

Therapeutic potential of dental stem cells

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Abstract

Stem cell biology has become an important field in regenerative medicine and tissue engineering therapy since the discovery and characterization of mesenchymal stem cells. Stem cell populations have also been isolated from human dental tissues, including dental pulp stem cells, stem cells from human exfoliated deciduous teeth, stem cells from apical papilla, dental follicle progenitor cells, and periodontal ligament stem cells. Dental stem cells are relatively easily obtainable and exhibit high plasticity and multipotential capabilities. The dental stem cells represent a gold standard for neural-crest-derived bone reconstruction in humans and can be used for the repair of body defects in low-risk autologous therapeutic strategies. The bioengineering technologies developed for tooth regeneration will make substantial contributions to understand the developmental process and will encourage future organ replacement by regenerative therapies in a wide variety of organs such as the liver, kidney, and heart. The concept of developing tooth banking and preservation of dental stem cells is promising. Further research in the area has the potential to herald a new dawn in effective treatment of notoriously difficult diseases which could prove highly beneficial to mankind in the long run.

Keywords

Dental stem cell, stem cell therapy, differentiation, regeneration, tissue engineering, tooth banking

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Introduction

The tooth is composed of distinct tissues including the outer mineralized enamel layer; the adjacent mineralized dentin layer; the dental pulp containing blood vessels, nerves, and mesenchymal tissue; and root structures composed of dentin, cementum, and periodontal ligament (PDL), which secure teeth to the underlying alveolar bone. Dentin contains characteristic and distinctive tubules, produced by neural crest derived dental mesenchymal stem cells called odontoblasts, which persist in mature teeth and exhibit limited regenerative capacities to form reparative dentin in response to injury or disease. The dental pulp is composed of dental mesenchymal cells, nerves, and blood vessels that thread through the root canal. Teeth develop through continuous and reciprocal interactions between cranial neural crest-derived mesenchymal stem cells (MSCs) and oral-derived epithelial stem cells during early embryogenesis.^{1,2}

Stem cells can be isolated from several oral tissues such as craniofacial bone, dental pulp, PDL, dental follicle, tooth germ, apical papilla, oral mucosa, gingival, and periosteum.³ The dental stem cells (DSCs) are post-natal

stem cell populations that have MSC-like qualities, including the capacity for self-renewal and multilineage differentiation potential. These cells are derived from the neural crest, and thus have a different origin from bone-marrow-derived mesenchymal stem cells (BMMSCs), which are derived from mesoderm.⁴ Among oral tissue-derived stem cells, human dental pulp stem cells (hDPSCs) have been

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widely studied due to their great clinical potential, easy accessibility, and less invasive harvesting. These cells were found to form dentin-like tissue and to differentiate into osteoblast-like cells that formed bone in vitro. In the presence of specific stimuli, these DPSCs differentiated into several cell types, including neurons, adipocytes, and chondrocytes. Interestingly, vascular endothelial cells and DPSCs were found to synergistically differentiate into osteoblasts and endothelial cells, respectively.^{5,6}

DSCs have been widely studied due to their great clinical potential, easy accessibility, and less invasive harvesting. Several preclinical investigations conducted so far indicated the extensive potential of the stem cell in tissue repair and regeneration of dental tissues, as well as other organs. This article focuses on the type of DSCs and its potential therapeutic applications in tissue engineering and regenerative medicine.

DSC

The dental pulp is a soft tissue of ectomesenchymal and mesenchymal origin, developing from the dental papilla. Stem cell populations can be isolated from different tissues of the oral and maxillofacial regions. They are stemmed from different developmental stages of the tooth. Around eight unique populations of dental tissue-derived MSCs have been isolated and characterized. Post-natal DPSCs were the first human dental MSCs to be identified from pulp tissue.⁷ Other dental MSC-like populations, such as stem cells from human exfoliated deciduous teeth (SHED),⁸ periodontal ligament stem cells (PDLSCs),⁹ dental follicle progenitor cells (DFPCs),¹⁰ alveolar bone-derived mesenchymal stem cells (ABMSCs),¹¹ stem cells from the apical part of the human dental papilla (SCAP),¹² tooth germ progenitor cells (TGPCs),¹³ and gingival mesenchymal stem cells (GMSCs),¹⁴ were also isolated and characterized (Figure 1).

Dental pulp-derived stem cells such as human adult DPSCs and SHED are self-renewing MSCs residing within the perivascular niche of the dental pulp.^{7,8} They are thought to originate from the cranial neural crest, which expresses early markers for both MSCs and neuroectodermal stem cells.¹⁵ DPSC and SHED have been reported to demonstrate the ability to regenerate into various tissues. Recently it has been shown that implantation of DPSCs or SHED promotes functional recovery after spinal cord injury (SCI).¹⁶ DPSC also protect against ischemic brain injury in neonatal mice.¹⁷ DSC have comparable therapeutic potential similar to BMMSCs, and DSC is another alternative noninvasive source to be used for future regenerative therapies.

DPSC

DPSCs were the first type of DSC derived from dental pulp and was isolated by enzymatic digestion of the pulp

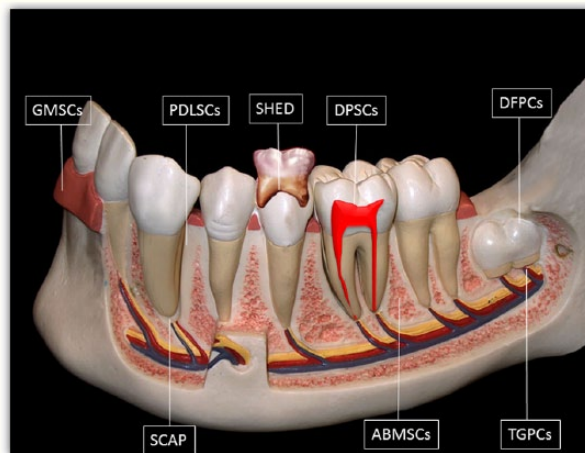


Figure 1. Schematic drawing illustrating sources of human dental tissue-derived MSCs.

ABMSCs: alveolar bone-derived mesenchymal stem cells; DFPCs: dental follicle progenitor cells; DPSCs: dental pulp stem cells; GMSCs: gingival mesenchymal stem cells; PDLSCs: periodontal ligament stem cells; SCAP: stem cells from the apical part of the human dental papilla; SHED: stem cells from human exfoliated deciduous teeth; TGPCs: tooth germ progenitor cells.

tissue of the human-impacted third molars. These multipotential cells exhibited a typical fibroblast-like morphology.⁷ Isolation of DPSC was performed and with various differentiation media, their dentinogenic, osteogenic, adipogenic, neurogenic, chondrogenic, and myogenic differentiation potential were demonstrated.^{18,19} Even though there is no specific biomarker available for the identification of DPSC, these cells express several markers including the mesenchymal and bone-marrow stem cell markers, STRO-1, and CD146, as well as the embryonic stem cell (ESC) marker, OCT4. The candidate markers of DPSCs include STRO-1, CD29, CD44, CD73, CD90, CD105, CD146, CD166, and CD271.²⁰

DPSC differentiate into adipogenic, chondrogenic, and osteogenic lineages, express epithelial markers and share common characteristics with neural stem cells; they are also able to differentiate, in vitro, into neural or vascular endothelial cells.^{21,22} DPSCs and endothelial cells have a synergistic effect in co-cultures, enhancing differentiation to osteogenic, odontogenic, and angiogenic phenotypes.²³ hDPSCs which are grown by explant culture method have better proliferative capacity and also differentiate into various cell types of osteogenic, adipogenic, and myogenic lineages.^{24,25} The hDPSC cultures contain multipotent neural crest stem cell (NCSC) which can differentiate into a number of neural crest-derived cell lineages including melanocytes.²⁶ Paino et al.²⁷ showed that DPSC spontaneously differentiate in vitro towards the melanocytic lineage. DPSCs have shown the greatest potential to produce a high volume of mineralized matrix, suggesting that these cells also show promise for use in regenerative dental

therapies.²⁸ Dental pulp progenitors have not been clearly identified but some data suggest that pericytes, which are able to differentiate into osteoblasts, could also differentiate into odontoblasts.²⁹

SHED. The stem cells isolated from the dental pulp of exfoliated deciduous teeth revealed their high proliferative and clonogenic nature. Miura et al.⁸ isolated mesenchymal progenitor stem cell from exfoliated deciduous teeth. These cells were named SHED (stem cells from human exfoliated deciduous teeth) and exhibited high plasticity since they could differentiate into neurons, adipocytes, osteoblasts, and odontoblasts. They differed from DPSCs as SHED were isolated from the pulp tissue of the crown of exfoliated deciduous teeth, and these stem cells did not grow as individual cells instead exhibited the growth in clusters forming several colonies which, after separation, grew as individual fibroblast-like cells. SHED demonstrated higher proliferation rate and a higher number of colony-forming cells compared to DPSCs with early expression of MSC markers (STRO-1 and CD146). They developed multiple cytoplasmic processes and expressed different neuronal and glial cell markers such as nestin when cultured with neurogenic inductive media, suggesting its neural crest origin. The transplantation of SHED into immunocompromised mice showed the formation of dentin-like tissues, which was immune-reactive to dentin-specific sialophospho protein antibody. This regenerated dentin was formed due to odontoblast-like cells indicating the odontogenic differentiation potential of SHED. SHED, unlike DPSCs, cannot be differentiated into osteoblasts or osteocytes but are able to induce the host cells to undergo osteogenic differentiation.⁸ SHED possess a higher proliferation rate, as well as high odontogenic and osteogenic differentiation potential, which make them distinct from the DPSC and represent the more immature form than DPSC.

PDLSC. The PDL is a specialized tissue located between the cementum and the alveolar bone and has a role of maintaining and supporting the teeth. Its continuous regeneration is thought to involve mesenchymal progenitors arising from the dental follicle. PDL contains different types of cells, which can be differentiated into cementoblasts and osteoblasts.³⁰ The isolated PDLSCs demonstrated their fibroblast-like morphology and exhibited clonogenic nature.³¹ These cells showed a high rate of proliferation than DPSCs and demonstrated expression of STRO-1, CD146, and a tendon-specific transcription factor.³¹ It is thus obvious that PDL itself contains progenitors, which can be activated to self-renew and regenerate other tissues such as cementum and alveolar bone.⁹ PDLSCs possessed multilineage differentiation capabilities and were able to undergo osteogenic, adipogenic, and chondrogenic differentiations when cultured with the suitable inductive medium.⁹

DFPC

The dental follicle is ectomesenchymal in origin and surrounds the unerupted tooth just like a protective sac. It controls the osteoclastogenesis and osteogenesis processes during the tooth eruption and differentiates into the periodontium.³² DFPCs isolated from follicle of human third molars displayed fibroblast-like morphology and expressed various biomarkers such as Notch1, STRO-1, and nestin.¹⁰ The in vitro studies demonstrated the multilineage potential of DFPCs to undergo osteogenic, adipogenic, and neurogenic differentiation.³³ These cells can be maintained in culture for at least 15 passages. STRO-1-positive dental follicle stem cells (DFSCs) can differentiate into cementoblasts in vitro and are able to form cementum in vivo.³⁴ DFPC showed their potential to differentiate and express cementoblast markers under stimulation by BMP-2 and BMP-7 and enamel matrix derivatives (EMDs).³⁴ Immortalized dental follicle cells are able to re-create a new PDL after in vivo implantation.³⁵

ABMSC

Successful isolation and culture of human alveolar bone-derived mesenchymal stem cells (hABMSCs) were performed by Matsubara et al.¹¹ The isolated cells exhibit a spindle-shaped fibroblast-like morphology, plastic adherence, and colony formation. These cells express the surface markers CD73, CD90, CD105, and STRO-1 but do not express the hematopoietic markers CD14, CD34, and CD45.^{11,36,37} ABMSCs can be differentiated into osteoblastic lineages with a high alkaline phosphatase (ALP) expression.¹¹ Studies have shown that treatment of hABMSCs with dichloromethane fraction of *Dipsaci Radix*³⁸ interferon-induced transmembrane protein 1³⁹ nicotine⁴⁰ low-frequency pulsed electromagnetic fields,⁴¹ low-intensity pulsed ultrasound,⁴² low fluid dynamic shear stress,⁴³ and orbital shear stress⁴⁴ could enhance osteogenesis in these cells. They have chondrogenic and adipogenic differentiation potentials similar to those of other stem cell populations.⁴⁵ Bioceramics may provide a good scaffold for ABMSC attachment, proliferation, migration, and differentiation for use in bone tissue engineering applications.⁴⁶

SCAP. During the tooth development, the dental papilla develops into the dental pulp and subsequently contributes to the development of the root. The dental papilla progresses apically where it is loosely attached to the developing root.¹² SCAP have been isolated and their potential to differentiate into odontoblasts was compared to that of the PDLSCs.¹² SCAP express the early mesenchymal surface markers especially CD24, which could be a unique marker for their population.^{12,47} These cells demonstrate their capacity to undergo osteogenic, adipogenic, chondrogenic, and neurogenic differentiation when they are cultured in

the suitable inductive media. After transplantation of SCAP into immunocompromised mice in an appropriate carrier matrix, a typical dentin-pulp-like structure was formed due to the presence of odontoblast-like cells.⁴⁸ SCAP exhibit a higher proliferative rate and appears more effective than PDLSC for tooth formation and are easily accessible since they can be isolated from human third molars.

TGPC

TGPCs are novel stem cell population that were identified in the dental mesenchyme of the third molar tooth germ during the late bell stage.¹³ They can be expanded and maintained for nearly 60 population doublings, during which they retain their spindle-shaped morphology and high proliferation rate. TGPC express the MSC-associated markers STRO-1 and CD45 and demonstrate a tendency for pluripotency-associated gene expression (*nanog*, *oct4*, *sox2*, *klf4*, and *c-myc*), indicating a mesenchymal phenotype.^{49,50} TGPC show a similar multilineage differentiation capacity to that of other dental MSCs, including the ability to differentiate into adipocytes, osteoblasts/odontoblasts, chondrocytes, and neurons. The hydroxyapatite (HA)/TGPC implants showed new bone formation in the presence of osteocytes in the newly formed bone matrix and a cuboid-shaped active osteoblast lining on the matrix surface. TGPCs can differentiate into cells with the morphological, phenotypic, and functional characteristics of hepatocytes in vitro suggesting that TGPC can be used to treat liver diseases.^{51,52}

GMSC

The gingiva is a unique oral tissue overlaying the alveolar ridges and retromolar region that is recognized as a biological mucosal barrier and a distinct component of the oral mucosal immunity. GMSC can be obtained from gingival tissue that are easily accessible from the oral cavity with minimal discomfort.⁵³ GMSC exhibit clonogenicity, self-renewal, and multipotent differentiation capacity, and these cells possess both stem cell-like- and immunomodulatory properties.^{14,54} GMSCs express CD45 and display positive signals for Oct4, Sox2, Nanog, Nestin, SSEA-4, and Stro-1.^{55,56} Gingival tissues also exhibit scarless wound-healing properties and a regenerative capability with rapid constitution of the tissue architecture. Interestingly, GMSCs display stable phenotype and telomerase activity in long-term cultures and are not tumorigenic.⁵⁷ Notably, GMSCs have demonstrated the capacity for self-renewal and the formation of connective tissue-like structures in vivo.¹⁴ GMSCs maintained the multilineage differentiation potential in the capacity to form mineral, fat, and cartilage-like matrix in vitro, compared with bulk-cultured growth factors. Studies also demonstrated that GMSCs possessed osteogenic potential in

vivo after incubation under osteoinductive medium in vitro.⁵⁶ These properties indicate that the clinical use of GMSCs is an attractive therapeutic option for tissue regeneration and repair.

Therapeutic potential of DSCs

The current understanding of the potential therapeutic applications of DSCs in various systems and diseases is summarized in Table 1.

The various cell types derived from DSCs are shown in Figure 2.

Neurological disorders

DPSCs originate from the cranial neural crest and have neural characteristics such as the expression of neurotrophins.⁸¹ These diffusible peptides, secreted by neurons and neuron-supporting cells, serve as growth factors for the development, maintenance, repair, and survival of specific neuronal populations: in particular, brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and glial-cell-derived neurotrophic factor (GDNF) produced by DPSCs have been shown to have crucial influence over neurons in the central nervous system (CNS), such as motor neurons and dopaminergic neurons of the substantia nigra.⁸² DPSCs are capable of influencing endogenous recruitment of neural stem cells and generated neurospheres.⁸³ Therefore, DPSCs may represent a promising source in cell therapy for neurological disorders.⁸⁴

The applications of DPSCs and SHEDs in SCI models have shown that the microenvironment of transplanted stem cells affects their capacity for differentiation; an injured spinal cord contains high levels of pro-inflammatory mediators which may activate the oligodendrocyte-specific differentiation cascade.^{15,85} Transplantation of hDPSCs into the completely transected adult rat spinal cord resulted in marked recovery of hind-limb locomotor functions. The hDPSCs exhibited neuroregenerative activities.¹⁵

The occlusion of a cerebral artery leads to ischemia in a restricted region of the CNS leading to stroke. Transplanting differentiated neural stem cells isolated from dental pulp improved motor disability and reduced infarct volume.⁸⁶ Therapeutic translation studies of DPSCs to stroke treatment in a rodent cerebral ischemic model showed promising observations.^{64,87,88} Transplantation of porcine CD31~/CD146~ side population (SP) cells accelerated neovascularization of the ischemic zone and enhanced neuronal regeneration.⁸⁷ Sugiyama et al.⁸⁸ transplanted porcine CD31~/CD146~ SP cells migrated to the peri-infarct area, released neurotrophic factors, and promoted migration and differentiation of neural progenitor cells in the subventricular zone. The intracerebral transplantation of hDPSC after focal cerebral ischemia in a rodent model resulted in

Table 1. Therapeutic potential of dental stem cells—systemic diseases.

No.	Author	System involved	Type of DSC	Outcome
1	Young et al. ⁵⁸	Neural system	DPSC	DPSC clones with high-nestin mRNA expression levels were found to successfully differentiate into Map2 and NF-positive neuronal-like cells.
2	Winderlich et al. ⁵⁹	Neural system	DPSC	Adult human dental pulp stem cells express vascular endothelial growth factor- α that increases permeability across an in-vitro model of the blood–brain barrier.
3	Mita et al. ⁶⁰	Neural system	SHED	Intranasal administration of SHED in mice resulted in substantially improved cognitive function in Alzheimer's disease through factors that improved neuroprotection, axonal elongation, neurotransmission, the suppression of inflammation, and microglial regulation.
4	Shimojima et al. ⁶¹	Neural system	SHED	EAE, a mouse model of multiple sclerosis, treated with a single injection of SHED-CM significantly improved disease scores, reduced demyelination and axonal injury, and reduced inflammatory cell infiltration and pro-inflammatory cytokine expression in the spinal cord.
5	Yang et al. ⁶²	Neural system	DPSC	Adult neuronal stem cells may be procured from third molars, and these cultured cells have potential for treatment of stroke-inflicted rats
6	Inoue et al. ⁶³	Neural system	SHED, DPSC	SHED-CM promoted the migration and differentiation of endogenous neural progenitor cells, induced vasculogenesis, and ameliorated ischemic brain injury after pMCAO, as well as transplantation of DPSC.
7	Leong et al. ⁶⁴	Neural system	DPSC	Intracerebral transplantation of human DPSC resulted in enhanced post-stroke functional recovery through non-neural replacement mechanisms.
8	Yamgata et al. ⁶⁵	Neural system	SHED	SHED transplantation into the hypoxia ischemic–injured brain resulted in remarkable neurological and pathophysiological recovery in mice.
9	Mead et al. ⁶⁶	Optic system	DPSC	Intravitreal transplants of DPSCs promoted significant neurotrophin-mediated retinal ganglion cell survival and axon regeneration after optic nerve injury in mice.
10	Gomes et al. ⁶⁷	Optic system	DPSC	Tissue-engineered DPSC sheet was successful for the reconstruction of corneal epithelium in an animal model of total LSCD.
11	Syed-Picard et al. ⁶⁸	Optic system	DPSC	DPSCs produced corneal stromal extracellular matrix without affecting corneal transparency or inducing immunological rejection when injected into mouse corneal stroma.
12	Yamaguchi et al. ¹⁷	Cardiac system	SHED	SHED-CM on myocardial injury in a mouse model of I/R decreased apoptosis and inflammatory cytokine levels, such as TNF- α , IL-6, and IL- β , thereby protecting the heart from acute ischemic injury.
13	Gandia et al. ⁶⁹	Cardiac system	DPSC	Evidence of cardiac repair was noted by improved cardiac function, increase in the number of vessels, and a reduction in infarct size when DPSC expanded ex vivo was injected in a mice model.
14	Iohara et al. ⁷⁰	Angiogenesis	DPSC	In models of mouse hind limb ischemia, local transplantation of this of side population derived from DPSC resulted in successful engraftment and an increase in the blood flow including high density of capillary formation.
15	Yang et al. ⁷¹	Muscular system	DPSC	Clones of DPSC aids in muscle regeneration by expressing human dystrophin and myosin heavy chain.
16	Tasli et al. ⁷²	Muscular system	TGPC	Pluronic F68 increases the myogenic and neurogenic differentiation of TGPC, useful in functional skeletal and neural tissue engineering applications.
17	Cho et al. ⁷³	Hepatic system	DPSC	Melatonin and DPSC when grafted in a liver cirrhosis mouse model significantly suppressed liver fibrosis and restored ALT, AST, and ammonia levels were observed
18	Ishikawa et al. ⁷⁴	Autoimmune diseases	SHED	Single intravenous administration of serum-free CM from human deciduous dental pulp stem cells (SHED-CM) into anti-CAIA, a mouse model of RA, markedly improved the arthritis symptoms and joint destruction.
19	Wakayama et al. ⁷⁵	Respiratory system	SHED	A single intravenous administration of either SHED or SHED-CM attenuated the lung injury and weight loss in BLM-treated mice that exhibit several pathogenic features associated with the human disease acute respiratory distress syndrome and improved their survival rate.
20	Kanafi et al. ⁷⁶	Endocrine	SHED	ICC derived from SHED reverse STZ diabetes in mice without immunosuppression.
21	Kanafi et al. ⁷⁷	Endocrine	DPSC and SHED	Observed a reversal of hyperglycemia to the normal level in the experimental diabetic mice.
22	Govindswamy et al. ⁷⁸	Endocrine	DPSC	Differentiated into pancreatic cell lineage resembling islet-like cell aggregates, glucose-dependently released insulin, and C-peptide.
23	Annibali et al. ⁷⁹	Bone	DPSC	Enhanced increase in the bone mineral density critical-sized bone defects in a rat calvarial critical defect model.
24	Honda et al. ⁸⁰	Bone	DFSC	Bone formation with evidence of vascular invasion similar to intramembranous ossification.

DPSC: dental pulp stem cell; SHED: stem cells from human exfoliated deciduous teeth; CM: conditioned medium; EAE: experimental autoimmune encephalomyelitis; pMCAO: permanent middle cerebral artery occlusion; LSCD: limbal stem cell deficiency; I/R: ischemia/reperfusion; TNF: tumor necrosis factor; IL: interleukin; TGPCs: tooth germ progenitor cells; CAIA: collagen type II antibody-induced arthritis; RA: rheumatoid arthritis; BLM: bleomycin; ICC: islet-like cell clusters; STZ: streptozotocin; DFSC: dental follicle stem cells.

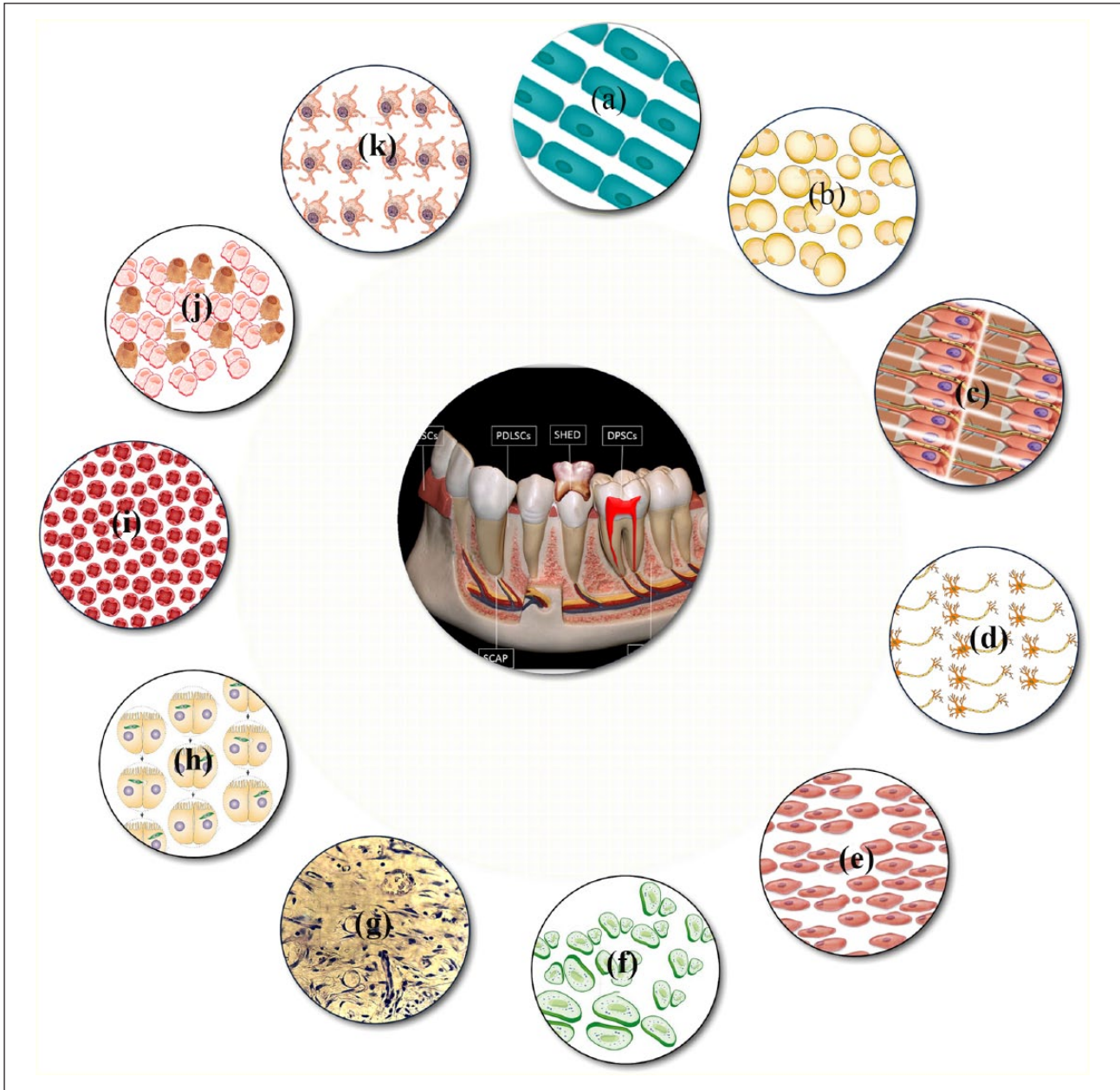


Figure 2. The figure enumerates various cell types derived from dental stem cells: (a) cementoblast, (b) adipocyte, (c) odontoblasts, (d) neuronal cells, (e) myoblast, (f) chondrocyte, (g) pulp cells, (h) hepatocyte, (i) endothelial cell, (j) osteoblast, and (k) melanocyte.

significant improvement in forelimb sensorimotor function. Improvements to function appeared to be mediated by DPSC-dependent paracrine effects.⁶⁴ The therapeutic benefit of implanted rat DPSCs into the vitreous body of the eye after a surgically induced optic nerve crush injury promoted neurotrophin-mediated survival of rat ganglion cells and axon regeneration.⁶⁶

Parkinson's disease is a neurodegenerative disorder characterized by the progressive death of substantia nigra dopaminergic neurons that results in a regional loss of striatal dopamine (DA) levels. Nesti et al.⁸⁹ investigated the

neuroprotective effects of DPSC against MPP⁺ and rotenone in an *in vitro* model of Parkinson's disease, using an indirect co-culture system with mesencephalic cell cultures. They found that the co-culture with DPSCs significantly attenuated MPP⁺ or rotenone-induced toxicity probably by the neuroprotection by the soluble factors such as BDNF and NGF, released by DPSC. Hence, DPSC can be viewed as possible candidates for studies on cell-based therapy in neurodegenerative disorders.

DPSC demonstrated that dental pulp-derived cell grafting promoted survival of injured motor neurons in a rat

model of SCI.⁸² DPSC from both rats and humans produced and secreted neurotrophic factors, including NGF, BDNF, and GDNF, and these promoted the survival of sensory and dopaminergic neurons.⁹⁰ DPSC exerted a neuroprotective effect in in-vitro models of Alzheimer's and Parkinson's diseases. The ability to produce and secrete growth factors is of prime importance, since these factors may induce the differentiation of endogenous cell types into those cells required at the place of injury or elicit secretion of other neurotrophic factors from endogenous cells to improve tissue regeneration.⁸⁴ DPSC showed their applicability also at peripheral nervous system level for nerve injury treatment: they were loaded on poly(DL-lactic-co-glycolic acid) (PLGA) collagen and the scaffold was inserted in a model of facial nerve injury. The system allowed the reconnection of damaged axons (Figure 3).⁹¹

Angiogenesis and vasculogenesis

Vasculogenesis is a potential treatment for ischemic heart disease, and it is an exciting area of research in regenerative medicine. SP cells have been identified in human PDL cells and porcine dental pulp tissues.⁹² SP cells are highly enriched for stem cell activity.^{93,94} Iohara et al.⁷⁰ have shown that SP of dental pulp cells has a property of vasculogenesis. They have isolated a highly vasculogenic subfraction of SP cells from dental pulp which are positive for CD31 and CD146 genes. Thus, they have further suggested that these SP cells are a new source of stem cells which stimulate angiogenesis/vasculogenesis in tissue and can be used in cell-based treatment of ischemic heart diseases.⁷⁰ Under steady condition, EphB/ephrin-B molecule restricts DPSCs cells for their attachment, migration, and to maintain within their stem cell niche and thus contributing to the localization and maintenance of DPSCs within adult human teeth. Following injury, the mobilization of DPSC to the dentin surfaces may be mediated by EphB/ephrin-B interactions within the adult dental pulp tissue. Therefore, this result suggests a role of EphB/ephrin-B molecule in dental pulp development and regeneration.⁹⁵

Liver disease

Liver cirrhosis, an irreversible fibrotic change to the liver, can lead to serious consequences such as impaired liver function, portal hypertension, and hepatocellular carcinoma. Liver transplantation is still the only treatment option to prevent more severe clinical course resulting from cirrhosis. Cell-based therapies have drawn attention as novel therapeutic alternatives to whole organ allograft⁹⁶ SHED are feasible cell source for MSC-based therapy for both pediatric and adult patients with liver dysfunction.⁹⁷ Stem cells from third molars were differentiated into hepatocytes in cell culture, and in an animal model of liver disease, they prevented liver fibrosis and increased levels

of albumin and bilirubin.^{13,73} Cho et al.⁷³ demonstrated that melatonin promotes hepatic differentiation of hDPSC by modulating the BMP, p38, ERK, and NF- κ B pathways. Hence, they concluded that the combined treatment of grafted hDPSCs and melatonin could be a viable approach for the treatment of liver cirrhosis.

Diabetes mellitus

In diabetes, the autoimmune destruction of pancreatic β -cells or decreased sensitivity to insulin develops persistent hyperglycemia. The use of differentiated stem cells or islet transplantation for replenishing the lost insulin-producing cells could be an alternative approach to the conventional insulin-based therapy for diabetes.⁹⁸ The potential of DPSC to differentiate into pancreatic cell lineage resembling islet-like cell aggregates was reported.⁷⁸ Carnevale et al.⁹⁹ reported that hDPSCs under appropriate stimuli express genes related to pancreatic β -cell development and function, including insulin, pancreatic, and duodenal homeobox-1. Kanafi et al.⁷⁶ demonstrated the transplantation of islet-like cell clusters (ICCs) derived from hDPSC and SHED in diabetic mice. They noticed the reversal of hyperglycemia to the normal level in experimental diabetic mice. These observations suggested the use of dental pulp as an autologous stem cell therapy in diabetic patients.^{76,100}

Regenerative ocular therapy

DSC, as an autologous stem cell source, has been successfully tested in corneal blindness. Since both cornea and DPSC share similar embryonic origin, DPSC differentiated effectively into keratocytes in vitro to generate a tissue-engineered corneal stromal-like tissue construct and to function as keratocytes in vivo without eliciting overt rejection.¹⁰¹ DPSC cultured on aligned nanofiber substrate-generated tissue-engineered, corneal stromal-like constructs, recaptulated the tightly packed, aligned, parallel fibrillar collagen of native stromal tissue in mouse corneal stroma. These findings demonstrate a potential for the clinical application of DPSC in cellular or tissue engineering therapies for corneal stromal blindness.⁶⁸ Studies have shown promising results using stem cells from exfoliated deciduous teeth (SHED) for corneal epithelium regeneration.^{67,102} SHED express markers similar to those of corneal limbal stem cells, and the delivery of cell sheets composed of SHED with and without the addition of amniotic membrane resulted in the regeneration of the corneal epithelium in total limbal stem cell deficiency rabbit models. Huang et al.¹⁰³ reported that the inhibition of Wnt and bone morphogenetic protein signaling induced retinal cell differentiation from stem cells isolated from the PDL. Adult dental pulp cells isolated from third molars has the capability to differentiate into keratocytes, cells of the

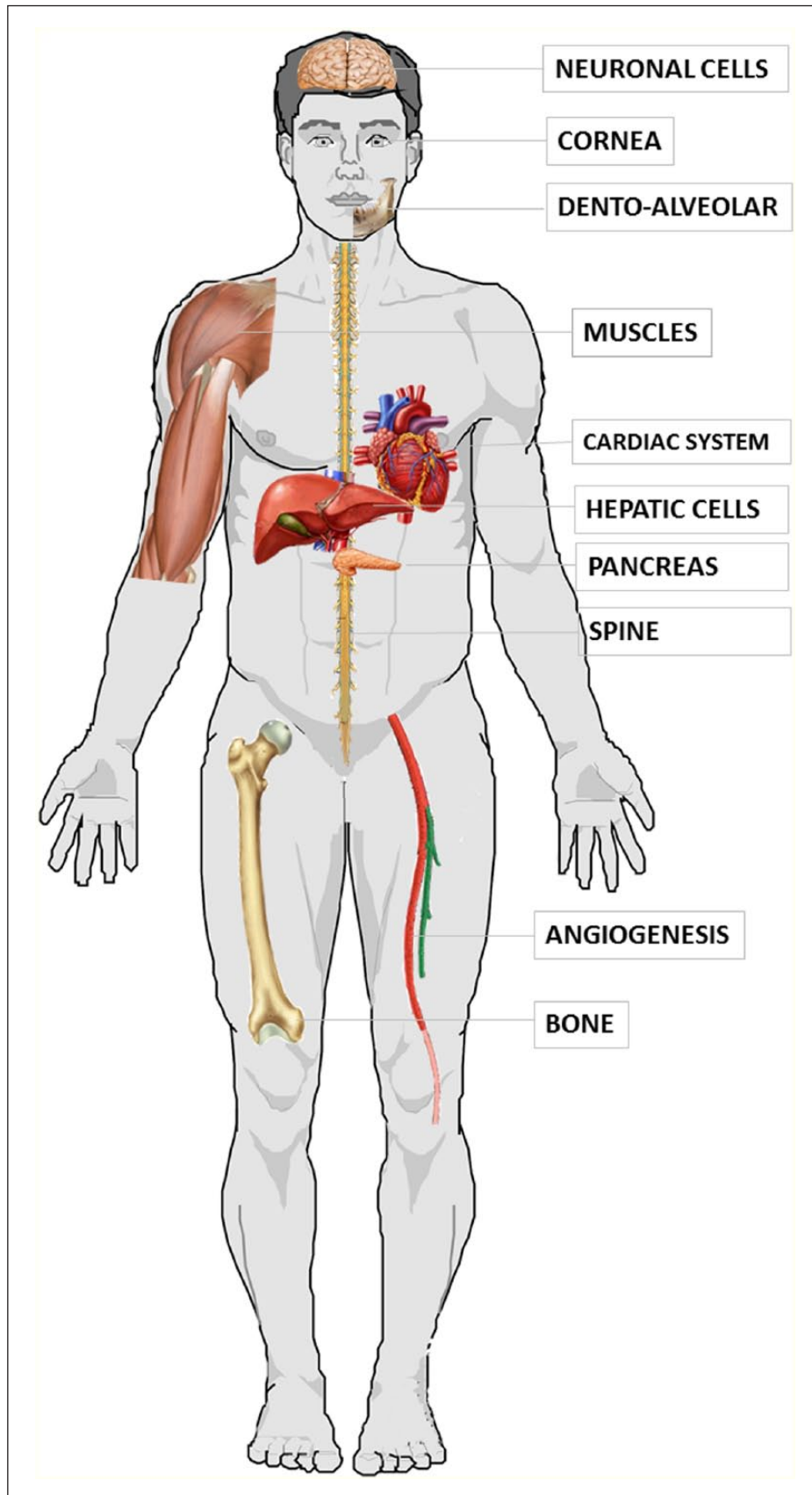


Figure 3. Multilineage differentiation capacity, tissue regeneration, and potential clinical applications of human dental stem cells.

Table 2. Therapeutic potential of dental stem cells in dentistry.

No.	Author	Type of DSC	Oral site	Outcome
1	Yu et al. ¹¹¹	GMSCs	Periodontal regeneration	GMSCs significantly enhanced the regeneration of the damaged periodontal tissue, including the alveolar bone, cementum, and functional PDL.
2	Li et al. ¹¹²	DPSCs	Periodontal regeneration	DPSCs from inflammatory site had a positive effect on regeneration of new bones to repair periodontal defects.
3	Zhu et al. ¹¹³	PDLSC + JBMSC	Periodontal regeneration	DLSCs and JBMSCs regenerated periodontal ligament-like fibers and mineralized matrix on the Ti scaffold surface, both in nude mice ectopic and minipig orthotropic transplantations.
4	Nagata et al. ¹¹⁴	PDLSC	Periodontal regeneration	PDLSC-CM enhanced periodontal regeneration by suppressing the inflammatory response via TNF- α production in mice model.
5	Lucaciu et al. ¹⁰⁷	DFSC	Osseointegration	Dental follicle stem cells have a spontaneous tendency for osteogenic differentiation and can be used for improving bone regeneration on titanium implant surfaces.
7	Zhang et al. ¹¹⁵	GMSCs	Oral mucositis	Preconditioned GMSCs enhance mitigation of oral mucositis.
8	Gao et al. ¹¹⁶	GMSCs	Odontogenic regeneration	GMSCs showed enhanced odontogenic differentiation potential when induced with embryonic tooth germ cell-CM.

DSC: dental stem cell; GMSC: gingival mesenchymal stem cell; DPSC: dental pulp stem cell; JBMSC: jaw bone mesenchymal stem cell; PDLSC: periodontal ligament stem cell; PDL: periodontal ligament; CM: conditioned medium; TNF: tumor necrosis factor.

corneal stoma. After inducing differentiation in vitro, DPSC expressed molecules' characteristic of keratocytes, keratocan, and keratan sulfate proteoglycans at both the gene and the protein levels. Intravitreal transplants of DPSC promoted significant neurotrophin-mediated retinal ganglion cell survival and axon regeneration after optic nerve injury in mice.⁶⁶

Bone tissue engineering

DPSCs showed differentiation profiles similar to those shown during bone differentiation, and this event make them very interesting as a model to study the osteogenesis and the relationship with scaffolds.^{104,105} The osteogenic differentiation capacity of DPSC has been well demonstrated in vitro and in vivo, with strong ALP results¹⁰⁶ and expression of bone-specific markers within newly formed bone.^{6,106} Enhanced mineralization, protein secretion, and an upregulated osteo-related gene profile resulted from immobilization and, interestingly, immobilization triggered osteogenic differentiation of DPSC without the use of induction factors in the medium.⁷⁷

A tendency to increase the bone mineral density was observed when DPSCs were implanted in the granular deproteinized bovine bone (GDPB) scaffold.⁷⁹ Rat DPSCs were utilized in a rat calvarial critical defect model in conjunction with a GDPB or beta tricalcium phosphate (β -TCP) scaffold. GDPB bone scaffolds with DPSC showed the potential to ameliorate bone regeneration process in the

reconstruction of the calvarial defects.⁷⁹ Lucaciu et al.¹⁰⁷ used DFSCs from impacted teeth to improve bone regeneration on titanium implants surfaces. They observed spontaneous tendency for osteogenic differentiation and concluded that DFSCs could be used for improving bone regeneration on titanium implant surfaces. Maraldi et al.¹⁰⁸ also utilized DPSCs in a rat calvarial critical-sized defect model. hDPSC-seeded collagen sponges showed almost complete bridging of the defect by 8 weeks. Regulation of DPSC differentiation is crucial for clinical use in cell therapies and regenerative medicine. The topographical design of biomaterials may be optimized to achieve this. The interaction of surface topographical parameters upon attachment, morphology, proliferation, and osteogenic differentiation of DPSCs with alterations in pillar topography revealed enhanced mineralization.¹⁰⁹ The observations from the in vivo and in vitro studies showed that the addition of DPSC to scaffolds offers a great potential for clinical application in bone reconstruction.¹¹⁰

Therapeutic applications in dentistry

DSCs can be used in the repair of damaged dentin, pulp revascularization and regeneration, and for periodontal disorders (Table 2). The combination of DSCs with novel scaffolding materials might enable us to reach the goal of engineering oral tissues in the near future. Understanding the molecular mechanisms of tooth development and repair, utilizing emerging technologies in tissue engineering and

biomaterials, and exploiting the potential of DSCs to form complex dental tissues are the foundations of regenerative dentistry. In tissue engineering, whole tooth regeneration, a current dental regenerative process under progress would reduce the difficulties associated with the presently offered dental treatments such as prosthesis, implants, and tooth transplantation.¹¹⁷

Regenerative endodontic therapy

The current treatment modality of the infected root pulp is the removal of necrotic pulp tissue and replacement with bioinert cements to obturate the root canals. Although the root canal treatment is effective in combating infection, it does not restore lost dental pulp tissue and the vitality of the tooth. The use of DPSC to regenerate healthy pulp tissue represents a simple and potentially very effective biological treatment. DPSC can be easily expanded *in vitro* and have been shown to reconstitute a pulp-like tissue *ex vivo* and *in vivo*.^{8,48,118–120}

Complete pulp regeneration with neurogenesis and vasculogenesis occurred in an adult canine model with pulpectomy and with autogenous transplantation of pulp CD105 + SP cells with stromal cell-derived factor-1 (SDF-1).¹²¹ Another preclinical trial using autologous “mobilized” DPSCs to the pulpectomized teeth of dogs showed regeneration of pulp tissue with no adverse effects and the treated teeth showed recovery of pulp.¹²² Rosa et al.¹²³ found that SHED survive and differentiate into odontoblasts when transplanted into full-length human root canals with injectable scaffolds. The pulp tissue generated under these experimental conditions contains functional odontoblasts capable of regenerating tubular dentin. Future regenerative endodontic protocol could be a combination of disinfection or debridement of infected root canal systems along with the use of stem cells, scaffolds, and growth factors to permit the revascularization of this pulp. The outcomes of the ongoing studies suggest that it might be feasible to restore viability in a necrotic young permanent tooth by engineering a new dental pulp. The potential impact of such therapy is immense and may allow for the completion and reinforcement of the tooth structure by biological regeneration in near future.¹²⁴

Dentin regeneration

Regenerative property of the pulp-dentin complex mainly depends on the formation of tertiary dentin, reactionary dentin, and reparative dentin. DPSCs, a unique group of cells with clonogenic ability, high reproductive activity, and multiple differentiation potentials, are extremely crucial element for tertiary dentinogenesis.^{7,125–127} The DPSCs migrate, proliferate, and differentiate into odontoblasts, which then synthesize matrix to form the tertiary dentin at the damaged sites.¹²⁸ There are two different approaches

implemented in regeneration of dentin by the use of tissue engineering techniques.^{129,130} The first approach includes a device which can be used as a filling material into a deep cavity of tooth with partial layer of dentin on top of the pulp. In this process, they used some growth factors or molecules that can form reparative dentin.^{131,132} The second approach is to put scaffold on open pulp along with odontoblast-like cells to grow on it.¹³³ These cells will synthesis reparative dentin. DPSCs have been cultured on a variety of scaffolds to engineer dentin tissues.^{134,135} However, the signaling pathways underlying the regulation of DPSCs in dentin regeneration remains largely unknown, limiting their effective application in dentin tissue engineering.

Regenerative periodontal therapy

The high prevalence of periodontal disease, the limited regenerative activity exhibited by the PDL, and the critical role of the PDL in maintaining tooth health and function have given more focus to the research on PDL tissue engineering. The challenges in regenerative periodontal therapy lie in the ability to induce the regeneration of a complex apparatus composed of different tissues, such as bone, cementum, and PDL. Despite the recent advancements in periodontal therapy, a complete regeneration of the damaged periodontium is still unattainable. Research findings established that the cells derived from the PDL can differentiate into osteoblasts or cementoblasts to contribute to periodontal regeneration.¹³⁶ The multipotent PDLSCs were first isolated from the PDL of extracted teeth. These cells express MSC markers, such as STRO-1 and MCAM (CD146), and were shown to be clonogenic and able to differentiate into adipocytes, osteoblasts, and cementoblast-like cells both *in vitro* and *in vivo*.^{9,137,138}

The multipotent differentiation properties of PDLSC for generating both hard and soft tissues were further demonstrated by constructing multilayered cell sheets supported by woven polyglycolic acid. Transplanted cell-seeded polyglycolic acid sheets regenerated new bone, cementum, and well-oriented collagen fibers when inserted into root surfaces. Efforts are under way to find an appropriate delivery system for engineering an efficient cell-based therapeutic tool for periodontal tissue regeneration.¹³⁹ Flores et al.¹⁴⁰ have found that fibrin gels carrying several layers of PDL cells can be successfully used as a delivery system for these cells. Collagen sponge scaffolds seeded with PDL cells were successfully tested for the regeneration of periodontal fenestration defects in beagle dogs.¹⁴¹ Another delivery system for PDLSC based on a combination of bovine bone with human dentin was shown to be effective because these stem cells formed a cementum-like complex in subcutaneous dorsum pockets of immunocompromised mice.¹⁴² Successful therapies for PDL tissue regeneration will not only facilitate the treatment of periodontal diseases but may also be used to improve current dental implant therapies.

Table 3. List of licensed dental stem cell banks.

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

Bioengineered tooth

The biological replacement tooth must include generation of a root and PDL with nerve and blood supplies. The bioengineering of a tooth from non-embryonic cells, one of the cell populations, either epithelial or mesenchymal, must be able to provide the inductive signals to the other.¹⁴³ Embryonic tooth primordia have been generated in vitro from adult stem cells combined with instructive embryonic oral epithelium, adult epithelial cells combined with embryonic tooth inducing mesenchyme, or by the development of tooth germ models utilizing harvested cells from tooth buds combined with materials.^{118,144,145}

The whole tooth regeneration by tissue engineering currently uses two methods: scaffold method and cell aggregates method. In scaffold method of tooth regeneration, the stem or precursor cells are arranged in proper spatial orientation using a biodegradable polymer membrane or collagen sponge scaffolds to generate an artificial tooth germ.^{143,146} The cell aggregates method, dental epithelial tissue, and mesenchymal cell pellets are dispersed in a well-controlled culture condition to create an artificial tooth germ. The tooth germ formed in this method mimics a tooth germ of the early inductive stage of tooth development where cell-to-cell and epithelial–mesenchymal interactions are predominant.^{147–150}

Hung et al.¹⁵¹ were able to utilize DPSC to form tooth-like structures in rabbit alveolar sockets but there was no visible tooth eruption in any of the graft sites. In vivo study showed crown-like structures derived from DPSC pellet associated with adult rat apical bud cells with distinct regions of enamel, dentin, pre-dentin, and both ameloblast and odontoblast layers.¹⁵² The induced pluripotent stem cells (IPSCs) could generate dental epithelial cells, with embryonic-like properties, that could be recombined with the autologous DPSC and transplanted to form a tooth. The use of IPSCs from DPSCs may indeed provide a future solution to this problem.¹⁵³ The bioengineered tooth germ primordium, isolated from a bioengineered tooth germ, could be transplanted into a tooth cavity after the extraction of a mandibular incisor and could develop into a tooth with typical spatial orientation of structures, such as enamel, dentin,

dental pulp, root, blood vessels, PDL, and alveolar bone. These findings suggest the future possibility of successful generation of whole teeth by transplantation of bioengineered tooth germs into the adult oral environment.^{145,154}

DSC banking

Since the DSCs have various clinical benefits and are applied in various fields of regenerative medicine, the preservation of DSCs for medical applications established the concept of “tooth bank.” Once stem-cell-containing tissues, such as pulp, apical papilla, PDL, follicle, gingiva, or the tooth itself, have been obtained from the patient, they can be preserved for many years to retain their regenerative potential for use in future regenerative therapies. The DSC is stored using either cryopreservation or magnetic freezing.^{155–157}

Although tooth banking is currently not very popular, the trend is gaining acceptance mainly in the developed countries (Table 3). BioEden (Austin, Texas, USA) has international laboratories in the United Kingdom and Thailand. Stem-cell banking companies like Store-A-Tooth (Provia Laboratories, Littleton, Massachusetts, USA) and StemSave (StemSave Inc., New York, USA) are also expanding their presence internationally. The first commercial tooth bank was established as a venture company at Hiroshima University and the company was named as “Three Brackets.”¹⁵⁸ Nagoya University (Kyodo, Japan) also came up with a tooth bank in 2007. Taipei Medical University in collaboration with Hiroshima University opened the nation’s first tooth bank in September 2008. The Norwegian Tooth Bank (a collaborative project between the Norwegian Institute of Public Health and the University of Bergen) set up in 2008 is collecting exfoliated primary teeth from 1,00,000 children in Norway. Stemade Biotech introduced the concept of DSC banking in India.

Potential limitations and challenges

Stem cell therapy represents a fascinating new approach for the repair of defective tissues or functions through the transplantation of live cells. However, multiple key

parameters need to be optimized through clinical research such as the required stem cell density and availability, as well as appropriate strategies, for their use. Another issue is the availability of the cells over time, that is, DPSCs or exfoliated deciduous tooth stem cells are not available throughout a patient's lifetime. Even though DSC banking may constitute a potential solution by cryopreserving them for future use, such a possibility is not only time-consuming and costly but limits their use in clinical applications.¹⁵⁹ Culture conditions, dose of cell infusion, number of infusions, and route of cell delivery need to be optimized. Appropriate double-blind randomized clinical trials are still need to be performed to confirm the true regenerative power of these stem cells. The immune rejection is a major risk for cell transplantation, so biosecurity is a crucial point for cell therapy, requiring control of cell transformation and a protocol for cellular biobanking. The risk of transmission of bacterial, viral, and fungal or prion pathogens may lead to life-threatening reactions.¹⁶⁰ Manufacturing of cell-based medicinal products inevitably does not include terminal sterilization, purification, viral removal, and inactivation. Hence, viral and microbial safety is a pivotal risk factor associated with the use of non-autologous cells including stem cells. Further research and understanding of the stem cell physiology may enhance development of novel and more competent therapeutic approaches and will help in fulfilling the huge impact that stem cell therapy will have for future healthcare.

Conclusion

Stem cell research has expanded at an exponential rate, though its therapeutic applications progress at a slower phase. Stem cell therapy has a promising future in tissue regeneration and the management of disease. It allows the repair of defective tissues or functions through the transplantation of autologous cells. Globally, the advances using stem cells in therapeutic, reconstructive, orthopedic, and cosmetic applications are the future of personalized and regenerative medicine. However, because stem cell technology is still in its infancy, interdisciplinary cooperation is needed to achieve successful clinical applications. DSCs have drawn attention in recent years because of their accessibility, plasticity, and high proliferative ability. Several types of DSCs have been identified, including DPSCs from adult human dental pulp, stem cells from human primary exfoliated deciduous teeth, PDLSCs, and DFSCs from human third molars. The DSCs can undergo self-renewal and have multipotent differentiation ability, but do not have the ethical issues associated with other sources of stem cells. The tissue engineering methodologies combined with an increased understanding of DSC biology will provide powerful tools for a wider spectrum of application of DSC in various therapeutic strategies. We anticipate that the next decade will bring great advances in stem cell and tissue engineering therapies.

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