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Review Article

Dental stem cells and their therapeutic role in oral tissue regeneration

Ashwini Savia Colaco*

Department of Conservative Dentistry and Endodontics,
A.J. Institute of Dental Sciences, Mangalore, 575004 India

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Abstract

A new direction in the field of tissue engineering aims to regenerate a functional tooth-tissue structure. The emerging scientific field combines the principles of biology, medicine, and engineering to repair and regenerate damaged tissue. It is a multidisciplinary approach that involves three basic key elements: stem cells, morphogens, and scaffolds. The dental tissues are considered a rich source of mesenchymal stem cells that are suitable for tissue engineering applications. It is known that these stem cells have the potential to differentiate into several cell types, including odontoblasts, neural progenitors, osteoblasts, chondrocytes, and adipocytes. Dental stem cell research has grown exponentially. Hence this article reviews and discusses the basic concepts, biology, and applied clinical applications of dental stem cells.

Keywords: mesenchymal stem cells, dental stem cells, molecular markers, regenerative dentistry

1. Introduction

The discovery and characterization of multipotent mesenchymal stem cells have played a vital role in the field of tissue regeneration. Tissue engineering consists of a triad of dental pulp progenitor/stem cells, morphogens, and scaffolds (Nakashima & Reddi, 2003). Stem cells are generally defined as clonogenic cells capable of both self-renewal and multi-lineage differentiation. The primary role of adult stem cells is to maintain and repair the tissues that are damaged by disease or injury. Stem cells may remain quiescent (non-dividing) for long periods of time until they are activated by a physiological need. These cells are thought to reside in specific areas termed stem cell niches (Scadden, 2006). Dental-tissue derived stem cells are among the many other stem cells that have been isolated and characterized. The other adult stem cells include cells from the brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, heart, gut, liver, ovarian epithelium, and testis (Bissels, Eckardt, & Bosio, 2013).

*Corresponding author
Email address: ashfern16@yahoo.co.in

2. Significance of Various Stem Cells and Their Role in Tissue Regeneration

The first type of dental stem cell to be isolated from the human pulp tissue was called 'postnatal dental pulp stem cells' (Gronthos, Mankani, Brahim, Robey, & Shi, 2000). This was followed by stem cells isolated from human exfoliated deciduous teeth (SHED) (Miura *et al.*, 2003), periodontal ligament stem cells (PDLSCs) (Seo *et al.*, 2004), and stem cells from apical papilla (SCAP) (Sonoyama *et al.*, 2008). Recent studies have identified newer dental-tissue-derived progenitor cell populations referred to as 'dental follicle pre-cursor cells' (DFPCs) (Morsczeck *et al.*, 2005) and induced pluripotent stem (iPS) cells (Takahashi, Okita, Nakagawa, & Yamanaka, 2007). These cells have the potential to give rise to multi-lineage differentiation and hence have potential applications in dentistry. Dental tissue engineering is a novel technique to restore teeth that are lost due to pathology, traumatic injuries, and congenital deformities. Replacements of missing teeth using prostheses are based on external materials. Dental caries can cause loss of the outer mineralized enamel layer and the adjacent dentin layer. Further progress of caries may affect the pulp causing loss of vitality.

Dental stem cells have been shown *in vitro* and *in vivo* to reconstitute enamel dentin as well as pulp. Insertion of

dental pulp stem cells (DPSCs) and SCAP into the canal space promoted regeneration of pulp-like tissue and mineralized dentine (Huang *et al.*, 2010). SCAP cells are also effective in regeneration of dentin (Wang *et al.*, 2016). Studies have shown that autologous DPSCs treated with growth factors and implanted into the space of a partially amputated pulp chamber were able to stimulate reparative dentin (Atalayin *et al.*, 2016). Thus stem cell-based tissue engineering not only restores the lost tooth but also reinstates tooth vitality.

Stem cell application is also a favorable method to repair root perforations, resorbed roots, promote periodontal regeneration, bone reinforcement, and whole tooth regeneration (Mitsiadis & Orsini, 2016). The periodontal apparatus lost due to degenerative diseases like periodontal diseases and necrosis of the mandible can be restored by implantation of a bio-complex of stem cells and various scaffolds (Bassir *et al.*, 2016).

Studies also revealed that PDLSC (Menicanin *et al.*, 2014), SCAP (Yang *et al.*, 2009), DPSC (Otaki *et al.*, 2007), and DFPC (Volponi, Pang, & Sharpe, 2010) resulted in the formation of cementum, periodontal ligament, and bone thus providing an innovative tool for the development of reconstructive treatments.

De novo tooth construction with an appropriate hardness necessary for chewing (measured by the Knoop hardness test) and responding to noxious stimuli, such as mechanical stress and pain challenge with various thermal and electrical stimuli has also been demonstrated using stem cells (Ikeda *et al.*, 2009). The whole tooth is regenerated by the scaffold and cell aggregate methods. In the scaffold method an artificial tooth germ is generated using stem cells and suitable scaffolds. While the cell aggregates method is comprised of a dispersion of epithelial tissue and mesenchymal cell pellets in a well-controlled microenvironment to create an artificial tooth germ. Remarkably, the advent of iPS cells has provided a good alternative source for implantable tooth germs and whole tooth regeneration. Stem cell application encourages rapid healing of oral ulcers and wounds. The use of gene-transfer methods to manipulate salivary proteins and oral microbial colonization patterns are also a promising approach (Bansal & Jain, 2015).

Currently dental stem cells are considered due to their numerous benefits such as easy surgical access and the simple painless procedure. The cells have low morbidity, can be safely cryopreserved, and can be combined with various scaffolds (Rai, Kaur, & Kaur, 2013). They possess immuno-privilege and are not subjected to the same ethical concerns as embryonic stem cells. They are highly proliferative and possess multi-differentiation potential, thus providing an autologous transplant to generate tissues within a short period. Another advantage is that stem cell banking is a reasonable and simple alternative to harvesting stem cells (Aly, 2015)

2.1 Dental pulp stem cells (DPSCs)

DPSCs are considered similar to bone marrow stem cells. They can be easily isolated from teeth and exhibit pluripotent mesenchymal stem cell characteristics with self-renewal multi-differentiation properties (Gronthos *et al.*, 2002). Two basic methods for the DPSCs isolation are the explant method and the enzymatic digestion of pulp tissue method. In the explant method, the pulp tissue is surgically removed and the cells are grown from tissue fragments, whereas in the enzymatic digestion technique, the pulp is digested using collagenase and dispase, after which the cells are seeded (Karamzadeh, Eslaminejad, & Aflatoonian, 2012).

DPSCs can be either multiple-colony-derived or single-colony-derived strains. Multiple-colony-derived strains have a growth potential of approximately more than 120 population-doublings (PD) while the single-colony-derived strains proliferate to 10-20 PD. DPSCs can be grown for long periods without compromising their plasticity and ability to regenerate (Table 1). It is generally accepted that a prolonged expansion period may induce senescence and decrease their biological activities. Interestingly, it was reported that DPSCs still had a normal karyotype and doubling period up to 40 doublings after reaching the maximum number of passages before entering senescence (Ledesma-Martínez, Mendoza-Núñez, & Santiago-Osorio, 2016). The differentiation of DPSCs to a specific cell lineage is determined by the components of the local microenvironment such as growth factors, receptor molecules, signaling molecules, transcription factors, and extracellular matrix protein (Estrela, Alencar, Kitten, Vencio, & Gava, 2011).

The most striking feature of DPSCs is their ability to regenerate a dentin-pulp-like complex. These cells express dentin sialophosphoprotein (DSPP) which helps in regeneration of mineralized matrix with tubules lined with odontoblasts (Gronthos, Mankani, Brahim, Robey, & Shi, 2000). They also express high levels of the alkaline phosphatase (ALP) gene and dentin matrix protein 1 (DMP-1) (Yamada, Fujimoto, Ito, Yoshimi, & Ueda, 2006). DMP is a non-collagen extracellular matrix protein that can significantly promote the odontoblastic differentiation of DPSC and formation of reparative dentin over the exposed pulp tissue (Almushayt, Narayanan, Zaki, & George, 2006). DPSC can be reprogrammed into multiple cell lineages, such as odontoblast phenotype, characterized by polarized cell bodies and accumulation of mineralized nodules (About *et al.*, 2000), osteoblasts, chondrocytes, myocytes, neurocytes, adipocytes, corneal epithelial cells, melanoma cells, and even iPS cells (Stevens *et al.*, 2008; Yan *et al.*, 2010). This subpopulation provides for a versatile use of these cells in regenerative medicine.

2.2 Stem cells from human exfoliated deciduous teeth (SHED)

SHED cells seeded onto synthetic scaffolds and seated into the pulp chamber space formed odontoblast-like cells which were directly associated with a dentin-like structure (Cordeiro *et al.*, 2008). They expressed putative markers of odontoblastic differentiation such as DSPP and DMP-1 and matrix extracellular phosphoglycoprotein (MEPE) (Rosa, Zhang, Grande, & Nör, 2013). The regenerated dentin expressed DSPP. When these cells were transplanted into immunocompromised mice, they resulted in human-specific odontoblast-like cells (Miura *et al.*, 2003). SHED proliferated faster with greater PDs than DPSCs and bone-marrow-derived mesenchymal stem cells. Another striking feature of SHED is the osteo-inductive capacity. They are capable of forming bone by inducing recipient murine cells to differentiate into an osteoinductive template. Hence SHED were used to repair critical calvarial defects with substantial bone formation (Seo

Table 1. Characteristics of dental stem cells and their clinical implication.

Dental stem cell	Cell source	PD	Tissue formation & Proliferation rate and	differentiation capacity	Clinical implication
DPSC dental pulp stem cells (Ledesma-Martínez, 2016)	Pulp of adult teeth	>120	Moderate	dentinogenic lineage, mesodermal lineage ectodermal lineage	Dentine-pulp regeneration, PDL regeneration, nonoral tissue regeneration
SHED stem cells isolated from human exfoliated deciduous teeth (Miura, 2003)	Pulp of deciduous teeth	>140	High	dentinogenic lineage, mesodermal lineage ectodermal lineage	Dentine-pulp regeneration, bone regeneration, neural tissue regeneration, and nonoral tissue regeneration
PDLSC periodontal ligament stem cells (Menicanin, 2014)	Periodontal ligament	not determined	High	dentinogenic lineage, mesodermal lineage ectodermal lineage	Periodontal regeneration
SCAP stem cells from apical papilla (Huang, 2008)	Apical papilla of developing root	>70	High	mesodermal lineage	Dentine regeneration bone regeneration, continued root formation, bioroot engineering
DFPCs dental follicle precursor cells (Aly, 2015)	Dental follicles of developing tooth	not determined	High	dentinogenic lineage, mesodermal lineage ectodermal lineage	Tooth root regeneration
iPS induced pluripotent stem cells (Malhotra, 2016)	human somatic cells	not determined	High	ectoderm lineage endoderm lineage mesoderm lineage	Whole tooth regeneration

et al., 2008). SHED are also capable of neurogenesis due to their expression of neuronal and glial cell markers such as neurofilament M, nestin, glial fibrillary acidic protein, double cortin, and neuronal nuclei. This phenomenon may be related to the neural-crest-cell origin of dental pulp (Chai *et al.*, 2000).

2.3 Stem cells from apical papilla (SCAP)

SCAP refer to the cell-rich zone lying between the apical papilla and the pulp. They form a rich source of primary odontoblasts that are responsible for the formation of root dentin (Huang *et al.*, 2008). Hertwig's epithelial root sheath along with SCAP is important for the continued root development of a tooth. SCAP display higher proliferation rates, number of population doublings, and dental tissue regeneration which can be attributed to two critical proteins, namely survivin and telomerase. In contrast to DPSC, SCAP have shown to express lower levels of DSPP, MEPE, transforming growth factor receptor II, and vascular endothelial growth factor receptor I (Bakopoulou & About, 2016)

It has been demonstrated that after transplantation of teeth, the odontoblast lineages required for continued root development can also be derived from SCAP (Sonoyama *et al.*, 2008). The highly proliferative potential of SCAP residing in the apical papilla is due to its proximity with the periapical tissues. Furthermore, collateral circulation enables them to survive during the process of pulp necrosis (Chueh & Huang, 2006). SCAP and PDLSCs are being used for bioroot engineering. A recent study demonstrated SCAP and SHED produced a more highly mineralized matrix in comparison with DPSCs but with lower crystallinity and carbonate substitution (Volponi *et al.*, 2015). The highly proliferative potential of SCAP makes this population of cells suitable for cell-based regeneration and preferentially for forming roots. They are also capable of forming odontoblast-like cells and produce dentin *in vivo* (Sonoyama *et al.*, 2006)

The discovery of SCAP may also explain a clinical phenomenon that was presented in a number of recent clinical case reports showing that apexogenesis can occur in infected immature permanent teeth with apical periodontitis or abscess. It is likely that SCAP residing in the apical papilla survived the infection due to their proximity to the periapical tissues. These tissues benefit from the collateral circulation which enables the tissues to survive during the process of pulp necrosis. Furthermore, after endodontic disinfection, these cells give rise to primary odontoblasts to complete the root formation (Chueh & Huang, 2006).

2.4 Periodontal ligament stem cells (PDLSCs)

The periodontal ligament (PDL) has been identified to contain a population of progenitor cells that are capable of differentiating along mesenchymal cell lineages. These progenitor cells maintain tissue homeostasis and regeneration of periodontal tissue. PDLSCs differentiate into cementoblasts, osteoblasts, adipocytes, and connective tissue rich in collagen (Seo *et al.*, 2004). The presence of multiple cell types within the PDL suggests that this tissue contains characteristics and differentiation potential similar to bone marrow stromal stem cells and DPSC. These cells display an array of cementoblastic and osteoblastic surface markers. PDLSCs express putative stem cell markers such as STRO-1, the best-known mesenchymal stem cell marker, and the perivascular cell marker. They also express CD44, CD90, CD105, CD166, and scleraxis, a transcription factor specific to tendon, which indicate their osteogenic, cementoblastic, and chondrogenic potential (Kadkhoda, Rafiei, Azizi, & Khoshzaban, 2016)

Implantation of SCAP and PDLSCs into sockets of the porcine jaw promoted the formation of a bio-root encircled with periodontal ligament in a short span of three months (Sonoyama *et al.*, 2006). In addition to this, PDLSCs help to repair periodontal defects and regenerate alveolar bone

heights (Liu *et al.*, 2008). All of these studies demonstrated characteristics such as multipotency, clonogenic ability, and high osteogenic, cementoblastic and chondrogenic proliferation of PDLSCs and hence can be used for regeneration.

2.5 Dental follicle precursor cells (DFPCs)

The enamel organ and the dental papilla of the developing tooth germ are surrounded by an ectomesenchymal tissue known as dental follicle. This tissue is a multipotent tissue that contains progenitor cells which have the ability to generate cementum, bone, and PDL. DFPCs have been isolated from human dental follicles of impacted third molars and show a typical fibroblast-like morphology. They show rapid attachment in tissue culture. These cells express nestin, Notch-1, collagen type I, bone sialoprotein, osteocalcin, and fibroblast growth factor receptor (Morsczeck *et al.*, 2005). They have the ability to form compact calcified nodules *in vitro* (Lin, Gronthos, & Mark Bartold, 2009).

2.6 Induced pluripotent stem (iPS) cells

The iPS cells are a new source of stem cells that have been generated from human somatic cells into a pluripotent stage (Takahashi, Okita, Nakagawa, & Yamanaka, 2007). iPS cells can differentiate into advanced derivates of all three primary germ layers. They resemble human embryonic stem cells (ESCs) but unlike ESCs, iPS cell technology can derive patient-specific stem cells allowing derivation of tissue-matched differentiation of donor cells. Hence they can be widely used in basic research, disease modeling, and regenerative medicine. This iPS technology has modernized treatment protocols in the field of dentistry by using a concept of autologous transplantation (Otsu *et al*., 2014). It was shown that re-aggregates of mouse embryonic cells can develop into a fully functional tooth with revascularization and root formation. The accelerated differentiation of iPS cells indicates a realistic future cell source for implantable tooth germs in dental practice (Cai *et al.*, 2013) and has provided great hopes in regenerative therapies for various diseases (Warren *et al.*, 2010). iPS cells have been utilized as a potential neuroprotectant for the prevention of chemotherapy-induced peripheral neuropathy (Wheeler, Wing, Delaney, Komatsu, & Dolan, 2015). This research can revolutionize the adverse effects of oral carcinoma treatments and promote extensive studies that focus on the multiple sides of iPS cells including method optimization, clinical application exploration, and the mechanisms of iPS cell reprogramming.

3. Potential Use, Challenges, and Future Perspective

Stem cell biology has become an important field in regenerative medicine (Potdar & Jethmalani, 2015). Dental stem cells promote osseous regeneration and in maxillofacial defects, due to congenital anomalies, infection, trauma, and tumor, can be effectively reconstructed. The bone-specific markers enable development, maintenance, repair, and survival of specific osteogenic cells (Yang *et al.*, 2017). It was suggested that the secretion of neurotrophic factors provoke neural regeneration. Several neurotrophic factors, like nerve growth factor, brain-derived neurotrophic factor, and glial cell line-derived neurotrophic factor, promote reconnection of damaged axons which can lead to the repair of spinal cord injury, facial nerve injury, and Alzheimer's and Parkinson's diseases (Ellis, O'Carroll, Lewis, Rychkov, & Koblar, 2014). Dental stem cells may represent a striking cell source for the treatment of stroke and myocardial infarction due to the expression of angiogenic factors such as platelet derived growth factor, vascular endothelial growth factor, and fibroblast growth factor (Yeasmin *et al.*, 2014). Cell-based therapies have drawn attention as novel treatment options for irreversible liver disease, ocular reconstruction, pancreatic β-cell development in diabetic patients, muscular dystrophy, salivary gland reconstitution, and whole tooth/organ regeneration (Chalisserry, Nam, Park, & Anil, 2017).

Despite its potential use, stem cell therapy encompasses many challenges such as clear identification, isolation, purification, and cell manipulations. A better understanding on the types of scaffold materials, delivery systems, and the influence of growth factors is essential. Biological evidence showing complete and predictable regeneration still remains an elusive clinical goal since the evidence in human clinical research is still scanty. Clinical challenges after administration include functional integration of transplanted tissues and oncogenic properties of stem cells (Feng & Lengner, 2013). Therapeutic surrogate modules using secretomes, which is a group of secreted trophic and immunomodulatory cytokines produced by stem cells, have been proposed as a safer alternative (Ranganath, Levy, Inamdar, & Karp, 2012). The concept of developing tooth banking and preservation of dental stem cells is promising. Further research in this area could prove highly beneficial to treat irreversible medical conditions.

4. Conclusions

Dental stem cells display multifactorial potential such as high proliferation rates, multi-differentiation ability, easy accessibility, high viability, and easy induction to distinct cell lineages. The discovery of stem cells has revolutionized regenerative therapies. Comprehensive research programs directed at growth and differentiation factors that accelerate and induce a natural biological regeneration will provide a niche for regenerative procedures. Rapid advancement in iPS cell technology may provide a new era of personalized medicine. The potential impact of such therapies is immense and may allow for the completion and reinforcement of the tooth structure by biological regeneration in the near future.

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