Dental Tissues Originated Stem Cells for Tissue Regeneration

Maryam Rezai Rad, Sepanta Hosseinpour, Qingsong Ye, and Shaomian Yao

1 Introduction

Stem cell-based therapy as a major field in regenerative medicine has attracted scientists in the field of tissue engineering [1]. Stem cells possess a remarkable capability for proliferation and for differentiation into various cell types, and such capabilities can be useful for regenerative therapies. Nowadays, although multipotent mesenchymal stem cells derived from bone marrow (BMMSC) are among the best-known and best-characterized cells for tissue engineering [2], the invasive procedure needed to isolate these from bone marrow, and variations in their potency according to the age of the donor [3, 4] impede their usefulness. Therefore, several alternative tissue origins for stem cells have been explored including skeletal muscle [5], dental tissues [6–9], and adipose tissue [10–12].

In clinical dentistry, teeth are extracted due to various reasons, such as impacted third molars for orthodontic reasons. Collecting these extracted teeth does not require additional procedures. In addition, deciduous human teeth naturally exfoliate. Hence, harvesting MSCs from dental tissues of such exfoliated teeth is convenient. Dental stem cells (DSCs) have demonstrated the capability to differentiate into various cell lineages, including osteogenic, odontogenic, neurogenic, and adipogenic pathways [13, 14].

Although because of their origin DSCs seem to be more efficient for developing odontogenic structures than other osseous tissues when compared with BMMSCs [15], the precise differences between DSCs and BMMSCs, and among each of the different types of DSCs remain unclear. These cells are derived from the neural crest (i.e., they have an ectomesenchymal origin) [16], which gives them a superior capability for regenerating a wide variety of tissues [17], when compared to BMMSCs that originate from mesoderm [18]. Adding to this greater usefulness, DSCs can be obtained without any major ethical concerns [19, 20].

The first report of MSCs in the dental pulp was in 1985 by Yamamura [21, 22]. To date, five main types of dental tissue-derived stem cells have been

M. Rezai Rad
Research Institute of Dental Sciences, Dental school Shahid Beheshti University of Medical Sciences, Tehran, Iran

S. Hosseinpour
School of Dentistry, The University of Queensland, Brisbane, QLD, Australia

Q. Ye
Skeletal Biology Research Center, Massachusetts General Hospital and Harvard School of Dental Medicine, Boston, MA, USA

Institute of Stem Cells and Tissue Engineering, Wenzhou Medical University, Wenzhou, China

S. Yao (✉)
Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, LA, USA
e-mail: shaomia@lsu.edu

S. Hosseinpour et al. (eds.), *Regenerative Approaches in Dentistry*,
https://doi.org/10.1007/978-3-030-59809-9_2

reported. The first type was termed as "postnatal dental pulp stem cell" (DPSC) by Gronthos et al. [23] in 2000. These can regenerate a "dentine–pulp complex like" tissue in vitro. Subsequently, in 2003, Miura et al. reported the isolation and characterization of stem cells from exfoliated deciduous teeth which had proliferated from remnants of living DPSCs. These are known as stem cells from human exfoliated deciduous teeth (SHED) [24]. Morsczeck et al. [25] and Kemoun et al. [26] reported undifferentiated mesenchymal progenitor cells from the human dental follicle, in the connective tissue sac surrounding developing teeth. They named these dental follicle stem cells (DFSCs). The dental follicle forms the tooth and the supporting structures, and the follicle develops into the periodontal ligament when tooth is erupting. Stem cells have also been reported as being present in the periodontal ligament [27]. In 2006, Sonoyama et al. described unique undifferentiated stem cells from the dental apical papilla of human immature permanent teeth (SCAP) with the ability to generate osteoblasts, odontoblasts, and adipocytes in vitro [28, 29].

Figure 1 schematically shows the potential sources of these various dental tissue-derived stem cells. Although these DSCs have been investigated in many studies [13, 14, 30, 31], only limited work has been done to characterize their biological properties and compared their in vivo applications. In this chapter, we have compiled information regarding the isolation, characterization, and potential applications of DSCs in tissue regeneration and tissue engineering.

2 Stem Cells Obtained from Human Permanent Teeth or Exfoliated Deciduous Teeth

As mentioned earlier, the first isolated DSCs were dental pulp stem cells (DPSCs). DPSCs were isolated initially from permanent third molar teeth. They demonstrated colony formation and a high proliferation rate, as well as the ability to form calcified nodules [23]. DPSCs derived from impacted third molars at the stage

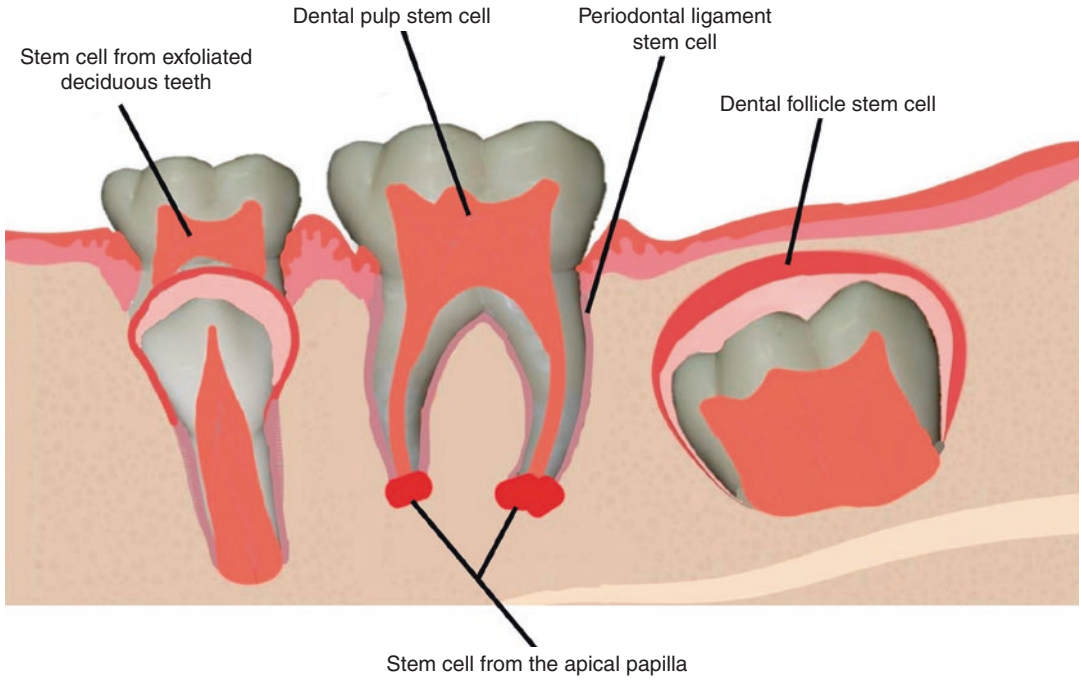

Fig. 1 Schematic view of various sources of stem cells from dental tissues

of root development can differentiate into active migratory odontoblast-like cells, which are able to produce a three-dimensional dentine-like mineralized structure [32]. The stem cell properties of DPSCs vary according to the donor's age when the specimens are obtained (Nakamura et al. 2009); i.e., the cells show a reduction in stem cell features as donor age is increased.

The transition of deciduous teeth in the primary dentition to the permanent dentition is a dynamic and distinctive process, in which the developing permanent teeth buds gradually resorb the roots of the overlying deciduous teeth [33]. As mentioned above, a unique population of DSCs can be harvested from the remaining living pulpal cells of exfoliated teeth, and cultivated in the laboratory. These cells, known as SHED, provide an easily accessible source of stem cells that can be preserved for future applications [24]. These cells differ from regular DPSCs because of their greater proliferation rate and enhanced population doubling rate, and their capability to form sphere-like cell clusters [34]. SHED express the minimum essential markers for stem cells defined by the International Society for Cellular Therapy criteria [15, 35]. They also express some embryonic stem cell markers [24], as listed in Table 1.

While SHED have been isolated using similar methods to those used to isolate DPSCs, there are two major differences between SHED and DPSCs. Firstly, the source of SHED is the dental pulp tissue of deciduous teeth, rather than permanent teeth. Secondly, the isolated cells do not grow as proliferative cells, but instead grow as clustered fibroblast-like cells [36]. It has been shown that 69.8% of SHED are in the S and G2

Table 1 Sources, surface markers, multipotentiality, and in vivo applications of dental stem cells[a]

Stem cell type	Source	Surface markers for characterization		In vitro multipotentiality	Regenerative in vivo application
		Positive	Negative		
Dental pulp stem cell (DPSC)	**Pulpal tissue of crown/root of** 1. Immature teeth that their pulp is exposed and accessible through the root 2. Extracted permanent or deciduous teeth; dental pulp extraction is accomplished through the dental crown by cutting the cementum–enamel junction using dental instruments 3. Carious teeth that its pulp exposed and removed during endodontic treatments	ALP, CD 9, CD 10, CD 13, CD 29, CD 44, CD 59, CD 73, CD 90, CD 105, CD 146, CD 166, CD 271, DSPP, DMP1, OPN, BSP, BBX, nestin, Oct4, STRO-1	CD 11b, CD 14, CD 19, CD 24, CD 31, CD 34, CD 45, CD 117, CD 133, HLA-DR	1. Odontogenic 2. Osteogenic 3. Chondrogenic 4. Neurogenic 5. Adipogenic 6. Myogenic 7. Melanogenic 8. Endothelial differentiation 9. Hepatogenic	• Bone regeneration • Dentin-pulp complex regeneration, regenerative endodontics, and dentin regeneration • Periodontal regeneration • Cornea regeneration • Angiogenic regeneration and blood vessel • Neural reconstruction • Hair follicle repair
Stem cell from human exfoliated deciduous teeth (SHED)	Pulpal tissue of crown/root of exfoliated deciduous teeth	CD 13, CD 29, CD 31, CD 44, CD 73, CD 90, CD 105, CD 146, CD 166, nestin, Oct4, STRO-1	CD 11b, CD 14, CD 19, CD 45, CD 34, CD 43, CD 45	1. Odontogenic 2. Osteogenic 3. Chondrogenic 4. Neurogenic 5. Adipogenic 6. Myogenic	• Dentin–pulp complex and regenerative endodontics • Cornea regeneration • Hepatocytes regeneration

(continued)

Table 1 (continued)

Stem cell type	Source	Surface markers for characterization		In vitro multipotentiality	Regenerative in vivo application
		Positive	Negative		
Stem cell from apical papilla (SCAP)	Apical papilla of immature root in unerupted teeth, especially impacted third molars	ALP, CD 13, **CD 24**[b], CD 29, CD 44, CD 53, CD 59, CD 61, CD 73, CD 90, CD 105, CD 106, CD 146, CD 166, nestin, STRO-1	CD 14, CD 18, CD 34, CD 45, CD 117, CD 150	1. Odontogenic 2. Osteogenic 3. Chondrogenic 4. Neurogenic 5. Adipogenic 6. Angiogenic 7. Hepatogenic	• Dentin–pulp complex regeneration • Pulpal tissue regeneration • Neural regeneration • Angiogenic regeneration
Dental follicle stem cell (DFSC)	Dental follicle tissue which surrounded the crown of unerupted teeth	CD 9, CD 10, CD 13, CD 29, CD 44, CD 53, CD 59, CD 73, CD 90, CD 105, CD 106, CD 146, CD 166, CD 271, nestin, notch-1, STRO-1	CD 31, CD 34, CD 45, CD 133, HLA-DR	1. Osteogenic 2. Cementogenic 3. Chondrogenic 4. Neurogenic 5. Adipogenic 6. Hepatogenic	• Bone regeneration • Periodontal tissue regeneration
Periodontal ligament stem cell (PDLSC)	Soft connective tissue which surrounded the root surface of the tooth between alveolar bone proper and cementum	ALP, CD 10, CD 13, CD 29, CD 44, CD 49c, CD 59, CD 73, CD 90, CD 97, CD 105, CD 106, CD 146, CD 166, SSEA-3 and 4, STRO-1, HLA-A, HLA-B, HLA-C, Oct4, Nanog, Scleraxis, Sox2, STRO-1	CD 11b, CD 14, CD 34, CD 40, CD 45, CD 80, CD 86, CD 106, HLA-DR	1. Osteogenic 2. Cementogenic 3. Chondrogenic 4. Neurogenic 5. Adipogenic 6. Pancreatic islet cell 7. Endothelial differentiation	• Periodontal regeneration • Angiogenic regeneration and blood vessel regeneration • Bone regeneration • Tendon and cartilage regeneration

Abbreviations: *ALP* alkaline phosphatase, *BBX* bobby sox homolog, BSP, bone sialoprotein, *CD* cluster differentiation, *DMP* dentin matrix protein, *DSPP* dentin sialophosphoprotein, *HLA-DR* human leukocyte antigen D related, *Oct* octamer-binding transcription factor, *OPN* osteopontin, *PDL* periodontal ligament, *STRO* stromal precursor antigen

[a]References were quoted in specific paragraphs in the text

[b]Specific marker in comparison to other dental stem cells

stages of the cell cycle; however, 56% of DPSCs are in those cell cycle phases. This explains the different proliferative capacity of these cells [37]. In the ensuing sections, the isolation, characterization, and various applications of DPSCs and SHED will be discussed.

2.1 Isolation and Characterization

There are many approaches to the collection and isolation of DPSCs. A key consideration is to maintain the quality and safety of the isolated cells. Generally, enzymatic digestion of related tissues (such as the dental pulp of an adult tooth or the remaining dental pulp of an exfoliated tooth) or an outgrowth of a tissue explant [38] are the major approaches that have been used for DPSCs and SHED. Enzymatic digestion usually involves placing the source tissues into an appropriate mixture of enzymes such as collagenase I and dispase for 30–60 min at 37 °C, to break the tissue down in order to obtain single-cell suspensions. The tissue remnants are filtered through a sieve or strainer. This is followed by colony-based cultivation of the stem cells, or by cell sorting using magnetic or fluorescent markers [39].

On the other hand, in the outgrowth method, the harvested tissue is diced into 1–2 mm pieces and placed into culture plates, to allow outgrowth of cells from the tissue pieces [40]. Comparing these two methods of isolation, several studies

have shown that DPSCs isolated by enzymatic digestion have a higher proliferation rate, higher expression of stromal precursor antigen (STRO-1) and CD 34, and higher rates of osteogenic or odontogenic differentiation [41–43].

After the isolation of DPSCs and SHED, the next step is to cultivate the cells to expand their number to reach the amount required for cell-based therapy. In addition, the pathway of differentiation can be adjusted by the choice of parameters used in the culture system [39, 44, 45]. Various culture systems have been used including serum-free versus serum-rich culture media, sphere-forming culture, and co-culture systems. Most commonly, a 10–20% concentration of fetal bovine serum (FBS) is included in the culture media for both isolation and expansion of DPSCs and SHED, to accelerate cell adhesion during the first stage of culture. Because of the risk of contamination of serum by bovine pathogens (and the presence of bacterial endotoxin) and an altered possibility of malignant transformation of the stem cells in a serum-containing culture system, the use of a chemically defined serum-free culture system has been recommended [46–49]. In this regard, a serum-free medium (Table 2) has been reported to be successful for obtaining DPSCs [50]. Cells from the adherent population appeared to be more engaged in the odontoblastic lineage than the adherent cells. The neural stem cells that are derived from various sources can proliferate in sphere-forming culture systems [51, 52], which are also recommended for differentiation of DSCs [53, 54].

Table 2 Serum-free medium for DPSC culture

Medium composition	Concentration	Source
Dulbecco's modified Eagle medium (DMEM)/Ham's F12		
Glucose	33 mM	
HEPES (pH 7.2)	5 mM	
N_2 supplement		Life Technologies, Invitrogen
Human EGF	10 ng/ml	
Human bFGF	5 ng/mL	Peprotech
Heparin solution[a]	0.2%	Peprotech
Streptomycin-penicillin	5 µg/mL–5 UI/mL	StemCell Technologies

[a]Final concentration in the medium is Heparin 5 µg/ml

Phenotypic markers expressed by DPSCs are summarized in Table 1. Expression of these markers relates to the unique capability of these cells [28, 55]. For example, STRO-1 positive cells show greater odontogenic or osteogenic differentiation, while CD34 and CD117 positive DPSCs show a greater ability for cell renewal and for mineralization [56]. Low expression of Class II HLA-DR surface antigen indicates that these cells may be immunologically privileged, which reduces concerns around antigenicity in the setting of tissue matching between the donor and the recipient. If the cells truly are not immunogenic then this raises the possibility of creating banks for DPSCs. This aspect is discussed in the last section of this chapter.

The phenotype of SHED differs from that of DPSCs. Pluripotency markers such as Pou5f1, Sox2, Oct3/Oct4, and Nanog are expressed more strongly in SHED than in DPSCs [57]. Nestin as a neuroepithelial marker is expressed less in SHED cells compared to DPSCs [58], which explains the reduced ability of SHED cells in comparison to DPSCs for neuronal regeneration [58]. However, due to the greater proliferative capability of SHED cells, they form sphere-like clusters in a neurogenic culture medium [24].

DPSCs have shown odonto/osteogenic, neurogenic, and adipogenic differentiation in preclinical studies [23, 59, 60] (as listed in Table 1). More recently, in vitro investigations have revealed osteogenic, chondrogenic, and myogenic differentiation of DPSCs [61–63]. Similar to DPSCs, SHED cells have also demonstrated the ability to undergo odonto/osteogenic, chondrogenic, neurogenic, adipogenic, and myogenic differentiation [13, 14, 30, 31] (Table 1).

2.2 Regenerative Applications

The concept of harvesting and banking dental tissue MSCs has opened up a new window for stem cell-based regenerative treatments. As already mentioned, DPSCs and SHED can differentiate

effectively to various cell lineages. In this section, the regenerative capacity of these cells will be discussed further.

2.2.1 Dentine–Pulp Complex Regeneration

Dental pulp is a specialized connective tissue, which consists of odontoblasts, endothelial cells, neurons, fibroblasts, and other cells. The peculiar internal anatomy of teeth and the unique blood flow may influence the survival of stem cells [64]. When the dental pulp is infected by bacterial pathogens, it is hard to remove or inactivate these pathogens through antibiotics alone. For a number of clinical reasons, extirpation of the whole pulp may need to be undertaken [65].

For regeneration, dental pulp stem cells can be expanded in the laboratory. When seeded onto hydroxyapatite/tricalcium phosphate (HA/TCP), the cells have demonstrated formation of a dentine-pulp-like complex in mice [23]. In addition, SHED implanted into mice can generate odontoblast-like cells and dentine-like mineralized nodules. However, SHED cells do not seem to be able to form a complete dentine-pulp-like complex like that seen with transplantation of DPSCs [24]. In contrast, DPSCs can form mineralized nodules, which are covered by a layer of dentine-like reparative tissue in vivo [66]. DPSCs that have been seeded onto calcium phosphate [67], hexafluoropropanol silk [68], and polylactic acid [69] scaffolds have demonstrated dentine–pulp complex formation in animal models.

In addition to dentine–pulp complex regeneration, DPSCs have also been used in periodontal regeneration [31]. DPSCs transplanted into immunocompromised mice have been shown to differentiate into collagen forming cells, with the capacity to form a cementum-like tissue [70]. The expression of particular phenotypic markers such as SCD-1, STRO-1, CD44, and CD146 on DPSCs and SHED cells sensibly linked to their role in periodontal regeneration [71, 72]. In addition, both SHED and DPSCs are capable of bone regeneration [31]. This aspect will be discussed further in the next section.

2.2.2 Bone Regeneration

Bone formation, including the aggregation of osteoprogenitor cells, is similar in some ways to tooth bud development, but without the epithelial invagination aspect. Intramembranous and endochondral bone formation are the two main processes of bone regeneration. In endochondral regeneration, the aggregated MSCs first undergo chondrogenesis followed by ossification of the cartilage into bone tissue [73]. In intramembranous bone formation, MSCs first become osteoprogenitor cells and then further differentiate into osteoblasts, that create extracellular matrix containing collagen fibrils and other bone components, and this is called osteoid.

Bone tissue possesses the intrinsic capability of regenerating itself through adulthood [74]. However, when bone defects or fractures are larger than the regenerative capacity of the bone, other interventions are required to rehabilitate the defect site in the bone tissue [74]. One alternative is using stem cell-based therapy with stem cells of dental origin [75]. In vitro studies have demonstrated the osteogenic and chondrogenic multipotency of both DPSCs and SHED [75–77]. An in vivo investigation showed that DPSCs can regenerate osteoblasts and endotheliocytes that eventually formed bone in immunocompromised rats [78].

The application of SHED in combination with a hydroxyapatite (HA)/tricalcium phosphate scaffold has been shown to regenerate calvarial bone defects in animal models [79]. These studies have also shown that a large amount of bone and bone marrow-like structures are formed in the regenerated mineralized matrix. Other investigations have reported that the application of DPSCs in conjunction with fibroin/collagen scaffolds can significantly enhance bone formation after 4–8 months in rats [80–82]. The application of DPSCs loaded onto a HA-based hydrogel showed superior bone healing in rat calvarial defects compared to the scaffold only and to untreated control groups [83]. In addition, DPSCs cultured for 13 days on poly(lactide-*co*-glycolide) scaffolds caused significant bone regeneration in the same types of defects [84]. Other studies have

reported the successful application of DPSCs and SHED in combination with HA/TCP in rats and mice in the treatment of calvarial defects [85].

The research team of d'Aquino et al. demonstrated that administration of DPSCs loaded onto a collagen sponge resulted in higher mineralization after 1 month and complete bone regeneration with a large amount of cortical bone formed after 3 months, in comparison to a scaffold only group [78]. Moreover, an increased level of clinical attachment was found in the gingiva overlying the defect site after transplantation of DPSCs loaded onto a collagen scaffold [86].

Several studies have investigated the use of DPSCs or SHED for bone regeneration in maxillary or mandibular bone defects [87–91]. Alkaisi et al. created a mandibular defect (from the first premolar to mental foramen) in rabbits, and assessed the formation of bone after applying SHED without using a scaffold [87]. Radiographic evaluations revealed that in SHED transplanted animals, partial bone defects were bridged after only 2. In addition, the regenerated bone in this group showed a bony ridge with higher radiopacity than insights treated with a scaffold only after 4 weeks. Finally, after 6 weeks, clear corticalization and strong radiodensity were observed in the defect gap in sites treated with SHED group. Histomorphometric analysis showed that the regenerated bone was significantly higher in the SHED group compared with the scaffold control.

Paino et al. created tissue-engineered woven bone tissue using DPSCs in vitro, and then transplanted the manufactured bone tissue into the mandibular defects in rats [88]. Their findings confirmed the remodeling capacity of the implanted tissue, and its integration into the surrounding normal bone with the passage of time. Lamellar bone tissue with vital osteocytes and vascularized Haversian canals were found after bone remodeling.

SHED have been administered intravenously in order to treat osteoporosis in animal models of this condition [79, 92]. Ma et al. have found that this type of administration route for SHED can ameliorate problems of low bone mineral density, and can improve the radiopacity of the trabecular bone structures [79]. Moreover, Liu et al. showed that systemic administration of SHED was able to enhance bone volume, and promote trabecular number, thickness, and density [92]. In general, SHED transplantation has been found to improve cortical bone parameters, including bone area, thickness, and cortical bone fraction [93]. Bone that has been regenerated using SHED has been found to express higher levels of Runx2, alkaline phosphatase, and osteocalcin than controls [94]. At the same time, systemic application of SHED markedly downregulates genes associated with bone resorption such as RANKL and C-terminal telopeptide, and upregulates osteoprotegrin (OPG) [79, 92, 95]. This is a powerful demonstration of the immunomodulatory effects of SHED.

According to a recent systematic review by Leyendecker et al., transplantation of DPSCs into cranial, maxillary, and mandibular bone defects gives superior regenerative outcomes compared to controls [93]. However, in one study Annibali et al. found no difference in bone regeneration between DPSCs and control groups [96], while in another study by Behnia et al. there was no difference after the application of SHED in combination with a collagen scaffold compared to a scaffold only for the treatment of mandibular defects in dogs [97].

The type of scaffold material that is used as the carrier for DSCs plays an important role in bone repair. For example, Zhang et al. did not find ectopic bone regeneration in DPSCs loaded on HA/TCP [98]. However, Kuo et al. demonstrated that DPSCs in combination with alpha-calcium sulfate hemihydrate/amorphous calcium phosphate could efficiently promote bone formation in comparison to DPSCs + calcium sulfate dihydrate or DPSCs + calcium sulfate dihydrate/TCP [90].

2.2.3 Neural Regeneration

Neural regeneration is a challenging objective for two major reasons. The first is the lack of neural progenitor cells, while the second reason is that the local microenvironment may impede regenerative processes [99]. DPSCs express markers of neural progenitors such as nestin and Pax6, due to the neural crest origin of these cells [100, 101].

Human DPSCs are able to form spheroids under serum-free neuronal stimulating culture conditions [102]. In addition, even without neural induction, DPSCs express neural markers such as CDH2, TUBB3, and NFM [103].

Transplantation of DPSCs and SHED into defect sites in the central nervous system (CNS) has been shown to enhance neural recovery [56, 101, 104]. DPSCs can coordinate axonal regrowth by secreting CXCR-4 and stimulating the SDF-1/CXCL12 axis that induces neuroplasticity [105]. It has been reported that the implantation of DPSCs into the hippocampus of immunocompromised mice can accelerate cell recruitment and proliferation, and the maturation of endogenous existing neural cells [118]. In fact, taken together all these findings suggest that DPSCs may have an application as a modulator and stimulator in neural recovery in the CNS [143].

In spinal cord trauma and cerebral ischemia models, DPSCs can significantly enhance neurological dysfunctions [57, 161]. Furthermore, in the case of a peripheral nerve injury, DPSCs can mediate neural tissue engineering with artificial nerve conduits, to regenerate myelinated neural fibers [122]. Luo et al. demonstrated that transplantation of a heparin–poloxamer hydrogel and DPSCs with fibroblast growth factor could significantly regenerate neurons in spinal cord injuries, and functionally repair nerve injury defects in rats after 28 days [101].

Despite several studies which have demonstrated that both DPSCs and SHED cells can (1) differentiate into functional neural cells which were voltage-sensitive in vitro, (2) express neural markers, and (3) migrate into the CNS in animal models [24, 105, 106], many in vivo investigations have reported that DPSCs and SHED are unable to differentiate into functional neurons that the sites of injuries to nerves [102]. The neural regenerative capacity of DPSCs and SHED seems to occur because of their production of neurotrophic products [107]. Furthermore, these cells impede axon growth inhibitor signals and improve the microenvironment for regeneration [108]. Further studies are necessary to clarify the neural regeneration capability of DPSCs and SHED.

2.2.4 Other Regenerative Applications

Muscle Regeneration

Arminan et al. reported the differentiation of DPSCs into cardiomyocytes in rats [109], while Yang et al. documented that DPSCs can differentiate into dystrophin-producing muscle cells in a mouse model of cardiac muscle injury [110]. Based on the observation that DPSCs infused into cardiac defect sites express dystrophin and myosin [111], it has been proposed that DPSCs can potentially be applied for muscle regeneration [110].

Corneal Regeneration

Some experimental evidence indicates that DPSCs are more similar to epithelial stem cells than BMMSCs [112, 113]. In fact, DPSCs can differentiate into keratinocytes and can express keratinocyte markers [114]. Gomez et al. reported that eye transparency in a rabbit corneal defect model was improved by the implantation of a sheet of tissue-engineered DPSCs into the defect sites [115]. In another study, DPSCs delivered by soft contact lenses in a clinical application were shown to promote corneal epithelial regeneration [116]. DPSCs transferred from the contact lenses to the corneal surface expressed the keratinocyte markers cytokeratin 3 and 12. Moreover, DPSCs can impede conjunctival cells from growing into the center of the cornea [117].

Cartilage Regeneration

As mentioned earlier, DPSCs can develop into dentine, cartilage, and bone tissues [118]. Yu et al. showed cartilage formation after 14 days after the implantation of pellets containing DPSCs into the renal capsule of rats [119]. In addition, Morito et al. reported similar results after the subcutaneous implantation of DPSCs in combination with FGF in immunocompromised mice [120].

Hair Follicle and Blood Vessel Regeneration

DPSCs have been transplanted to the surgically compromised hair follicles, where they have been shown to cause the formation of new head bolts and the regeneration of hair fibers [121].

DPSCs can promote angiogenesis and vasculogenesis in sites where there is peripheral nerve injury [122]. DPSCs can differentiate into endotheliocytes in rat models [78].

Endocrine Regenerative Potential

Previous studies have documented the possibility of using DPSCs and SHED in the treatment of diabetes mellitus using a regenerative approach, due to their multipotent capabilities, since they can differentiate into insulin-producing cells [13]. DPSCs are capable of differentiating into pancreatic cells and also into insulin-producing islet-like cells [123, 124]. Kanafi et al. implanted islet-like cell aggregates derived from DPSCs or SHED cells into diabetic mice, and found that the SHED group was superior to the group treated with DPSCs in terms of maintaining normal blood glucose levels [50].

SHED have also been studied for the treatment of injuries to the kidney, where they influence the proliferation of tubular epithelial cells [125], through a paracrine effect that induce cell migration and facilitates the repair of acute kidney damage.

Regenerative Therapy of Various Systemic Disease

Previous investigations demonstrated the positive impact of DPSCs and SHED when used in the treatment of various systemic diseases [126]. The enormous capacity of these DSCs makes them an attractive source for regenerative therapies in medicine. Both DPSCs and SHED can differentiate into hepatic cells [127, 128], which makes them promising in the treatment of liver cirrhosis [129].

As already discussed, due to their myogenic multipotency, DPSCs and SHED cells can be of value in the treatment of muscle injuries, including to the myocardium. Both cell types have been used experimentally to treat myocardial infarction [111] and Duchene muscular dystrophy [130, 131]. Moreover, the immunomodulatory and anti-inflammatory effects exerted by these cells have been applied successfully in the treatment of a range of inflammatory and autoimmune diseases including systemic lupus erythematosus [132], rheumatoid arthritis [133], autoimmune encephalomyelitis [134], Alzheimer's disease, and Parkinson's disease [135, 136].

There have been few systematic comparisons between BMMSCs and DSCs in terms of their immunophenotype, gene expression profile, and regenerative potentials [34]. Collectively, in vitro investigations show that DPSCs share a similar pattern of gene expression with BMMSCs [15]. In addition, signaling pathways of odontoblastic differentiation of DPSCs are similar to the pathways whereby bone marrow-derived stem cells take on osteoblastic features [15].

Shi et al. evaluated gene expression in DPSCs and BMMSCs, and showed that more than 4000 known human genes were similar between these cells [137]. However, they have found that collagen type 18, insulin-like growth factor-2, and cyclin-dependent kinase 6 were much more highly expressed in DPSCs, while insulin-like growth factor binding protein-7 and collagen types I and II were expressed more in BMMSCs [137].

Yamada et al. characterized and compared DPSCs and BMMSCs using a cDNA microarray system, including 12814 genes and a clustering algorithm [138]. They demonstrated that after osteoinduction DPSCs expressed alkaline phosphatase, DSPP, and DMP-1 at levels that were higher than BMMSCs. However, in the clustering assessment, it became apparent that both cells share similar gene regulation pathways for signaling, cell metabolism, and communication [138].

Despite DPSCs and BMMSCs having many similarities in regulating roles, in signaling factors, and in their expression profile, these two types of stem cells are very different in their proliferative capacity and their differentiation potential, and this has led to distinct patterns of use for tissue regeneration in preclinical studies [15]. For instance, the chondrogenic potential of DPSC, and the adipogenic capacity of both SCAP and DPSCs are weaker than those of BMMSCs. Conversely, the neurogenic capabilities of DSCs are far more potent than those of BMMSCs. In addition, SHED and PDLSCs have a much higher growth potential compared to BMMSCs [139]. These differences may be due to the neural crest origin of DSCs.

3 Stem Cells from the Apical Papilla

The apical part of dental papilla is a cell-rich zone containing stem cells (Fig. 1). The apical papilla can be harvested from an immature extracted tooth, and used for isolation of SCAP [140]. The main difference between SCAP and DPSCs is that SCAP is the precursor of the radicular pulp. The characteristic differences between these cells are listed in Table 1. In general, SCAP derives from the developing dental tissues, which consists of early progenitor cells that are distinctive from the cells of mature tissues (DPSCs) [141].

3.1 Isolation and Characterization

After harvesting the apical papilla of a developing root, the tissue is diced into smaller pieces and subjected to enzymatic digestion with collagenase and dispase [140] as already described for the isolation of DPSCs and SHED.

Cultivated SCAP possess low immunogenicity, as seen by lymphocyte assays in the laboratory [142]. Flow-cytometry analysis shows that SCAP express typical cell markers including CD73, 90, and 105 (Table 1) [143]. In addition, the perivascular location of SCAP reflected by their expression of STRO-1 and CD146, which gradually fades with extended passaging of the cells [144–146]. Although expressed at a relatively low amount CD24 seems to be exclusively positive in SCAP compared to other DSCs and other MSCs [147, 148]. Expression of CD24 expression reduces to zero after the 10th passage [149, 150]. If CD 24 is a specific marker for determining the "stemness" of SCAP, these findings imply that loss of stemness in SCAP occurs after the 10th passage.

3.2 Regenerative Applications

SCAP can differentiate into various cell lineages [149], which make these cells an attractive source for tissue engineering.

3.2.1 Pulp Regeneration and Angiogenesis

Regenerative endodontic therapy includes the process of regenerating the dentine–pulp complex [151]. SCAP are one of the most promising stem cell sources for such therapy due to their known odontogenic potency and their expression of dentine-related differentiation markers such as dentine sialphosphoprotein (DSPP) [152].

Nowadays, with the application of scaffolds and the inclusion of growth factors such as vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF), there is increasing optimism regarding the possibility of regeneration of dentin-pulp complex [153–155]. Cell homing therapies have determined that chemotactic factors for SCAP include SDF-1, TGF-β, and granulocyte-colony stimulating factor (G-CSF). These not only can improve the migration of SCAP, but they also can promote their differentiation [156].

Sonoyama et al. reported that human SCAP can develop into a functional root in an animal model [29]. De novo dentine–pulp complex regeneration by SCAP begins with the migration of these cells onto the dentine surface, followed by odontoblastic differentiation and the expression of phenotypic markers of dentinogenesis [157].

Hikens et al. demonstrated that SCAP are able to express a number of markers of angiogenesis including VEGF), thrombospondin-1, angiopoietin-1, endostatin, matrix metalloproteinases, and FGF [153]. Revascularization is a critical requirement for pulpal tissue engineering [158]. SCAP cells appear to be a good candidate for promoting revascularization because of their original niche in the perivascular location [159].

Moreover, in repairing dentine–pulp complex, the administration of scaffolds can be challenging, as some of the biomaterials that are used (e.g., HA and TCP) are osteoinductive, and thus there is a risk of generalized calcification occurring in the pulp space [157]. Thus, the properties of the scaffold need to be adjusted to suit the requirements of pulp tissue regeneration. Amirkia et al. used a three-dimensional silk fibroin as a natural

scaffold, and found that this improved the attachment of SCAP and their differentiation [160]. In addition, chitosan-based scaffolds seem to be an appropriate carrier for SCAP as they improve their odontogenic potential [161].

3.2.2 Neural Regeneration

The neurogenic potential of SCAP has been exploited for neural tissue engineering [158]. SCAP originated from the neural crest, and they express high level of the neural marker nestin [152]. In addition, SCAP can drive a neuroprotective mechanism, by decreasing inflammation and inducing differentiation of oligodendrocytes [155]. SCAP also express various markers (genes) for neurogenesis (Table 1). In vitro and in vivo investigations show SCAP can participate in neurite outgrowth and in axonal induction [158].

Under neurogenic induction, SCAP start to mimic spindle-shaped neurocytes, with long cellular process [152, 162, 163]. In a study by De Berdt et al., the entire apical papilla was transplanted into an area of artificial spinal cord damage in an animal model. The apical papilla functioned as a scaffold for SCAP to regenerate neural tissue in the original niche of the cells [164]. Interestingly, this study revealed that hypoxic conditions could stimulate SCAP to express neural-specific genes and to secrete growth factors [164].

3.2.3 Bone Regeneration

The osteogenic potency of SCAP has been confirmed by studies which have shown differentiation of these cells into osteoblasts, as determined by alizarin red staining of calcium deposition and the expression of osteogenic markers, including bone sialoprotein, alkaline phosphatase, gamma-carboxyglutamate protein, runt-related transcription factor-2 (RUNX2), and bone morphogenetic proteins (BMPs) [32]. The osteogenic differentiation capability of SCAP is comparable to that of BMMSCs [148].

The cultivation of SCAP in an osteogenic medium results in the formation of osteoblast-like cells that are able to produce mineralized nodules [165]. Nada et al. observed that SCAP isolated from different teeth showed different differentiation patterns. For instance, SCAP isolated from third molars tended to produce diffuse mineralization, whereas SCAP isolated from premolar teeth showed a localized pattern of calcific deposits [165]. To date, no in vivo investigations have been conducted to evaluate the bone regeneration capacity of SCAP.

3.2.4 Other Regenerative Applications

The chondrogenic potential of SCAP has been assessed using Alcian Blue staining of the chondrocytes that have formed in vitro [166], but to date no study has been carried out to evaluate the molecular evidence for the chondrogenic potential of SCAP, such as the expression of chondrogenic genes in SCAP under chondrogenic induction. Thus far, no in vivo study of cartilage formation by SCAP has been reported.

Several studies have demonstrated the adipogenic capacity of SCAP [165]. SCAP can express lipoprotein and lipase, suggesting the adipogenic capability of these cells [152]. However, in comparison to BMMSCs, their adipogenic potential is low [148, 153, 167].

4 Dental Follicle Stem Cells

The dental follicle is a loose connective tissue sac surrounding the tooth bud. It plays an important role in tooth development and eruption. The dental follicle is involved in tooth eruption by controlling osseous remodeling through the timely production of various secreted mediators [168]. Stem cells have been harvested from dental follicle of different species in various developmental stages [169, 170]. DFSCs are multipotent, and can differentiate to form periodontium, bone, and cementum [169, 171].

4.1 Isolation and Characterization

Human extracted third molar teeth are the major tissue source that has been used to isolate human DFSCs. The method for DFSCs isolation is similar to that described for other DSCs.

In culture, DFSCs have a typical fibroblast-like morphology. They express CD9, CD10, CD13, notch-1, and nestin, but they do not express CD31, CD34, CD45, and HLA-DR [172, 173] (Table 1). DFSCs also express cementum attachment protein and cementum protein-23 (CP-23), which are two putative cementoblast markers [15]. DFSCs also express STRO-1 and BMP receptors in vivo [26].

4.2 Regenerative Applications

DFSCs can differentiate into osteoblasts, cementoblasts [30], and adipocytes in the appropriate inducing culture media [170]. Although experimental investigations have revealed mineralized tissue formation by DFSCs, DPSCs have a greater capacity for all hard tissue formation. Yagyuu et al. reported hard tissue formation of DFSC at preclinical studies [176], while Yokoi et al. demonstrated that DFSCs are capable of regenerating soft tissue and PDL in vivo [174].

4.2.1 Bone Regeneration

DFSCs have the capacity to create calcification, as seen both in cell culture [25] and in animal [175] studies. Several investigations have reported the osteogenic differentiation of DFSCs in appropriate osteogenic medium [176–179]. In vivo bone formation by DFSCs has been demonstrated in critical size bone defects in rat calvaria [180]. Moreover, in vitro investigations have indicated that BMP-6 and BMP-9 promote the osteogenic differentiation of DFSCs [176].

Rezai Rad et al. showed that a temperature of 37–40 °C was optimal for inducing osteogenesis of DFSCs in vitro [181]. Honda et al. reported the results of two different animal studies to evaluate the bone regenerative capacity of DFSCs [180, 182]. The findings of both studies suggest that DFSCs supported bone regeneration, although it was not clear whether the transplanted stem cells had in fact differentiated into osteoblasts.

4.2.2 Periodontal Regeneration

DFSCs are capable of generating osteoblasts, cementoblasts, and PDL [14]. Honda et al. isolated DFSCs from the third molar teeth of 6-month-old pigs [180]. DFCSs were seeded in the bottom of a tube and DPSCs and the enamel organ epithelium (which originated from same pigs) were added in order to produce a recombination which mimicked the tooth primordia. The whole mixture was then transplanted into the omentum of immunocompromised rats (Fig. 2). A thick layer of dentine with viable odontoblasts and a layer of cementum-like tissue were found at 24 weeks post-surgery. Moreover, collagen fibers were present in a pattern that resembled the PDL, and they were attached to the cementum-like layer.

Another histological evaluation also confirmed the possibility of whole periodontium regeneration via expanded DFSCs [182]. A further investigation reported that DFSCs could differentiate into PDL cells, and could regenerate PDL-like structures including cementum-like tissues [183]. Other researchers have indicated that DFSCs used in combination with a treated dentine matrix could regenerate root-like tissues with a dentine–pulp complex [184].

5 Stem Cells from the Periodontal Ligament

Although early studies provided some evidence to support the differentiation capability of periodontal ligament (PDL) cells, such as the ability to differentiate into cementum-forming cells and osteoblasts [185, 186], Seo et al., conclusively identified a population of MSCs within the periodontal ligament that can express stem cell markers and that have the capability to differentiate into various cell lines [27].

5.1 Isolation and Characterization

To isolate PDLSCs, extracted teeth with an intact PDL are immersed into a digestion solution consisting of collagenase and trypsin [187]. The resultant cells from the enzymatic digestion can then be cultured in various media such as serum-

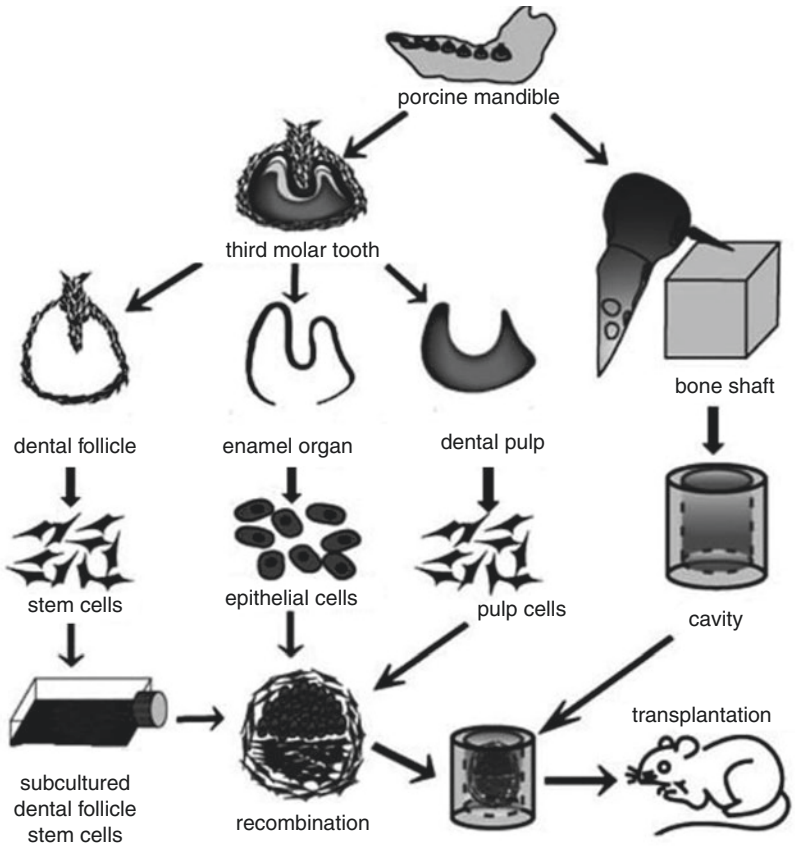

Fig. 2 Schematic diagram of procedure used to regenerate engineered dental root analogue in Honda et al. study [180]

containing media and neurosphere-forming medium, according to the intended therapeutic purpose [53].

PDLSCs possess low immunogenicity, and they can modulate behavior of peripheral blood mononuclear cells by secreting TGF-β and HGF [188]. PDLSCs isolated from inflamed periodontium can significantly reduce the activity and proliferation of T lymphocytes [189]. They express high levels of interleukin (IL)-10, and IL-17 in comparison to PDLSCs derived from healthy tissues [190].

PDLSCs express MSC-related markers and tendon specific transcription factor (scleraxis) (Table 1). Scleraxis expression is significantly higher in PDLSCs than in DPSCs and in BMMSCs [15]. PDLSCs do not express hematopoietic markers such as CD14, CD19, CD34, and CD45 [15], or markers associated with hematopoietic cells including CD40, CD80, and CD86 [188].

5.2 Regenerative Applications

The current literature report that PDLSCs can differentiate into cementum-like structures, and along osteogenic, adipogenic, and chondrogenic cell lineages (Table 1).

5.2.1 Periodontal Regeneration

PDLSCs were firstly applied in animal models and used to reconstruct cementum-PDL-like structures [27]. Subsequently, several studies investigated the capability of PDLSCs for periodontal regeneration [191–193]. Ninomiya et al. implanted a HA scaffold loaded with PDLSCs into the dorsal muscle of rats, and demonstrated bone-like tissue formation [194]. PDLSCs seeded on HA/TCP successfully formed PDL and cementum-like tissues surrounding the scaffold [195, 196]. Complete PDL regeneration was achieved, with formation of Sharpey's fibers

between the newly formed cementum and the fibers of the PDL [197].

Likewise, when transplanting PDLSCs that were treated with recombinant human plasminogen activator inhibitor-1, and then seeded on HA/TCP scaffold, into the dorsal region of immunodeficient mice, cementum-like tissue surrounded by PDL-like tissue was observed after 10 weeks [198].

Using stem cell all sheets derived from PDLSCs for tissue engineering has been attempted. HA/TCP wrapped with PDLSCs cell sheets has been shown to generate PDL/cementum-like structures in rats and mice [199, 200]. Moreover, adding platelet-rich fibrin (PRF) to a HA/TCP scaffold wrapped with PDLSCs sheets was shown to regenerate not only cementum and PDL, but also blood vessels [201].

Transplantation of human PDLSCs sheets into infra-bony mandibular defects in dogs showed new cementum regeneration, with the formation of collagen and nerve fibers around the roots of the teeth after 8 weeks [202]. When PDLSCs and gelatin sponge scaffolds were grafted onto fenestration defects in rats, complete healing of bone, cementum, and PDL was seen after only 3 weeks [203]. These findings suggested that there is considerable potential for regeneration of the periodontium using PDLSCs, and that this approach is likely to be successful when used in the clinic to manage real bony defects [204].

However, a significant point is that the regenerative capability of PDLSCs is affected considerably by the presence of inflammation and by the age of the PDL tissue from which the cells are harvested [200, 204, 205]. Gao et al. compared various PDLSCs from donors of different ages [200]. They found that PDLSCs from younger donors had greater cementum/PDL formation potential than those from older donors. Other studies have reported that PDLSCs derived from donors with periodontitis have a significantly lower capacity to form bone than PDLSCs derived from healthy sites or healthy donors [205].

5.2.2 Bone Regeneration

The osteogenic potential of PDLSCs has been shown in several in vitro studies, which have reported the formation of mineralized nodules [27, 206–208]. Although PDLSCs typically form cementum-like structures [15], PDLSCs implanted into periodontal defects of animal models appear to accelerate the regeneration of trabecular bone next to PDL-like structures, thus demonstrating their capacity for alveolar bone regeneration [27, 209].

Transplantation of human PDLSCs encapsulated in a RGD-modified alginate has been shown to enhance bone formation in critical-sized calvarial defects in rats [210]. Several studies have observed a lower bone regenerative potential of PDLSCs compared to BMMSCs. By way of comparison, BMMSCs were reported to be more effective for alveolar bone repair in canine models [211]. Another study confirmed the lower osteogenic capability of PDLSCs compared to BMMSCs [212]. The reason for this may be the presence of more end-differentiated cells in the PDLSCs population [213]. However, there are a few contrary reports in the literature suggesting similar or even better osteogenic potentials of PDLSCs compared to BMMSCs [214], and other DSCs [80, 215]. This point needs further research to resolve it.

5.2.3 Tendon and Cartilage Regeneration

Gronthos et al. showed that PDLSCs can express a tendon-specific marker (scleraxis) in vitro [206]. A combination of human PDLSCs with an RGD-coupled alginate could form a tendon-like tissue in mice. When used for tendon regeneration, compared with BMMSCs, PDLSCs gave a more highly organized tissue with a greater amount of collagen fibers [216].

Moshavernia et al. showed that cartilage healing could be achieved by applying encapsulated PDLSCs in an alginate hydrogel [216]. Moreover, several animal studies have successfully used PDLSCs for cartilage tissue engineering. Ectopic cartilage formation was observed at PDLSC transplantation sites [102, 217, 218]. It is well-known that cartilage has a very restricted ability for self-renewal and regeneration [219]. In this regard, the chondrogenic potential of PDLSCs is noteworthy, and it is likely that they will be of interest for cartilage repair [102].

Table 3 Licensed dental tissue-derived stem cells bank all around the world[a]

Name	Website	Country
BioEDEN	http://www.bioeden.com/	United States
Store-A-Tooth	http://www.store-atooth.com/	
StemSave	http://www.stemsave.com/	
Three brackets (Hiroshima University)	http://www.teethbank.jp/	Japan
Teeth Bank Co.	http://www.teethbank.jp/	
Advanced Center for Tissue Engineering	http://www.acte-group.com/	
MoBaTann: Tooth biobank	http://www.uib.no/en/rg/biomaterial/64723/mobatann-tooth-biobank	Norway
The Norwegian Tooth Bank	http://www.fhi.no/morogbarn	
Stemade Biotech Pvt.	http://www.stemade.com/	India

[a]All information gathered from Chalisserry et al. and Liu et al. systematic reviews

5.2.4 Other Regenerative Applications

Recently, it has been shown that PDLSCs have both neurogenic and angiogenic differentiation potentials [53]. PDL-derived spheres are able to differentiate into mesodermal and neural cells. MSCs originated from the PDL can form Schwann cells by inducing the Erk1 signaling pathway [220]. Furthermore, these cells can regenerate retinal ganglion-like cells via functional synapses and respond to calcium [221]. Cen et al. demonstrated that human PDLSCs ameliorate ganglion cells and axonal regeneration when used in the retina of animals with a traumatized optic nerve [222].

Another novel application of PDLSCs is its possible use for cardiogenic differentiation. PDLSCs express some cardiac cell markers, such as sarcomeric actin and cardiac troponin T [223].

6 Banking of DSCs

According to the diversity of DSCs and their beneficial aspects, the potential uses of stem cell-based treatments using DSCs in both dentistry and medicine are significant. The administration of a patient's own DSCs during therapy may not often be practical, since they may have more conditions requiring treatment in their later years, but have higher numbers of stem cells in their tissues when they are in their childhood years. Banking of DSCs is one way of easily maintaining a suitable supply of DSCs to meet the needs of patients later in their life. Such an approach can pave a new road for progress in healthcare by maintaining a promising source of autologous cells for personalized regenerative treatments.

Given these considerations, the ability to harvest and safely preserve DSCs becomes more important. Nowadays, DSCs can be cryopreserved for a long period of time [224–226]. In a number of developed countries, licensed tooth banks have been founded (Table 3) [30, 34, 227]. When such banks have been established there are a number of ethical controversies, as well as social, and legal issues that need to be considered. Consistent and well-documented laboratory procedures are needed to evaluate and preserve the cells. There is also a need for appropriate regulations or legislation regarding stem cell banking.

7 Limitations

Although stem cell-based therapeutic approaches have shown much promise with an appealing path to their use in tissue regeneration and functional repair, multiple factors need to be optimized. This will require thorough clinical investigations as well as cell culture studies to enhance methods to grow up and maintain a large quantity of cells. One must bear in mind that the availability of the dental tissues over a lifetime

will alter, and the time of tooth extraction may not match the time when the patient needs therapy with dental stem cells. Banking of dental stem cells may partially address this issue, but more work is needed to optimize preservation methods, and make such banks less expensive and easier to use for clinical applications. Cell banks must address quality and safety issues such as the stability of the cell phenotype over time, and the possible risks of contamination with endotoxins or with pathogens [30, 228].

8 Conclusions and Future Direction

DSCs as one of the more versatile MSCs have been used widely utilized in preclinical studies for tissue engineering purposes. DSCs exert a range of immunomodulatory activities, and these have not yet been characterized fully. DSCs have a low immunogenicity and may also have immunosuppressive actions. This makes dental tissues a promising source for stem cells for the repair of bone, dental pulp, periodontium, nerves, and other tissues. The various recognized DSCs not only have the potential to differentiate into different cell lines and undergo self-renewal, but their collection could be done as part of normal dental treatment, harvesting them from extracted teeth. Isolation of DSCs usually does not require additional surgical procedures, as exfoliated and extracted teeth are often discarded as medical waste. The collection of DSCs does not pose any major ethical concerns, unlike the use of embryonic stem cells. Nonetheless, in future, it is important to optimize DSC cryopreservation protocols, and address issues including donor-related diversity, and the influence of cell culture conditions [34, 229, 230]. Because of their potential use in a range of cell-based therapies, the ability to expand stem cells of dental origin while also maintaining their original stemness properties is critical. Thus, development of safe and efficient cell expansion strategies should be a focus for research in the future.

References

1. Volarevic V, Markovic BS, Gazdic M, Volarevic A, Jovicic N, Arsenijevic N, et al. Ethical and safety issues of stem cell-based therapy. Int J Med Sci. 2018;15(1):36.
2. Charbord P. Bone marrow mesenchymal stem cells: historical overview and concepts. Hum Gene Ther. 2010;21(9):1045–56.
3. Zhou S, Greenberger JS, Epperly MW, Goff JP, Adler C, LeBoff MS, et al. Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell. 2008;7(3):335–43.
4. Zomorodian E, Baghaban EM. Mesenchymal stem cells as a potent cell source for bone regeneration. Stem Cells Int. 2012;2012:980353.
5. Bosch P, Musgrave DS, Lee JY, Cummins J, Shuler F, Ghivizzani SC, et al. Osteoprogenitor cells within skeletal muscle. J Orthop Res. 2000;18(6):933–44.
6. Morad G, Kheiri L, Khojasteh A. Dental pulp stem cells for in vivo bone regeneration: a systematic review of literature. Arch Oral Biol. 2013;58(12):1818–27.
7. Bartold PM, Shi S, Gronthos S. Stem cells and periodontal regeneration. Periodontol 2000. 2006;40(1):164–72.
8. Khojasteh A, Nazeman P, Rad MR. Dental stem cells in oral, maxillofacial and craniofacial regeneration. Dental stem cells. New York: Springer; 2016. p. 143–65.
9. Rezai-Rad M, Bova JF, Orooji M, Pepping J, Qureshi A, Del Piero F, et al. Evaluation of bone regeneration potential of dental follicle stem cells for treatment of craniofacial defects. Cytotherapy. 2015;17(11):1572–81.
10. Gimble J, Rad MR, Yao S. Adipose tissue–derived stem cells and their regeneration potential. Stem Cells Craniofacial Dev Regen. 2013;241–58.
11. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng. 2001;7(2):211–28.
12. Salehi-Nik N, Rezai Rad M, Kheiri L, Nazeman P, Nadjmi N, Khojasteh A. Buccal fat pad as a potential source of stem cells for bone regeneration: a literature review. Stem Cells Int. 2017;2017:8354640.
13. Marei MK, El Backly RM. Dental mesenchymal stem cell-based translational regenerative dentistry: from artificial to biological replacement. Front Bioeng Biotechnol. 2018;6:49.
14. Zhai Q, Dong Z, Wang W, Li B, Jin Y. Dental stem cell and dental tissue regeneration. Front Med. 2018;13:1–8.
15. Huang G-J, 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(9):792–806.

16. Ibarretxe G, Crende O, Aurrekoetxea M, García-Murga V, Etxaniz J, Unda F. Neural crest stem cells from dental tissues: a new hope for dental and neural regeneration. Stem Cells Int. 2012;2012:103503.
17. Yu J, Wang Y, Deng Z, Tang L, Li Y, Shi J, et al. Odontogenic capability: bone marrow stromal stem cells versus dental pulp stem cells. Biol Cell. 2007;99(8):465–74.
18. Sheng G. The developmental basis of mesenchymal stem/stromal cells (MSCs). BMC Dev Biol. 2015;15(1):44.
19. Rezai Rad M. Characteristics of dental follicle stem cells and their potential application for treatment of craniofacial defects. 2014. doi: etd-07052014-002034.
20. Jaquery A. Implantation of dental pulp stem cells in a biodegradable scaffold for dental pulp tissue engineering; 2007.
21. Ferro F, Spelat R, D'Aurizio F, Puppato E, Pandolfi M, Beltrami AP, et al. Dental pulp stem cells differentiation reveals new insights in Oct4A dynamics. PLoS One. 2012;7(7):e41774.
22. Rodríguez-Lozano FJ, Bueno C, Insausti CL, Meseguer L, Ramirez M, Blanquer M, et al. Mesenchymal stem cells derived from dental tissues. Int Endod J. 2011;44(9):800–6.
23. 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):13625–30.
24. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA. 2003;100(10):5807–12.
25. Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, et al. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol. 2005;24(2):155–65.
26. Kémoun P, Laurencin-Dalicieux S, Rue J, Farges J-C, Gennero I, Conte-Auriol F, et al. 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):283–94.
27. Seo B-M, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet. 2004;364(9429):149–55.
28. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo B-M, Zhang C, et al. Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One. 2006;1(1):e79.
29. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod. 2008;34(2):166–71.
30. Chalisserry EP, Nam SY, Park SH, Anil S. Therapeutic potential of dental stem cells. J Tissue Eng. 2017;8:2041731417702531.
31. Hollands P, Aboyeji D, Orcharton M. Dental pulp stem cells in regenerative medicine. Br Dent J. 2018;224(9):747.
32. Bakopoulou A, Leyhausen G, Volk J, Tsiftsoglou A, Garefis P, Koidis P, 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(7):709–21.
33. Parner E, Heidmann JM, Kjaer I, Væth M, Poulsen S. Biological interpretation of the correlation of emergence times of permanent teeth. J Dent Res. 2002;81(7):451–4.
34. Liu J, Yu F, Sun Y, Jiang B, Zhang W, Yang J, et al. Concise reviews: characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells. 2015;33(3):627–38.
35. Pivoriūnas A, Surovas A, Borutinskaitė V, Matuzevičius D, Treigytė G, Savickienė J, et al. Proteomic analysis of stromal cells derived from the dental pulp of human exfoliated deciduous teeth. Stem Cells Dev. 2009;19(7):1081–93.
36. Jamal M, Chogle S, Goodis H, Karam SM. Dental stem cells and their potential role in regenerative medicine. J Med Sci. 2011;4(2):53–61.
37. Suchánek J, Visek B, Soukup T, El-Din Mohamed S, Ivancakova R, Mokry J, et al. Stem cells from human exfoliated deciduous teeth-isolation, long term cultivation and phenotypical analysis. Acta Med (Hradec Kralove). 2010;53(2):93–9.
38. Karamzadeh R, Eslaminejad MB. Dental-related stem cells and their potential in regenerative medicine. InTech: Regenerative Medicine and Tissue Engineering; 2013.
39. Yan M, Yu Y, Zhang G, Tang C, Yu J. A journey from dental pulp stem cells to a bio-tooth. Stem Cell Rev Rep. 2011;7(1):161–71.
40. Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Massironi SMG, Pereira LV, et al. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs. 2006;184(3–4):105–16.
41. Huang GT-J, Sonoyama W, Chen J, Park SH. In vitro characterization of human dental pulp cells: various isolation methods and culturing environments. Cell Tissue Res. 2006;324(2):225.
42. Bakopoulou A, Leyhausen G, Volk J, Tsiftsoglou A, Garefis P, Koidis P, et al. Assessment of the impact of two different isolation methods on the osteo/odontogenic differentiation potential of human dental stem cells derived from deciduous teeth. Calcif Tissue Int. 2011;88(2):130–41.
43. Karamzadeh R, Eslaminejad MB, Aflatoonian R. Isolation, characterization and comparative differentiation of human dental pulp stem cells derived from permanent teeth by using two different methods. J Vis Exp. 2012;69:4372.
44. Morsczeck C, Völlner F, Saugspier M, Brandl C, Reichert TE, Driemel O, et al. Comparison of human dental follicle cells (DFCs) and stem cells from human exfoliated deciduous teeth (SHED) after neural differentiation in vitro. Clin Oral Investig. 2010;14(4):433–40.

45. Kim S-H, Kim Y-S, Lee S-Y, Kim K-H, Lee Y-M, Kim W-K, et al. Gene expression profile in mesenchymal stem cells derived from dental tissues and bone marrow. J Periodontal Implant Sci. 2011;41(4): 192–200.
46. Tseng P-Y, Chen C-J, Sheu C-C, Yu C-W, Huang Y-S. Spontaneous differentiation of adult rat marrow stromal cells in a long-term culture. J Vet Med Sci. 2007;69(2):95–102.
47. Torsvik A, Røsland GV, Svendsen A, Molven A, Immervoll H, McCormack E, et al. Spontaneous malignant transformation of human mesenchymal stem cells reflects cross-contamination: putting the research field on track–letter. Cancer Res. 2010;70(15):6393–6.
48. Gou S, Wang C, Liu T, Wu H, Xiong J, Zhou F, et al. Spontaneous differentiation of murine bone marrow-derived mesenchymal stem cells into adipocytes without malignant transformation after long-term culture. Cells Tissues Organs. 2010;191(3): 185–92.
49. Ren Z, Wang J, Zhu W, Guan Y, Zou C, Chen Z, et al. Spontaneous transformation of adult mesenchymal stem cells from cynomolgus macaques in vitro. Exp Cell Res. 2011;317(20):2950–7.
50. Kanafi MM, Rajeshwari YB, Gupta S, Dadheech N, Nair PD, Gupta PK, et al. Transplantation of islet-like cell clusters derived from human dental pulp stem cells restores normoglycemia in diabetic mice. Cytotherapy. 2013;15(10):1228–36. https://doi.org/10.1016/j.jcyt.2013.05.008.
51. Reynolds BA, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science. 1992;255(5052):1707–10.
52. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci USA. 1999;96(19):10711–6.
53. Techawattanawisal W, Nakahama K, Komaki M, Abe M, Takagi Y, Morita I. Isolation of multipotent stem cells from adult rat periodontal ligament by neurosphere-forming culture system. Biochem Biophys Res Commun. 2007;357(4):917–23.
54. Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Okano T, et al. Neurosphere generation from dental pulp of adult rat incisor. Eur J Neurosci. 2008;27(3):538–48.
55. Espagnolle N, Guilloton F, Deschaseaux F, Gadelorge M, Sensébé L, Bourin P. CD 146 expression on mesenchymal stem cells is associated with their vascular smooth muscle commitment. J Cell Mol Med. 2014;18(1):104–14.
56. Yang K-L, Chen M-F, Liao C-H, Pang C-Y, Lin P-Y. A simple and efficient method for generating Nurr1-positive neuronal stem cells from human wisdom teeth (tNSC) and the potential of tNSC for stroke therapy. Cytotherapy. 2009;11(5): 606–17.
57. Govindasamy V, Abdullah AN, Ronald VS, Musa S, Aziz ZACA, Zain RB, et al. Inherent differential propensity of dental pulp stem cells derived from human deciduous and permanent teeth. J Endod. 2010;36(9):1504–15.
58. Dahlstrand J, Lardelli M, Lendahl U. Nestin mRNA expression correlates with the central nervous system progenitor cell state in many, but not all, regions of developing central nervous system. Dev Brain Res. 1995;84(1):109–29.
59. Gronthos S, Zannettino AC. A method to isolate and purify human bone marrow stromal stem cells. Mesenchymal stem cells. New York: Springer; 2008. p. 45–57.
60. Gronthos S, Brahim J, Li W, Fisher L, Cherman N, Boyde A, et al. Stem cell properties of human dental pulp stem cells. J Dent Res. 2002;81(8):531–5.
61. Laino G, d'Aquino R, Graziano A, Lanza V, Carinci F, Naro F, et al. A new population of human adult dental pulp stem cells: a useful source of living autologous fibrous bone tissue (LAB). J Bone Miner Res. 2005;20(8):1394–402.
62. d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, et al. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ. 2007;14(6): 1162.
63. Zhang W, Walboomers XF, Shi S, Fan M, Jansen JA. Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. Tissue Eng. 2006;12(10):2813–23.
64. Bhaskar S. Orban's oral histology and embryology, vol. 178. 11th ed. St Louis: CV Mosby; 1991.
65. Cipolleschi MG, Sbarba PD, Olivotto M. The role of hypoxia in the maintenance of hematopoietic stem cells. Blood. 1993;82(7):2031–7.
66. Batouli S, Miura M, Brahim J, Tsutsui T, Fisher L, Gronthos S, et al. Comparison of stem-cell-mediated osteogenesis and dentinogenesis. J Dent Res. 2003;82(12):976–81.
67. Takeda T, Tezuka Y, Horiuchi M, Hosono K, Iida K, Hatakeyama D, et al. Characterization of dental pulp stem cells of human tooth germs. J Dent Res. 2008;87(7):676–81.
68. Zhang W, Ahluwalia IP, Literman R, Kaplan DL, Yelick PC. Human dental pulp progenitor cell behavior on aqueous and hexafluoroisopropanol based silk scaffolds. J Biomed Mater Res Part A. 2011;97(4):414–22.
69. Wang J, Ma H, Jin X, Hu J, Liu X, Ni L, et al. The effect of scaffold architecture on odontogenic differentiation of human dental pulp stem cells. Biomaterials. 2011;32(31):7822–30.
70. Kinaia BM, Chogle SM, Kinaia AM, Goodis HE. Regenerative therapy: a periodontal-endodontic perspective. Dent Clin. 2012;56(3):537–47.
71. Du L, Yang P, Ge S. Stromal cell-derived factor-1 significantly induces proliferation, migration, and collagen type I expression in a human periodontal ligament stem

cell subpopulation. J Periodontol. 2012;83(3):379–88. https://doi.org/10.1902/jop.2011.110201.
72. Rettori E, De Laurentiis A, Zorrilla Zubilete M, Rettori V, Elverdin JC. Anti-inflammatory effect of the endocannabinoid anandamide in experimental periodontitis and stress in the rat. Neuroimmunomodulation. 2012;19(5):293–303. https://doi.org/10.1159/000339113.
73. Rodan GA. Introduction to bone biology. Bone. 1992;13(Suppl 1):S3–6.
74. Dimitriou R, Jones E, McGonagle D, Giannoudis PV. Bone regeneration: current concepts and future directions. BMC Med. 2011;9(1):66. https://doi.org/10.1186/1741-7015-9-66.
75. Graziano A, d'Aquino R, Laino G, Papaccio G. Dental pulp stem cells: a promising tool for bone regeneration. Stem Cell Rev. 2008;4(1):21–6. https://doi.org/10.1007/s12015-008-9013-5.
76. Yamada Y, Ito K, Nakamura S, Ueda M, Nagasaka T. Promising cell-based therapy for bone regeneration using stem cells from deciduous teeth, dental pulp, and bone marrow. Cell Transplant. 2011;20(7):1003–13. https://doi.org/10.3727/096368910x539128.
77. Mori G, Brunetti G, Oranger A, Carbone C, Ballini A, Lo Muzio L, et al. Dental pulp stem cells: osteogenic differentiation and gene expression. Ann NY Acad Sci. 2011;1237:47–52. https://doi.org/10.1111/j.1749-6632.2011.06234.x.
78. d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, et al. Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ. 2007;14(6):1162–71. https://doi.org/10.1038/sj.cdd.4402121.
79. Ma L, Makino Y, Yamaza H, Akiyama K, Hoshino Y, Song G, et al. Cryopreserved dental pulp tissues of exfoliated deciduous teeth is a feasible stem cell resource for regenerative medicine. PLoS One. 2012;7(12):e51777. https://doi.org/10.1371/journal.pone.0051777.
80. de Mendonca CA, Bueno DF, Martins MT, Kerkis I, Kerkis A, Fanganiello RD, et al. Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. J Craniofac Surg. 2008;19(1):204–10. https://doi.org/10.1097/scs.0b013e31815c8a54.
81. Pisciotta A, Riccio M, Carnevale G, Beretti F, Gibellini L, Maraldi T, et al. Human serum promotes osteogenic differentiation of human dental pulp stem cells in vitro and in vivo. PLoS One. 2012;7(11):e50542. https://doi.org/10.1371/journal.pone.0050542.
82. Riccio M, Maraldi T, Pisciotta A, La Sala GB, Ferrari A, Bruzzesi G, et al. Fibroin scaffold repairs critical-size bone defects in vivo supported by human amniotic fluid and dental pulp stem cells. Tissue Eng Part A. 2012;18(9–10):1006–13. https://doi.org/10.1089/ten.TEA.2011.0542.
83. Petridis X, Diamanti E, Trigas G, Kalyvas D, Kitraki E. Bone regeneration in critical-size calvarial defects using human dental pulp cells in an extracellular matrix-based scaffold. J Craniomaxillofac Surg. 2015;43(4):483–90. https://doi.org/10.1016/j.jcms.2015.02.003.
84. Asutay F, Polat S, Gul M, Subasi C, Kahraman SA, Karaoz E. The effects of dental pulp stem cells on bone regeneration in rat calvarial defect model: micro-computed tomography and histomorphometric analysis. Arch Oral Biol. 2015;60(12):1729–35. https://doi.org/10.1016/j.archoralbio.2015.09.002.
85. Seo BM, Sonoyama W, Yamaza T, Coppe C, Kikuiri T, Akiyama K, et al. SHED repair critical-size calvarial defects in mice. Oral Dis. 2008;14(5):428–34.
86. d'Aquino R, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A, 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.
87. Alkaisi A, Ismail AR, Mutum SS, Ahmad ZA, Masudi S, Abd Razak NH. Transplantation of human dental pulp stem cells: enhance bone consolidation in mandibular distraction osteogenesis. J Oral Maxillofac Surg. 2013;71(10):1758.e1-13. https://doi.org/10.1016/j.joms.2013.05.016.
88. Paino F, La Noce M, Giuliani A, De Rosa A, Mazzoni S, Laino L, et al. Human DPSCs fabricate vascularized woven bone tissue: a new tool in bone tissue engineering. Clin Sci (Lond). 2017;131(8):699–713. https://doi.org/10.1042/cs20170047.
89. Cao Y, Liu Z, Xie Y, Hu J, Wang H, Fan Z, et al. Adenovirus-mediated transfer of hepatocyte growth factor gene to human dental pulp stem cells under good manufacturing practice improves their potential for periodontal regeneration in swine. Stem Cell Res Ther. 2015;6:249. https://doi.org/10.1186/s13287-015-0244-5.
90. Kuo TF, Lee SY, Wu HD, Poma M, Wu YW, Yang JC. An in vivo swine study for xeno-grafts of calcium sulfate-based bone grafts with human dental pulp stem cells (hDPSCs). Mater Sci Eng C Mater Biol Appl. 2015;50:19–23. https://doi.org/10.1016/j.msec.2015.01.092.
91. Jahanbin A, Rashed R, Alamdari DH, Koohestanian N, Ezzati A, Kazemian M, et al. Success of maxillary alveolar defect repair in rats using osteoblast-differentiated human deciduous dental pulp stem cells. J Oral Maxillofac Surg. 2016;74(4):829.e1-9. https://doi.org/10.1016/j.joms.2015.11.033.
92. Liu Y, Wang L, Liu S, Liu D, Chen C, Xu X, et al. Transplantation of SHED prevents bone loss in the early phase of ovariectomy-induced osteoporosis. J Dent Res. 2014;93(11):1124–32. https://doi.org/10.1177/0022034514552675.
93. Leyendecker Junior A, Gomes Pinheiro CC, Lazzaretti Fernandes T, Franco BD. The use of human dental pulp stem cells for in vivo bone tissue engineering: a systematic review. J Tissue Eng. 2018;9:2041731417752766. https://doi.org/10.1177/2041731417752766.

94. Kukowska-Latallo JF, Bielinska AU, Johnson J, Spindler R, Tomalia DA, Baker JR. Efficient transfer of genetic material into mammalian cells using Starburst polyamidoamine dendrimers. Proc Natl Acad Sci USA. 1996;93(10):4897–902.
95. Ma L, Aijima R, Hoshino Y, Yamaza H, Tomoda E, Tanaka Y, et al. Transplantation of mesenchymal stem cells ameliorates secondary osteoporosis through interleukin-17-impaired functions of recipient bone marrow mesenchymal stem cells in MRL/lpr mice. Stem Cell Res Ther. 2015;6:104. https://doi.org/10.1186/s13287-015-0091-4.
96. Annibali S, Cicconetti A, Cristalli MP, Giordano G, Trisi P, Pilloni A, et al. A comparative morphometric analysis of biodegradable scaffolds as carriers for dental pulp and periosteal stem cells in a model of bone regeneration. J Craniofac Surg. 2013;24(3):866–71. https://doi.org/10.1097/SCS.0b013e31827ca530.
97. Behnia A, Haghighat A, Talebi A, Nourbakhsh N, Heidari F. Transplantation of stem cells from human exfoliated deciduous teeth for bone regeneration in the dog mandibular defect. World J Stem Cells. 2014;6(4):505–10. https://doi.org/10.4252/wjsc.v6.i4.505.
98. Zhang W, Walboomers XF, van Osch GJ, van den Dolder J, Jansen JA. Hard tissue formation in a porous HA/TCP ceramic scaffold loaded with stromal cells derived from dental pulp and bone marrow. Tissue Eng Part A. 2008;14(2):285–94. https://doi.org/10.1089/tea.2007.0146.
99. Horner PJ, Gage FH. Regenerating the damaged central nervous system. Nature. 2000;407(6807):963.
100. Xiao L, Tsutsui T. Human dental mesenchymal stem cells and neural regeneration. Hum Cell. 2013;26(3):91–6.
101. Luo L, He Y, Wang X, Key B, Lee BH, Li H, et al. Potential roles of dental pulp stem cells in neural regeneration and repair. Stem Cells Int. 2018;2018:1731289. https://doi.org/10.1155/2018/1731289.
102. Xiao L, Nasu M. From regenerative dentistry to regenerative medicine: progress, challenges, and potential applications of oral stem cells. Stem Cells Cloning. 2014;7:89.
103. Xiao L, Tsutsui T. Characterization of human dental pulp cells-derived spheroids in serum-free medium: stem cells in the core. J Cell Biochem. 2013;114(11):2624–36.
104. Yamagata M, Yamamoto A, Kako E, Kaneko N, Matsubara K, Sakai K, et al. Human dental pulp-derived stem cells protect against hypoxic-ischemic brain injury in neonatal mice. Stroke. 2013;44(2):551–4.
105. Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells. 2008;26(7):1787–95.
106. Wang J, Wei X, Ling J, Huang Y, Gong Q, Huo Y. Identification and characterization of side population cells from adult human dental pulp after ischemic culture. J Endod. 2012;38(11):1489–97.
107. Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, et al. Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Invest. 2012;122(1):80–90.
108. Mead B, Logan A, Berry M, Leadbeater W, Scheven BA. Intravitreally transplanted dental pulp stem cells promote neuroprotection and axon regeneration of retinal ganglion cells after optic nerve injury. Invest Ophthalmol Vis Sci. 2013;54(12):7544–56.
109. Arminan A, Gandia C, Bartual M, Garcia-Verdugo JM, Lledo E, Mirabet V, et al. Cardiac differentiation is driven by NKX2.5 and GATA4 nuclear translocation in tissue-specific mesenchymal stem cells. Stem Cells Dev. 2009;18(6):907–18. https://doi.org/10.1089/scd.2008.0292.
110. Yang R, Chen M, Lee CH, Yoon R, Lal S, Mao JJ. Clones of ectopic stem cells in the regeneration of muscle defects in vivo. PLoS One. 2010;5(10):e13547. https://doi.org/10.1371/journal.pone.0013547.
111. Gandia C, Arminan A, Garcia-Verdugo JM, Lledo E, Ruiz A, Minana MD, 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(3):638–45. https://doi.org/10.1634/stemcells.2007-0484.
112. Garzon I, Martin-Piedra MA, Alaminos M. Human dental pulp stem cells. A promising epithelial-like cell source. Med Hypotheses. 2015;84(5):516–7. https://doi.org/10.1016/j.mehy.2015.02.020.
113. Karaoz E, Demircan PC, Saglam O, Aksoy A, Kaymaz F, Duruksu G. Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow-derived mesenchymal stem cells. Histochem Cell Biol. 2011;136(4):455–73. https://doi.org/10.1007/s00418-011-0858-3.
114. Syed-Picard FN, Du Y, Lathrop KL, Mann MM, Funderburgh ML, Funderburgh JL. Dental pulp stem cells: a new cellular resource for corneal stromal regeneration. Stem Cells Transl Med. 2015;4(3):276–85. https://doi.org/10.5966/sctm.2014-0115.
115. Gomes JA, Geraldes Monteiro B, Melo GB, Smith RL, Cavenaghi Pereira da Silva M, Lizier NF, et al. Corneal reconstruction with tissue-engineered cell sheets composed of human immature dental pulp stem cells. Invest Ophthalmol Vis Sci. 2010;51(3):1408–14. https://doi.org/10.1167/iovs.09-4029.
116. Kushnerev E, Shawcross SG, Sothirachagan S, Carley F, Brahma A, Yates JM, et al. Regeneration of corneal epithelium with dental pulp stem cells using a contact lens delivery system. Invest Ophthalmol Vis Sci. 2016;57(13):5192–9. https://doi.org/10.1167/iovs.15-17953.
117. Yam GH, Peh GS, Singhal S, Goh BT, Mehta JS. Dental stem cells: a future asset of ocular cell

therapy. Expert Rev Mol Med. 2015;17:e20. https://doi.org/10.1017/erm.2015.16.
118. Nakashima M, Iohara K, Murakami M. Dental pulp stem cells and regeneration. Endodontic Topics. 2013;28(1):38–50. https://doi.org/10.1111/etp.12027.
119. Yu J, He H, Tang C, Zhang G, Li Y, Wang R, et al. Differentiation potential of STRO-1+ dental pulp stem cells changes during cell passaging. BMC Cell Biol. 2010;11:32. https://doi.org/10.1186/1471-2121-11-32.
120. Morito A, Kida Y, Suzuki K, Inoue K, Kuroda N, Gomi K, et al. Effects of basic fibroblast growth factor on the development of the stem cell properties of human dental pulp cells. Arch Histol Cytol. 2009;72(1):51–64.
121. Reynolds AJ, Jahoda CA. Cultured human and rat tooth papilla cells induce hair follicle regeneration and fiber growth. Differentiation. 2004;72(9–10):566–75. https://doi.org/10.1111/j.1432-0436.2004.07209010.x.
122. Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Okano T, et al. Tubulation with dental pulp cells promotes facial nerve regeneration in rats. Tissue Eng Part A. 2008;14(7):1141–7. https://doi.org/10.1089/ten.tea.2007.0157.
123. Govindasamy V, Ronald VS, Abdullah AN, Nathan KRG, Ab Aziz ZAC, Abdullah M, et al. Differentiation of dental pulp stem cells into islet-like aggregates. J Dent Res. 2011;90(5):646–52. https://doi.org/10.1177/0022034510396879.
124. Carnevale G, Riccio M, Pisciotta A, Beretti F, Maraldi T, Zavatti M, et al. In vitro differentiation into insulin-producing beta-cells of stem cells isolated from human amniotic fluid and dental pulp. Dig Liver Dis. 2013;45(8):669–76. https://doi.org/10.1016/j.dld.2013.02.007.
125. Hattori Y, Kim H, Tsuboi N, Yamamoto A, Akiyama S, Shi Y, et al. Therapeutic potential of stem cells from human exfoliated deciduous teeth in models of acute kidney injury. PLoS One. 2015;10(10):e0140121. https://doi.org/10.1371/journal.pone.0140121.
126. Botelho J, Cavacas MA, Machado V, Mendes JJ. Dental stem cells: recent progresses in tissue engineering and regenerative medicine. Ann Med. 2017;49(8):644–51. https://doi.org/10.1080/07853890.2017.1347705.
127. Ishkitiev N, Yaegaki K, Imai T, Tanaka T, Nakahara T, Ishikawa H, et al. High-purity hepatic lineage differentiated from dental pulp stem cells in serum-free medium. J Endod. 2012;38(4):475–80. https://doi.org/10.1016/j.joen.2011.12.011.
128. Okada M, Ishkitiev N, Yaegaki K, Imai T, Tanaka T, Fukuda M, et al. Hydrogen sulphide increases hepatic differentiation of human tooth pulp stem cells compared with human bone marrow stem cells. Int Endod J. 2014;47(12):1142–50. https://doi.org/10.1111/iej.12262.
129. Hirata M, Ishigami M, Matsushita Y, Ito T, Hattori H, Hibi H, et al. Multifaceted therapeutic benefits of factors derived from dental pulp stem cells for mouse liver fibrosis. Stem Cells Transl Med. 2016;5(10):1416–24. https://doi.org/10.5966/sctm.2015-0353.
130. Kerkis I, Ambrosio CE, Kerkis A, Martins DS, Zucconi E, Fonseca SA, 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. https://doi.org/10.1186/1479-5876-6-35.
131. Pisciotta A, Riccio M, Carnevale G, Lu A, De Biasi S, Gibellini L, et al. Stem cells isolated from human dental pulp and amniotic fluid improve skeletal muscle histopathology in mdx/SCID mice. Stem Cell Res Ther. 2015;6(1):156. https://doi.org/10.1186/s13287-015-0141-y.
132. Yamaza T, Kentaro A, Chen C, Liu Y, Shi Y, Gronthos S, et al. Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. Stem Cell Res Ther. 2010;1(1):5. https://doi.org/10.1186/scrt5.
133. Ishikawa J, Takahashi N, Matsumoto T, Yoshioka Y, Yamamoto N, Nishikawa M, et al. Factors secreted from dental pulp stem cells show multifaceted benefits for treating experimental rheumatoid arthritis. Bone. 2016;83:210–9. https://doi.org/10.1016/j.bone.2015.11.012.
134. Mita T, Furukawa-Hibi Y, Takeuchi H, Hattori H, Yamada K, Hibi H, et al. Conditioned medium from the stem cells of human dental pulp improves cognitive function in a mouse model of Alzheimer's disease. Behav Brain Res. 2015;293:189–97. https://doi.org/10.1016/j.bbr.2015.07.043.
135. Gnanasegaran N, Govindasamy V, Simon C, Gan QF, Vincent-Chong VK, Mani V, et al. Effect of dental pulp stem cells in MPTP-induced old-aged mice model. Eur J Clin Investig. 2017;47(6):403–14. https://doi.org/10.1111/eci.12753.
136. Wang J, Wang X, Sun Z, Wang X, Yang H, Shi S, et al. Stem cells from human-exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. Stem Cells Dev. 2010;19(9):1375–83. https://doi.org/10.1089/scd.2009.0258.
137. Shi S, Robey P, Gronthos S. Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. Bone. 2001;29(6):532–9.
138. Yamada Y, Fujimoto A, Ito A, Yoshimi R, Ueda M. Cluster analysis and gene expression profiles: a cDNA microarray system-based comparison between human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs) for tissue engineering cell therapy. Biomaterials. 2006;27(20):3766–81.
139. Liu H, Gronthos S, Shi S. Dental pulp stem cells. Methods in enzymology. London: Elsevier; 2006. p. 99–113.
140. Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod. 2008;34(6):645–51. https://doi.org/10.1016/j.joen.2008.03.001.

141. Huang GT, Yamaza T, Shea LD, Djouad F, Kuhn NZ, Tuan RS, 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(2):605–15. https://doi.org/10.1089/ten.TEA.2009.0518.
142. Ding G, Liu Y, An Y, Zhang C, Shi S, Wang W, et al. Suppression of T cell proliferation by root apical papilla stem cells in vitro. Cells Tissues Organs. 2010;191(5):357–64. https://doi.org/10.1159/000276589.
143. Hilkens P, Bronckaers A, Ratajczak J, Gervois P, Wolfs E, Lambrichts I. The angiogenic potential of DPSCs and SCAPs in an in vivo model of dental pulp regeneration. Stem Cells Int. 2017;2017:2582080. https://doi.org/10.1155/2017/2582080.
144. Schneider R, Holland GR, Chiego D Jr, Hu JC, Nor JE, Botero TM. White mineral trioxide aggregate induces migration and proliferation of stem cells from the apical papilla. J Endod. 2014;40(7):931–6. https://doi.org/10.1016/j.joen.2013.11.021.
145. Liu C, Xiong H, Chen K, Huang Y, Huang Y, Yin X. Long-term exposure to pro-inflammatory cytokines inhibits the osteogenic/dentinogenic differentiation of stem cells from the apical papilla. Int Endod J. 2016;49(10):950–9. https://doi.org/10.1111/iej.12551.
146. Bakopoulou A, Leyhausen G, Volk J, Koidis P, Geurtsen W. Effects of resinous monomers on the odontogenic differentiation and mineralization potential of highly proliferative and clonogenic cultured apical papilla stem cells. Dent Mater. 2012;28(3):327–39. https://doi.org/10.1016/j.dental.2012.01.002.
147. Ding G, Wang W, Liu Y, An Y, Zhang C, Shi S, et al. Effect of cryopreservation on biological and immunological properties of stem cells from apical papilla. J Cell Physiol. 2010;223(2):415–22. https://doi.org/10.1002/jcp.22050.
148. Dong R, Yao R, Du J, Wang S, Fan Z. Depletion of histone demethylase KDM2A enhanced the adipogenic and chondrogenic differentiation potentials of stem cells from apical papilla. Exp Cell Res. 2013;319(18):2874–82. https://doi.org/10.1016/j.yexcr.2013.07.008.
149. Zhang W, Zhang X, Ling J, Liu W, Zhang X, Ma J, et al. Proliferation and odontogenic differentiation of BMP2 genetransfected stem cells from human tooth apical papilla: an in vitro study. Int J Mol Med. 2014;34(4):1004–12. https://doi.org/10.3892/ijmm.2014.1862.
150. Zhang J, Wang Z, Jiang Y, Niu Z, Fu L, Luo Z, et al. Nuclear factor I-C promotes proliferation and differentiation of apical papilla-derived human stem cells in vitro. Exp Cell Res. 2015;332(2):259–66. https://doi.org/10.1016/j.yexcr.2015.01.020.
151. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. J Endod. 2007;33(4):377–90.
152. Bakopoulou A, Leyhausen G, Volk J, Koidis P, Geurtsen W. Comparative characterization of STRO-1neg/CD146pos and STRO-1pos/CD146pos apical papilla stem cells enriched with flow cytometry. Arch Oral Biol. 2013;58(10):1556–68.
153. Hilkens P, Fanton Y, Martens W, Gervois P, Struys T, Politis C, et al. Pro-angiogenic impact of dental stem cells in vitro and in vivo. Stem Cell Res. 2014;12(3):778–90.
154. He L, Zhong J, Gong Q, Cheng B, Kim SG, Ling J, et al. Regenerative endodontics by cell homing. Dent Clin. 2017;61(1):143–59.
155. De Berdt P, Bottemanne P, Bianco J, Alhouayek M, Diogenes A, Llyod A, et al. Stem cells from human apical papilla decrease neuro-inflammation and stimulate oligodendrocyte progenitor differentiation via activin-A secretion. Cell Mol Life Sci. 2018:1–14.
156. Fayazi S, Takimoto K, Diogenes A. Comparative evaluation of chemotactic factor effect on migration and differentiation of stem cells of the apical papilla. J Endod. 2017;43(8):1288–93.
157. Huang GT-J, Yamaza T, Shea LD, Djouad F, Kuhn NZ, Tuan RS, 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. 2009;16(2):605–15.
158. de Almeida JFA, Chen P, Henry MA, Diogenes A. Stem cells of the apical papilla regulate trigeminal neurite outgrowth and targeting through a BDNF-dependent mechanism. Tissue Eng Part A. 2014;20(23–24):3089–100.
159. de Souza LEB, Malta TM, Kashima Haddad S, Covas DT. Mesenchymal stem cells and pericytes: to what extent are they related? Stem Cells Dev. 2016;25(24):1843–52.
160. Amirikia M, Shariatzadeh SMA, Jorsaraei SGA, Mehranjani MS. Impact of pre-incubation time of silk fibroin scaffolds in culture medium on cell proliferation and attachment. Tissue Cell. 2017;49(6):657–63.
161. Bellamy C, Shrestha S, Torneck C, Kishen A. Effects of a bioactive scaffold containing a sustained transforming growth factor-β1–releasing nanoparticle system on the migration and differentiation of stem cells from the apical papilla. J Endod. 2016;42(9):1385–92.
162. Bakopoulou A, Kritis A, Andreadis D, Papachristou E, Leyhausen G, Koidis P, et al. Angiogenic potential and secretome of human apical papilla mesenchymal stem cells in various stress microenvironments. Stem Cells Dev. 2015;24(21):2496–512.
163. Yuan C, Wang P, Zhu L, Dissanayaka WL, Green DW, Tong EH, et al. Coculture of stem cells from apical papilla and human umbilical vein endothelial cell under hypoxia increases the formation of three-dimensional vessel-like structures in vitro. Tissue Eng Part A. 2014;21(5–6):1163–72.
164. De Berdt P, Vanacker J, Ucakar B, Elens L, Diogenes A, Leprince J, et al. Dental apical papilla

as therapy for spinal cord injury. J Dent Res. 2015;94(11):1575–81.
165. Nada OA, El Backly RM. Stem cells from the apical papilla (SCAP) as a tool for endogenous tissue regeneration. Front Bioeng Biotech. 2018;6:103.
166. Cao Y, Xia D, Qi S, Du J, Ma P, Wang S, et al. Epiregulin can promote proliferation of stem cells from the dental apical papilla via MEK/Erk and JNK signalling pathways. Cell Prolif. 2013;46(4):447–56.
167. Lin LM, Kim SG, Martin G, Kahler B. Continued root maturation despite persistent apical periodontitis of immature permanent teeth after failed regenerative endodontic therapy. Aust Endod J. 2018;44(3):292–9.
168. Wise G, King G. Mechanisms of tooth eruption and orthodontic tooth movement. J Dent Res. 2008;87(5):414–34.
169. Honda MJ, Imaizumi M, Tsuchiya S, Morsczeck C. Dental follicle stem cells and tissue engineering. J Oral Sci. 2010;52(4):541–52.
170. Yao S, Pan F, Prpic V, Wise G. Differentiation of stem cells in the dental follicle. J Dent Res. 2008;87(8):767–71.
171. Nanci A, editor. Ten Cate's oral histology: development, structure and function. St. Louis: MOSBY E; 2008.
172. Lindroos B, Mäenpää K, Ylikomi T, Oja H, Suuronen R, Miettinen S. Characterisation of human dental stem cells and buccal mucosa fibroblasts. Biochem Biophys Res Commun. 2008;368(2):329–35.
173. Yagyuu T, Ikeda E, Ohgushi H, Tadokoro M, Hirose M, Maeda M, et al. Hard tissue-forming potential of stem/progenitor cells in human dental follicle and dental papilla. Arch Oral Biol. 2010;55(1):68–76.
174. Yokoi T, Saito M, Kiyono T, Iseki S, Kosaka K, Nishida E, et al. Establishment of immortalized dental follicle cells for generating periodontal ligament in vivo. Cell Tissue Res. 2007;327(2):301–11.
175. Handa K, Saito M, Tsunoda A, Yamauchi M, Hattori S, Sato S, et al. Progenitor cells from dental follicle are able to form cementum matrix in vivo. Connect Tissue Res. 2002;43(2–3):406–8.
176. Ramamoorthi M, Bakkar M, Jordan J, Tran SD. Osteogenic potential of dental mesenchymal stem cells in preclinical studies: a systematic review using modified ARRIVE and CONSORT guidelines. Stem Cells Int. 2015;2015:378368.
177. Xu L-L, Liu H-C, Wang D-S, Ling-Ling E, Xu L, Jin Z-L, et al. Effects of BMP-2 and dexamethasone on osteogenic differentiation of rat dental follicle progenitor cells seeded on three-dimensional β-TCP. Biomed Mater. 2009;4(6):065010.
178. Tsuchiya S, Ohshima S, Yamakoshi Y, Simmer JP, Honda MJ. Osteogenic differentiation capacity of porcine dental follicle progenitor cells. Connect Tissue Res. 2010;51(3):197–207.
179. Chen X, Zhang T, Shi J, Xu P, Gu Z, Sandham A, et al. Notch1 signaling regulates the proliferation and self-renewal of human dental follicle cells by modulating the G1/S phase transition and telomerase activity. PLoS One. 2013;8(7):e69967.
180. Honda MJ, Imaizumi M, Suzuki H, Ohshima S, Tsuchiya S, Satomura K. Stem cells isolated from human dental follicles have osteogenic potential. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011;111(6):700–8.
181. Rezai Rad M, Wise GE, Brooks H, Flanagan MB, Yao S. Activation of proliferation and differentiation of dental follicle stem cells (DFSCs) by heat stress. Cell Prolif. 2013;46(1):58–66.
182. Honda MJ, Tsuchiya S, Shinohara Y, Shinmura Y, Sumita Y. Recent advances in engineering of tooth and tooth structures using postnatal dental cells. Jpn Dent Sci Rev. 2010;46(1):54–66.
183. Handa K, Saito M, Yamauchi M, Kiyono T, Sato S, Teranaka T, et al. Cementum matrix formation in vivo by cultured dental follicle cells. Bone. 2002;31(5):606–11.
184. 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):459–70.
185. Isaka J, Ohazama A, Kobayashi M, Nagashima C, Takiguchi T, Kawasaki H, et al. Participation of periodontal ligament cells with regeneration of alveolar bone. J Periodontol. 2001;72(3):314–23. https://doi.org/10.1902/jop.2001.72.3.314.
186. McCulloch CA, Bordin S. Role of fibroblast subpopulations in periodontal physiology and pathology. J Periodontal Res. 1991;26(3 Pt 1):144–54.
187. Kaneda T, Miyauchi M, Takekoshi T, Kitagawa S, Kitagawa M, Shiba H, et al. Characteristics of periodontal ligament subpopulations obtained by sequential enzymatic digestion of rat molar periodontal ligament. Bone. 2006;38(3):420–6. https://doi.org/10.1016/j.bone.2005.08.021.
188. Wada N, Menicanin D, Shi S, Bartold PM, Gronthos S. Immunomodulatory properties of human periodontal ligament stem cells. J Cell Physiol. 2009;219(3):667–76. https://doi.org/10.1002/jcp.21710.
189. Ding G, Liu Y, Wang W, Wei F, Liu D, Fan Z, et al. Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. Stem Cells. 2010;28(10):1829–38. https://doi.org/10.1002/stem.512.
190. Liu D, Xu J, Liu O, Fan Z, Liu Y, Wang F, et al. Mesenchymal stem cells derived from inflamed periodontal ligaments exhibit impaired immunomodulation. J Clin Periodontol. 2012;39(12):1174–82. https://doi.org/10.1111/jcpe.12009.
191. Tomokiyo A, Yoshida S, Hamano S, Hasegawa D, Sugii H, Maeda H. Detection, characterization, and clinical application of mesenchymal stem cells in periodontal ligament tissue. Stem Cells Int. 2018;2018:5450768.

192. Liu Z, Yin X, Ye Q, He W, Ge M, Zhou X, et al. Periodontal regeneration with stem cells-seeded collagen-hydroxyapatite scaffold. J Biomater Appl. 2016;31(1):121–31. https://doi.org/10.1177/0885328216637978.
193. Sun X, Xu C, Wu G, Ye Q, Wang C. Poly(lactic-co-glycolic acid): applications and future prospects for periodontal tissue regeneration. Polymers. 2017;9(6):189.
194. Ninomiya T, Hiraga T, Hosoya A, Ohnuma K, Ito Y, Takahashi M, et al. Enhanced bone-forming activity of side population cells in the periodontal ligament. Cell Transplant. 2014;23(6):691–701.
195. Park JC, Kim JM, Jung IH, Kim JC, Choi SH, Cho KS, et al. Isolation and characterization of human periodontal ligament (PDL) stem cells (PDLSCs) from the inflamed PDL tissue: in vitro and in vivo evaluations. J Clin Periodontol. 2011;38(8):721–31.
196. Wang H, Li J, Zhang X, Ning T, Ma D, Ge Y, et al. Priming integrin alpha 5 promotes the osteogenic differentiation of human periodontal ligament stem cells due to cytoskeleton and cell cycle changes. J Proteome. 2018;179:122–30.
197. Feng F, Akiyama K, Liu Y, Yamaza T, Wang TM, Chen JH, et al. Utility of PDL progenitors for in vivo tissue regeneration: a report of 3 cases. Oral Dis. 2010;16(1):20–8.
198. Jin H, Choung H-W, Lim K-T, Jin B, Jin C, Chung J-H, et al. Recombinant human plasminogen activator inhibitor-1 promotes cementogenic differentiation of human periodontal ligament stem cells. Tissue Eng Part A. 2015;21(23–24):2817–28.
199. Zhang J, An Y, Gao L-N, Zhang Y-J, Jin Y, Chen F-M. The effect of aging on the pluripotential capacity and regenerative potential of human periodontal ligament stem cells. Biomaterials. 2012;33(29):6974–86.
200. Gao L-N, An Y, Lei M, Li B, Yang H, Lu H, et al. The effect of the coumarin-like derivative osthole on the osteogenic properties of human periodontal ligament and jaw bone marrow mesenchymal stem cell sheets. Biomaterials. 2013;34(38):9937–51.
201. Wang Z-S, Feng Z-H, Wu G-F, Bai S-Z, Dong Y, Chen F-M, et al. The use of platelet-rich fibrin combined with periodontal ligament and jaw bone mesenchymal stem cell sheets for periodontal tissue engineering. Sci Rep. 2016;6:28126.
202. Tsumanuma Y, Iwata T, Washio K, Yoshida T, Yamada A, Takagi R, et al. Comparison of different tissue-derived stem cell sheets for periodontal regeneration in a canine 1-wall defect model. Biomaterials. 2011;32(25):5819–25.
203. Han J, Menicanin D, Marino V, Ge S, Mrozik K, Gronthos S, et al. Assessment of the regenerative potential of allogeneic periodontal ligament stem cells in a rodent periodontal defect model. J Periodontal Res. 2014;49(3):333–45.
204. Pereira L, Rubini M, Silva J, Oliveira D, Silva I, Poças-Fonseca M, et al. Comparison of stem cell properties of cells isolated from normal and inflamed dental pulps. Int Endod J. 2012;45(12):1080–90.
205. Tang HN, Xia Y, Yu Y, Wu RX, Gao LN, Chen FM. Stem cells derived from "inflamed" and healthy periodontal ligament tissues and their sheet functionalities: a patient-matched comparison. J Clin Periodontol. 2016;43(1):72–84.
206. Gronthos S, Mrozik K, Shi S, Bartold P. Ovine periodontal ligament stem cells: isolation, characterization, and differentiation potential. Calcif Tissue Int. 2006;79(5):310–7.
207. Fujita T, Iwata T, Shiba H, Igarashi A, Hirata R, Takeda K, et al. Identification of marker genes distinguishing human periodontal ligament cells from human mesenchymal stem cells and human gingival fibroblasts. J Periodontal Res. 2007;42(3):283–6. https://doi.org/10.1111/j.1600-0765.2006.00944.x.
208. Lekic P, Rojas J, Birek C, Tenenbaum H, McCulloch CA. Phenotypic comparison of periodontal ligament cells in vivo and in vitro. J Periodontal Res. 2001;36(2):71–9.
209. Lekic PC, Rajshankar D, Chen H, Tenenbaum H, McCulloch CA. Transplantation of labeled periodontal ligament cells promotes regeneration of alveolar bone. Anat Rec. 2001;262(2):193–202.
210. Moshaverinia A, Xu X, Chen C, Akiyama K, Snead ML, Shi S. Dental mesenchymal stem cells encapsulated in an alginate hydrogel co-delivery microencapsulation system for cartilage regeneration. Acta Biomater. 2013;9(12):9343–50. https://doi.org/10.1016/j.actbio.2013.07.023.
211. Kim SH, Kim KH, Seo BM, Koo KT, Kim TI, Seol YJ, et al. Alveolar bone regeneration by transplantation of periodontal ligament stem cells and bone marrow stem cells in a canine peri-implant defect model: a pilot study. J Periodontol. 2009;80(11):1815–23. https://doi.org/10.1902/jop.2009.090249.
212. Xu J, Wang W, Kapila Y, Lotz J, Kapila S. Multiple differentiation capacity of STRO-1+/CD146+ PDL mesenchymal progenitor cells. Stem Cells Dev. 2009;18(3):487–96. https://doi.org/10.1089/scd.2008.0113.
213. Gay IC, Chen S, MacDougall M. Isolation and characterization of multipotent human periodontal ligament stem cells. Orthod Craniofac Res. 2007;10(3):149–60. https://doi.org/10.1111/j.1601-6343.2007.00399.x.
214. Houshmand B, Behnia H, Khoshzaban A, Morad G, Behrouzi G, Dashti SG, et al. Osteoblastic differentiation of human stem cells derived from bone marrow and periodontal ligament under the effect of enamel matrix derivative and transforming growth factor-beta. Int J Oral Maxillofac Implants. 2013;28(6):e440–50. https://doi.org/10.11607/jomi.te24.
215. Chadipiralla K, Yochim JM, Bahuleyan B, Huang CY, Garcia-Godoy F, Murray PE, et al. Osteogenic differentiation of stem cells derived from human

periodontal ligaments and pulp of human exfoliated deciduous teeth. Cell Tissue Res. 2010;340(2):323–33. https://doi.org/10.1007/s00441-010-0953-0.
216. Moshaverinia A, Xu X, Chen C, Ansari S, Zadeh HH, Snead ML, et al. Application of stem cells derived from the periodontal ligament or gingival tissue sources for tendon tissue regeneration. Biomaterials. 2014;35(9):2642–50. https://doi.org/10.1016/j.biomaterials.2013.12.053.
217. Ebihara G, Sato M, Yamato M, Mitani G, Kutsuna T, Nagai T, et al. Cartilage repair in transplanted scaffold-free chondrocyte sheets using a minipig model. Biomaterials. 2012;33(15):3846–51. https://doi.org/10.1016/j.biomaterials.2012.01.056.
218. Yan H, Yu C. Repair of full-thickness cartilage defects with cells of different origin in a rabbit model. Arthroscopy. 2007;23(2):178–87. https://doi.org/10.1016/j.arthro.2006.09.005.
219. Tuan RS, Chen AF, Klatt BA. Cartilage regeneration. J Am Acad Orthop Surg. 2013;21(5):303–11. https://doi.org/10.5435/JAAOS-21-05-303.
220. Dapeng L, Xiaojie L, Ping G, Yan D, Gang S. Erk1/2 signalling is involved in the differentiation of periodontal ligament stem cells to Schwann cells in dog. Arch Oral Biol. 2014;59(5):487–91.
221. Ng TK, Yung JS, Choy KW, Cao D, Leung CK, Cheung HS, et al. Transdifferentiation of periodontal ligament-derived stem cells into retinal ganglion-like cells and its microRNA signature. Sci Rep. 2015;5:16429.
222. Cen LP, Ng TK, Liang JJ, Zhuang X, Yao X, Yam GH, et al. Human periodontal ligament-derived stem cells promote retinal ganglion cell survival and axon regeneration after optic nerve injury. Stem Cells. 2018;36(6):844–55. https://doi.org/10.1002/stem.2812.
223. Pelaez D, Torres ZA, Ng TK, Choy KW, Pang CP, Cheung HS. Cardiomyogenesis of periodontal ligament-derived stem cells by dynamic tensile strain. Cell Tissue Res. 2017;367(2):229–41.
224. Gioventù S, Andriolo G, Bonino F, Frasca S, Lazzari L, Montelatici E, et al. A novel method for banking dental pulp stem cells. Transfus Apher Sci. 2012;47(2):199–206.
225. Chen YK, Huang AHC, Chan AWS, Shieh TY, Lin LM. Human dental pulp stem cells derived from different cryopreservation methods of human dental pulp tissues of diseased teeth. J Oral Pathol Med. 2011;40(10):793–800.
226. Perry BC, Zhou D, Wu X, Yang F-C, Byers MA, Chu T-MG, et al. Collection, cryopreservation, and characterization of human dental pulp–derived mesenchymal stem cells for banking and clinical use. Tissue Eng Part C Methods. 2008;14(2):149–56.
227. Arora V, Arora P, Munshi AK. Banking stem cells from human exfoliated deciduous teeth (SHED): saving for the future. J Clin Pediatr Dent. 2009;33(4):289–94.
228. Almela T, Brook IM, Moharamzadeh K. The significance of cell-related challenges in the clinical application of tissue engineering. J Biomed Mater Res Part A. 2016;104(12):3157–63.
229. Hilkens P, Driesen RB, Wolfs E, Gervois P, Vangansewinkel T, Ratajczak J, et al. Cryopreservation and banking of dental stem cells. Adv Exp Med Biol. 2016;951:199–235. https://doi.org/10.1007/978-3-319-45457-3_17.
230. Hilkens P, Meschi N, Lambrechts P, Bronckaers A, Lambrichts I. Dental stem cells in pulp regeneration: near future or long road ahead? Stem Cells Dev. 2015;24(14):1610–22. https://doi.org/10.1089/scd.2014.0510.