

## Dental stem cells and their role in clinical medicine and dentistry

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### ABSTRACT

Advances have been made in identifying dental stem cells and their differentiation potential. Five different types of dental stem cells have been isolated from dental soft tissues: dental pulp, apical papilla, dental follicle and periodontal ligament. The characteristic features of these cells have been explored. They express various arrays of biomarkers including those specific for mesenchymal and/or embryonic stem cells. *In vitro* and *in vivo* studies have revealed that these stem cells varied in their proliferation and differentiation potential. Recent studies have demonstrated their wide range of plasticity and their potential use for regenerative medicine and dentistry. This review summarizes current knowledge, barriers, and challenges in the clinical use of dental stem cells.

**Keywords:** dentistry, stem cell, medicine

### Introduction

Stem cells are generally defined as clonogenic cells capable of both self renewal and multi-lineage differentiation. Dental derived stem cells have been isolated and identified as the cell sources for tooth repair and regeneration. Current research indicates that the dental SCs may have the potential to regenerate bone, the periodontal ligament, and possibly the teeth. Dental SCs have been found in several tissues and can be divided into dental mesenchymal SCs (MSCs) and dental epithelial SCs. DPSCs are SCs derived from dental pulp. They show a multipotent differentiation ability, which is similar to that of MSCs. A population of high quality human stem cells was found in the exfoliated human primary teeth (SHED) [23]. The SHEDs have the osteoinductive capacity *in vivo*, but failed to reconstitute a dentin-pulp-like complex. Stem cell fractions are called side population (SP). The adult pulp tissue contains side population (SP) cells that have tissue stem cell activities, self-renewal and multilineage potential.

Many tissues of the human body undergo normal physiological renewal. These renewing tissues have some capacity to repair damage due to disease or trauma. Recent therapeutic modalities of some diseases have taken advantage of this phenomenon and included tissue engineering in which biologic materials are employed to replace, repair, maintain, and/or enhance tissue function. The materials required for tissue engineering include stem cells, morphogens (or growth factors) and a scaffold to guide cell growth. Stem cells have unique characteristics. They are unspecialized cells with the ability of self renewal and differentiation in response to the appropriate signal. Adult stem cells can be categorized based on their origin into two main groups: germline and somatic stem cells. Several types of somatic stem cells have been discovered in different locations. The mesenchymal stem cells (MSCs) are found in the stroma of adult bone marrow. They are multipotential cells with ability to differentiate into osteoblasts, chondrocytes, adipocytes and even non-mesodermal tissues such as endoderm. Due to their multipotency search for MSCs or MSCs-like cells in different tissues was carried out and resulted in the discovery of a variety of cells in many organs of the body including the tooth. Dental stem cells have been isolated from different soft tissues of the tooth. The tooth is mainly made of hard tissues which are connected to soft tissues. The hard tissues include the dentin which is covered by enamel in the crown and

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cementum in the root. The dentin encloses the dental pulp which is a richly innervated, highly vascularized soft (loose connective) tissue. The tooth is attached to

its bony socket by another kind of soft (dense connective) tissue, the periodontal ligament (PDL).

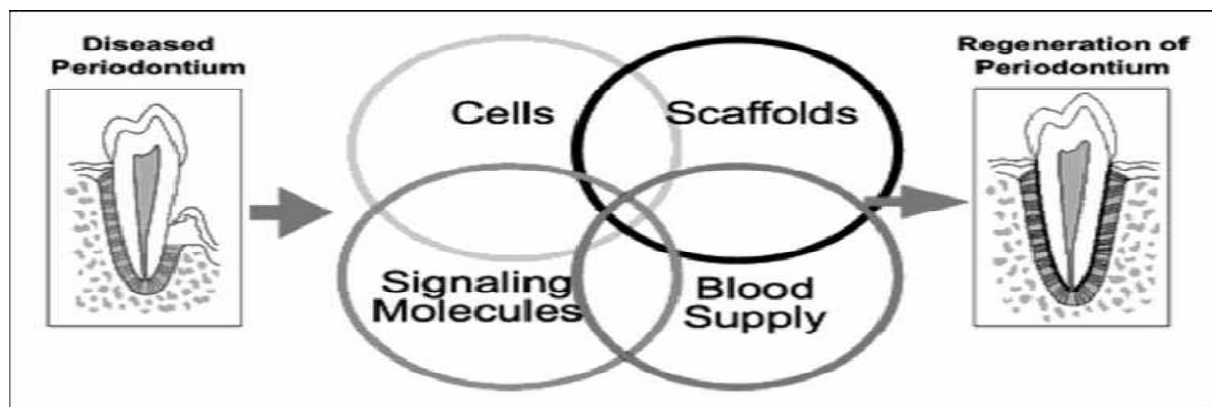


Figure 1: Different types of dental stem cells

#### Dental Pulp Stem Cells (DPSCs)

DPSCs were the first type of dental stem cells to be isolated. These cells were obtained by enzymatic digestion of the pulp tissue of the human impacted third molar tooth. DPSCs have a typical fibroblast-like morphology. They are clonogenic in nature and can maintain their high proliferation rate even after extensive subculturing. There is no specific biomarker to identify the DPSCs.[12] However, DPSCs express several markers including the mesenchymal and bone marrow stem cell markers, STRO-1 and CD146 as well as the embryonic stem cell marker. Culturing DPSCs with various differentiation media demonstrated their dentinogenic, osteogenic, adipogenic, neurogenic, chondrogenic and myogenic differentiation capabilities. Following their transplantation in animal models, DPSCs were able to maintain their self renewal and to form pulp-like tissue, odontoblast-like cells, ectopic dentin as well as reparative dentin-like and bone-like tissues. The characteristic features and multilineage differentiation potential of DPSCs have established their stem cell nature and indicated their promising role in regenerative therapy.

#### Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

In 2003, Miura *et al.* 8 isolated cells from the dental pulp which were highly proliferative and clonogenic. The isolation technique was similar to those used in the isolation of DPSCs. However, there were two differences:

- i) the source of cells was the pulp tissue of the crown of exfoliated deciduous teeth
- ii) the isolated SHEDs did not grow as individual cells, but clustered into several colonies which, after separation, grew as individual fibroblast-like cells.

SHEDs have a higher proliferation rate and a higher number of colony forming cells than DPSCs. SHEDs were found to express early mesenchymal stem cell markers (STRO-1 and CD146). In addition, embryonic stem cell markers such as, Nanog, stage-specific embryonic antigens (SSEA-3, SSEA-4), and tumor recognition antigens (TRA-1-60 and TRA-1-81) were found to be expressed by SHEDs[16].

The multilineage differentiation potential of SHEDs was demonstrated under different inductive conditions. SHEDs showed the capacity to undergo osteogenic and adipogenic differentiation. When SHEDs were cultured with neurogenic inductive media, they formed sphere-like clusters and changed their fibroblastic morphology into cells with multiple cytoplasmic processes. Under the same neurogenic culturing condition, SHEDs expressed different neuronal and glial cell markers such as nestin. [9] The expression of these markers may suggest a neural crest origin of these cells. When SHEDs were transplanted into immunocompromised mice, dentin-like structure was formed, and was immunoreactive to dentin specific sialoprophosphoprotein antibody. Odontoblast-like cells were found to be associated with this regenerated dentin structure, which indicate the odontogenic differentiation potential of SHEDs. However, unlike DPSCs, SHEDs did not form a dentin-pulp complex after *in vivo* transplantation.

This indicates that SHEDs have different odontogenic differentiation potential than DPSCs. In regard to the osteogenic differentiation potential, it was interesting to find that SHEDs, unlike DPSCs, were not able to differentiate into osteoblast or osteocyte, but were able to induce the host cells to undergo osteogenic differentiation. This is another difference in the differentiation potential of SHEDs and DPSCs which demonstrates that SHEDs, unlike DPSCs, have an osteoinductive potential rather than a differentiation potential. Due to their higher proliferation rate, unlike DPSCs, and their odontogenic and osteogenic differentiation potential, SHEDs appear to be distinct from the DPSCs and might indicate a more immature form than DPSCs[22].

### Periodontal Ligament Stem Cells (PDLSCs)

The PDL does not only anchor the tooth, but also contributes to its nutrition, homeostasis, and repair. PDL contains different types of cells including cells which can differentiate into cementoblast and osteoblasts. Heterogeneity and continuous remodeling of PDL is an indication for the presence of progenitor cells which can give rise to specialized cell types. In 2004, this speculation led to the discovery of the third type of dental stem cells which was referred to as PDLSCs. PDLSCs were isolated using the same methodology for DPSCs and SHED, but this time, the tissue used was from the separated PDL of the roots of impacted human third molar. The isolated cells were fibroblast-like and clonogenic, but showed a high rate of proliferation, comparable to that of the DPSCs but more prolific than the bone marrow MSCs. PDLSCs were found to express STRO-1, CD146 and scleraxis (tendon specific transcription factor). Scleraxis is expressed at a higher level in PDLSCs than in DPSCs and bone marrow MSCs. This finding was expected as the PDL and tendon exhibit similar structural contents of dense collagen fibers and similar ability to absorb mechanical stress during normal physiological activity. PDLSCs have a multilineage differentiation potential. They were able to undergo osteogenic, adipogenic and chondrogenic differentiation when they were cultured with the appropriate inductive medium. [9,21] When PDLSCs were transplanted into immunocompromised mice, a typical cementum-PDL structure was formed, which was not produced in the case of DPSCs or bone marrow MSCs. The newly formed PDL-like tissue was composed of type I collagen and interestingly it was connected to the cementum in the same way Sharpey's fibers of the PDL attach to the cementum of the tooth.

### Dental Follicle Precursor Cells (DFPCs)

The dental follicle (DF), is a loose connective tissue of an ectomesenchymal origin and it is present as a sac surrounding the unerupted tooth. During tooth development it has been found that DF plays an important role in the eruption process by controlling the osteoclastogenesis and osteogenesis needed for eruption. It is also believed that DF differentiates into the periodontium as the tooth is erupting and becomes visible in the oral cavity. [10] As the periodontium is composed of several cell types, it is reasonable to propose the presence of stem cells within the dental follicle which are able to give rise to the periodontium. In 2005, Morsczech *et al.* were successfully able to isolate stem cells from the dental follicle of the human impacted third molar, using the same methodology of DPSCs isolation and culture. The cells were fibroblast-like and expressed various markers, such as nestin and Notch. [3] The potential of DFPCs to undergo osteogenic, adipogenic and neurogenic differentiation was demonstrated using *in vitro* studies.

When the neurogenic differentiation potential of DFPCs was compared with that of SHEDs, different neural cell marker expression patterns were revealed suggesting different neuronal differentiation potential. [29] DFPCs were also able to differentiate and express cementoblast markers (cementum attachment protein and cementum protein-23) after being induced with enamel matrix derivatives, or BMP-1 and BMP-7.

### Stem Cells of Apical Papilla (SCAPs)

During tooth development, the dental papilla evolves into the dental pulp, and contributes to the development of the root. The apical part of the dental papilla is loosely attached to the developing root, and it is separated from the differentiated pulp tissue by a cell rich zone. [10] It contains less blood vessels and cellular components than the pulp tissue and the separating cell rich zone. In 2006, Sonoyama *et al.* isolated a new population of dental stem cells, and called them SCAPs. SCAPs are clonogenic fibroblast-like cells, but have a higher proliferation rate than DPSCs. As other dental stem cells, SCAPs express the early mesenchymal surface markers, STRO-1 and CD146. However, SCAPs also express CD24, which could be a unique marker for this cell population. The capacity of SCAPs to differentiate into functional dentinogenic cells has been verified by the same approaches as for the abovementioned dental stem cells. SCAPs have the capacity to undergo osteogenic, adipogenic, chondrogenic and neurogenic differentiation, when they are cultured in the appropriate inductive media. [15] As in the case of

DPSCs, when SCAPs were transplanted into immunocompromised mice in an appropriate carrier matrix, a typical dentin-pulp like structure was formed, with odontoblast-like cells.

## Discussion

### Role of dental stem cells

#### 1) Dental Pulp Regeneration

Since the discovery and isolation of the different types of dental stem cells, there have been many attempts to use them in the regeneration of the dental pulp tissue. Using a tooth slice model, pulp-like tissue was engineered using SHEDs seeded onto synthetic biodegradable scaffolds. SHEDs were able to differentiate into odontoblast-like cells, and also endothelial-like cells.[1] In another study using the same tooth slice model, DPSCs were seeded on collagen scaffold supplemented with dentin matrix protein (DMP-1) and were able to regenerate pulp-like tissue. These findings suggest that SHED and DPSCs can be considered as reliable sources of stem cells for dental pulp tissue engineering and regeneration.

#### 2) Bio-Root Engineering

Sonoyama *et al.* demonstrated the use of combined mesenchymal stem cell populations for root/periodontal tissue regeneration. They loaded root shaped hydroxyapatite/tricalcium phosphate (HA/TCP) block with swine SCAPs. They then coated the HA/TCP block with gelfoam containing swine PDLSCs and inserted the block in the central incisor socket of swine. Three months post-implantation, histological and computerized tomography scan revealed a HA/SCAP-gelfoam/PDLSC structure growing inside the socket with mineralized root-like tissue formation and periodontal ligament space. These findings suggest the ability of combined autologous SCAP/PDLSCs generating a bio-root, which can be an alternative to dental implants in replacing missing teeth.[2]

#### 3) Neural Regeneration

Cranial neural crest (CNC) cells represent an ideal source for neuronal differentiation and regeneration. The migrating CNC cells contribute to the formation of dental papilla, dental pulp, PDL and other tissues in the tooth and mandible.<sup>35</sup> Therefore, it is reasonable to consider that the different types of dental stem cells are of CNC origin. Different dental stem cells expressed neural and neural crest markers with or without induction.[7,8] *In vitro* and *in vivo* studies of SHEDs

demonstrated that these cell populations were able to differentiate into neurons based on cellular morphology and the expression of early neuronal markers.<sup>8,36</sup> Other studies on DPSCs, demonstrated that these cells responded to neuronal inductive conditions both *in vitro* and *in vivo* and acquired a neuronal morphology, and expressed neuronal-specific markers at both the gene and protein levels. They also exhibited the capacity to produce a sodium current consistent with functional neuronal cells when exposed to neuronal inductive media. In a recent study and using an animal model, it was found that avian trigeminal ganglion axons migrated toward the implanted DPSCs, and this was due to CXCL12 expression by the DENTAL STEM CELL PROPERTIES. DPSCs. This expression pattern assisted in the homing of endogenous neural stem cells to the site of DPSCs transplantation. These findings provided an evidence that DPSCs might be able to induce neuroplasticity within a receptive host nervous system. All these findings indicate that dental tissues represent an alternative, promising and less invasive stem cell source for neuronal regenerative-based therapy.

#### 4) Cardiac Repair

It was found that DPSCs can help cardiac repair after myocardial infarction. In an experimental model of acute myocardial infarction, the left coronary artery was ligated in nude rats. Then DPSCs were transplanted to the border of the infarction zone. Four weeks after transplantation, evidence of cardiac repair was noted by improved cardiac function, increase in the number of vessels and a reduction in infarct size. The cardiac repair occurred in the absence of any evidence of DPSCs differentiation into cardiac or smooth muscle cells. [5] This suggests that DPSCs induced cardiac repair due to its secretion of different growth factors and cytokines such as vascular endothelial growth factor, insulin-like growth factor-1 and -2 and stem cell factor, which helped in inducing angiogenesis and cardiac regeneration at the infarction zone.<sup>40</sup> Therefore, it seems that DPSCs have the potential to be used as a novel and alternative source for treatment of not only dental but also some other ischemic diseases.

#### Tooth banking

It is a future step for preliminary future tissue regeneration. Because of the opportunity to preserve dental SCs for medical applications, the term "tooth bank" was first raised in 1966. With the rapid development of advanced cryopreservation technology,

the first commercial tooth bank was established as a venture company at National Hiroshima University in Japan in 2004.

### Conclusion

The discovery of a dental source for stem cells could very well prove to be a milestone in the regenerative medicine. The minimal intervention required to obtain dental soft tissues within the oral cavity provides an advantage and may help avoid rejection by recipients. Advances in the isolation and understanding of dental stem cells have opened areas of research into the possibility to 'regrow' lost dental tissues. This may not only prevent tooth loss but also fundamentally change the concept and definition of a dental caregiver. The impact of this source of stem cells is even more far-reaching for the medical field. Current research is exploring the capability of the dental stem cells to differentiate into non-dental tissues such as cardiac muscle. Previously untreatable patients may now be improved by using stem cells harvested from their own teeth. The relative minimal intervention required to obtain the cells and the absence of rejection issues may be the prime advantages to support such therapies in the near future. Although many studies have to be conducted before applying such therapeutic modalities, the dental stem cells represent a powerful tool which holds a significant potential for advancement in the field of regenerative dentistry and medicine.

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