and differentiation *in vitro* and *in vivo*.

Reference

Bluteau G, Luder HU, De Bari C, Mitsiadis TA (2008). Stem cells for tooth engineering. *Eur Cell Mater*, 16:

1–9.

Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, *et al*. (2008). Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. *J Endod*, 34(8): 962–969.

Coura GS, Garcez RC, de Aguiar CB, Alvarez-Silva M, Magini RS, Trentin AG (2008). Human periodontal ligament: a niche of neural crest stem cells. *J Periodontal Res*, 43(5): 531–536.

Duailibi MT, Duailibi SE, Young CS, Bartlett JD, Vacanti JP, Yelick PC (2004). Bioengineered teeth from cultured rat tooth bud cells. *J Dent Res*, 83(7): 523–528.

Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, *et al*. (2002). Stem cell properties of human dental pulp stem cells. *J Dent Res*, 81(8): 531–535.

Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000). Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. *Proc Natl Acad Sci USA*, 97(25): 13625–13630.

Hicok KC, Du Laney TV, Zhou YS, Halvorsen YD, Hitt DC, Cooper LF, *et al*. (2004). Human adipose-derived adult stem cells produce osteoid *in vivo*. *Tissue Eng*, 10(3–4): 371–380.

Honda MJ, Shimodaira T, Ogaeri T, Shinohara Y, Hata K, Ueda M (2006). A novel culture system for porcine odontogenic epithelial cells using a feeder layer. *Arch Oral Biol*, 51(4): 282–290.

Honda MJ, Shinohara Y, Hata KI, Ueda M (2007). Subcultured odontogenic epithelial cells in combination with dental mesenchymal cells produce enamel-dentin-like complex structures. *Cell Transplant*, 16(8): 833–847.

Honda MJ, Tsuchiya S, Sumita Y, Sagara H, Ueda M (2007). The sequential seeding of epithelial and mesenchymal cells for tissue-engineered tooth regeneration. *Biomaterials*, 28(4): 680–689.

Hu B, Nadiri A, Kuchler-Bopp S, Perrin-Schmitt F, Peters H, Lesot H (2006). Tissue engineering of tooth crown, root, and periodontium. *Tissue Eng*, 12(8): 2069–2075.

Hu B, Unda F, Bopp-Kuchler S, Jimenez L, Wang XJ, Haïkel Y, *et al*. (2006). Bone marrow cells can give rise to ameloblast-like cells. *J Dent Res*, 85(5): 416–421.

Jing W, Wu L, Lin Y, Liu L, Tang W, Tian W (2008). Odontogenic differentiation of adipose-derived stem cells for tooth regeneration: necessity, possibility, and strategy. *Med Hypotheses*, 70(3): 540–542.

Kakudo N, Shimotsuma A, Miyake S, Kushida S, Kusumoto K (2008). Bone tissue engineering using human adipose-derived stem cells and honeycomb collagen scaffold. *J Biomed Mater Res A*, 84(1): 191–197.

Kawaguchi H, Hirachi A, Hasegawa N, Iwata T, Hamaguchi H, Shiba H, *et al*. (2004). Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. *J Periodontol*, 75(9): 1281–1287.

Kémoun P, Laurencin-Dalicieux S, Rue J, Farges JC, Gennero I, Conte-Auriol F, *et al*. (2007). Human dental follicle cells acquire cementoblast features under stimulation by BMP-2/-7 and enamel matrix derivatives (EMD) *in vitro*. *Cell Tissue Res*, 329(2): 283–294.

Langer R, Vacanti JP (1993). Tissue engineering. *Science*, 260(5110): 920–926.

Li ZY, Chen L, Liu L, Lin YF, Li SW, Tian WD (2007). Odontogenic potential of bone marrow mesenchymal stem cells. *J Oral Maxillofac Surg*, 65(3): 494–500.

Liu TM, Martina M, Hutmacher DW, Hui JH, Lee EH, Lim B (2007). Identification of common pathways mediating differentiation of bone marrow- and adipose tissue-derived human mesenchymal stem cells into three mesenchymal lineages. *Stem Cells*, 25(3): 750–760.

Luan X, Ito Y, Dangaria S, Diekwisch TG (2006). Dental follicle progenitor cell heterogeneity in the developing mouse periodontium. *Stem Cells Dev*, 15(4): 595–608.

Ma Z, Li S, Song Y, Tang L, Ma D, Liu B, *et al*. (2008). The biological effect of dentin noncollagenous proteins (DNCPs) on the human periodontal ligament stem cells (HPDLSCs) *in vitro* and *in vivo*. *Tissue Eng Part A*, 14(12): 2059–2068.

MacArthur BD, Oreffo ROC (2005). Bridging the gap. *Nature*, 433(7021): 19.

Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, *et al*. (2003). SHED: Stem cells from human exfoliated deciduous teeth. *Proc Natl Acad Sci USA*, 100(10): 5807–5812.

Morsczeck C, Gotz W, Schierholz J, Zeilhofer F, Kuhn U, Mohl C, *et al*. (2005). Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. *Matrix Biol*, 24(2): 155–165.

Murray PE, Garcia-Godoy F (2004). Stem cell responses in tooth regeneration. *Stem Cell Dev*, 13(3): 255–262.

Nakao K, Morita R, Saji Y, Ishida K, Tomita Y, Ogawa M, *et al*. (2007). The development of a bioengineered organ germ method. *Nat Methods*, 4(3): 227–230.

Nör JE (2006). Tooth regeneration in operative dentistry. *Oper Dent*, 31(6): 633– 642.

Ohazama A, Modino SA, Miletich I, Sharpe PT (2004). Stem-cell-based tissue engineering of murine teeth. *J Dent Res*, 83(7): 518–522

Orciani M, Trubiani O, Vignini A, Mattioli-Belmonte M, Di Primio R, Salvolini E (2009). Nitric oxide pro-

duction during the osteogenic differentiation of human periodontal ligament mesenchymal stem cells. *Acta Histochem*, 111(1): 15–24.

Otaki S, Ueshima S, Shiraishi K, Sugiyama K, Hamada S, Yorimoto M, *et al*. (2007). Mesenchymal progenitor cells in adult human dental pulp and their ability to form bone when transplanted into immunocompromised mice. *Cell Biol Int*, 31(10): 1191–1197.

Papaccio G, Graziano A, d'Aquino R, Graziano MF, Pirozzi G, Menditti D, *et al*. (2006). Long-term cryopreservation of dental pulp stem cells (SBP-DPSCs) and their differentiated osteoblasts: A cell source for tissue repair. *J Cell Physiol*, 208(2): 319–325.

Pereira RF, O'Hara MD, Laptev AV, Halford KW, Pollard MD, Class R, *et al*. (1998). Marrow stromal cells as a source of progenitor cells for nonhematopoietic tissues in transgenic mice with a phenotype of osteogenesis imperfecta. *Proc Natl Acad Sci USA*, 95(3): 1142–1147.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, *et al*. (1999). Multilineage potential of adult human mesenchymal stem cells. *Science*, 284 (5411): 143–147.

Sloan AJ, Smith AJ (2007). Stem cells and the dental pulp: Potential roles in dentine regeneration and repair. *Oral Dis*, 13(2): 151–157.

Trubiani O, Orsini G, Zini N, Di Iorio D, Piccirilli M, Piattelli A, *et al*. (2008). Regenerative potential of human periodontal ligament derived stem cells on three-dimensional biomaterials: A morphological report. *J Biomed Mater Res A*, 87(4): 986–993.

Tsuchiya S, Honda MJ, Shinohara Y, Saito M, Ueda M (2008). Collagen type Ⅰ matrix affects molecular and cellular behavior of purified porcine dental follicle cells. *Cell Tissue Res*, 331(2): 447–459.

Wu J, Jin F, Tang L, Yu J, Xu L, Yang Z, *et al*. (2008). Dentin non-collagenous proteins (dNCPs) can stimulate dental follicle cells to differentiate into cementoblast lineages. *Biol Cell*, 100(5): 291–302.

Wu L, Zhu F, Wu Y, Lin Y, Nie X, Jing W, *et al*. (2008). Dentin sialophosphoprotein-promoted mineralization and expression of odontogenic genes in adipose-derived stromal cells. *Cells Tissues Organs*, 187(2): 103–112.

Yang ZH, Zhang XJ, Dang NN, Ma ZF, Xu L, Wu JJ, *et al*. (2008). Apical tooth germ cell-conditioned medium enhances the differentiation of periodontal ligament stem cells into cementum/periodontal ligament-like tissues. *J Periodontal Res*, Jun 25. [Epub ahead of print].

Yao S, Pan F, Prpic V, Wise GE (2008). Differentiation of stem cells in the dental follicle. *J Dent Res*, 87(8): 767–771.

Yokoi T, Saito M, Kiyono T, Iseki S, Kosaka K, Nishida E, *et al*. (2007). Establishment of immortalized dental follicle cells for generating periodontal ligament *in vivo*. *Cell Tissue Res*, 327(2): 301–311.

Young CS, Terada S, Vacanti JP, Honda M, Bartlett JD, Yelick PC (2002). Tissue engineering of complex tooth structures on biodegradable polymer scaffolds. *J Dent Res*, 81(10): 695–700.

Zhang W, Walboomers XF, van Kuppevelt TH, Daamen WF, Bian Z, Jansen JA (2006). The performance of human dental pulp stem cells on different three-dimensional scaffold materials. *Biomaterials*, 27(33): 5658–5668.

Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang JI, Mizuno H, *et al*. (2002). Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell*, 13(12): 4279–429.

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