

## Dental stem cells: Progress and perspectives

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### Abstract

Dental pulp stem cells (DPSCs) are thought to contribute to reparative dentin formation, and that they may correspond to heterogenous populations of precursor cells or represent distinct differentiation stages along the odontoblastic lineage. DPSCs share many similarities with mesenchymal stem cells of the bone marrow (BMSCs). It appears that the distribution of tissue stem cells is not random and, within the dental pulp, there are potentially several distinct niches of stem/progenitor cells. In addition to DPSCs, other dental stem cell populations have been isolated. As for DPSCs, further studies are still needed to evaluate their potential of differentiation and their regenerative activity. Up today, (1) the formal demonstration that pulpal resident stem cells are actually the reparative dentin-forming cells recruited in response to injury is still lacking; and (2) the origin, localization and precise identity of odontogenic stem cells remain largely unknown. Dental clonal cell lines may represent valuable tool to answer some fundamental questions concerning the dental stem cell biology. Altogether, the presence of dental cell populations displaying stem cell properties has opened new paths for considering regenerative therapies. This might be a

prerequisite to design alternative strategies for capping and endodontic treatment, using stem cells.

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**Key words:** Dental pulp; Stem cells; Dentin repair; Niche

**Core tip:** The presence of cell populations displaying stem cell properties within the pulp has opened new paths for considering more conservative therapies. Still, dental stem cells research is still confronted with the lack of precise knowledge related to the location and identity of the cells participating to reparative dentin formation. Clonal cell lines derived from the dental sphere may represent valuable tool to answer some questions that are of fundamental importance to stem cell biology and clinical applications. This review discusses some fundamental concepts of dental stem cell biology within the context of regenerative dentistry.

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### INTRODUCTION

Tooth development requires a series of sequential and reciprocal interactions between the ectodermally derived oral epithelium at the origin of ameloblasts and neural crest-derived ectomesenchyme at the origin of odontoblasts. Tooth patterning proceeds through sequential morphogenetic events (bud, cap, bell) leading to crown and subsequently to root formation. During embryogenesis, morphogenesis is coupled to differentiation of committed cells that progressively elaborate enamel and dentin and in turn reach the terminal stages of amelogenic and odontogenic lineages. This cascade of events relies on

epithelial-mesenchymal interactions that progressively lead to transformation of the tooth germ into complex mineralized structures<sup>[1]</sup>.

Ameloblasts are lost following enamel maturation and tooth eruption, and hence enamel cannot be regenerated. Dental papilla ectomesenchymal cells give rise to the embryonic pulp and odontoblasts. Dental pulp cells maintain tooth homeostasis and odontoblasts synthesize dentin extracellular matrix. Dentin and pulp are related embryologically, histologically, and are functionally associated although the term of dentin-pulp complex is a notion underlying an oversimplification.

Odontoblasts are polarized postmitotic cells. These terminally differentiated cells cannot undergo further cell division and proliferate to replace irreversibly injured odontoblasts. Only the postmitotic cells forming the sub-odontoblastic Hoehl's cell layer, have the capacity to acquire a polarized phenotype and become functional odontoblasts. Odontoblasts are responsible for the secretion of primary and secondary dentin. They have a natural regenerative potential leading to the formation of reactionary dentin<sup>[2]</sup>. Odontoblasts can be up-regulated to secrete a reactionary dentin matrix when a mild injury occurs, such as attrition or early caries. However, injury of greater intensity, such as deep caries or restorative procedures, may lead to the death of the pre-existing odontoblasts and Hoehl's cells<sup>[3]</sup>. In such cases, recruitment of stem/precursor cells within the pulp will give rise to a new generation of odontoblast-like cells capable to elaborate reparative dentin.

The process of dental tissue repair may share many similarities with the embryological mechanisms of tooth development. It is assumed that many genes and signaling pathways involved in odontogenesis are also implicated in the tooth repair. Still, the mechanisms underlying reparative dentin formation are "open research areas" and offer exciting opportunities for possible tooth regeneration and dental tissue engineering.

## DENTAL PULP STEM CELLS

The post-natal dental pulp contains heterogeneous cell populations responsible for its maintenance, defence and capacity of repair: stromal fibroblasts, odonto-osteoprogenitors, neuronal and vascular cells as well as inflammatory and immune system cells such as dendritic cells, neutrophils, macrophages, lymphocytes<sup>[4]</sup>. The ability of the pulp to respond to a variety of pathological conditions and injuries by deposition of a reparative dentin by pulp "progenitors" is well recognized<sup>[5]</sup> but the origin, localization and precise identity of odontogenic stem cells remain largely unknown. Identifying cells mobilized in response to pulp injury is a prerequisite to design alternative strategies for capping and endodontic treatment, using stem cells.

A decade ago, a population of odontogenic progenitors, inferred as dental pulp stem cells (DPSCs), was isolated from the pulp of human permanent third molars<sup>[6]</sup>. Upon subcutaneous transplantation into immuno-

compromised mice, *in vitro* expanded DPSCs mixed with hydroxyapatite formed dentin/pulp like complexes at an ectopic site. Populations of DPSCs possess (1) generic mesenchymal stem cells-like properties (MSCs); (2) colony forming ability; and (3) were shown to express *in vitro* osteoblastic, adipogenic, chondrogenic or even neuronal markers<sup>[7-9]</sup>. DPSCs share many similarities with mesenchymal stem cells of the bone marrow (BMSCs) which are the most studied stromal stem cell populations. More than 4000 human genes are expressed either by BMSCs or DPSCs<sup>[10]</sup>. Dental stem cell populations also express different panels of stem cell surface markers such as 3G5, STRO-1, CD44, CD106, CD146, CD90 and Sca-1 used to characterize hematopoietic stem cells. However, it is important to note that DPSCs and BMPCs have not the same embryonic origin and that cells derived from the human or animal dental pulps have not been able to support hematopoiesis in transplantation assays<sup>[11]</sup>. DPSCs are thought to contribute to reparative dentin formation, and it appears that they may correspond to heterogeneous populations of precursor cells or represent distinct differentiation stages along the odontoblastic lineage.

The presence of cell populations displaying stem cell properties within the pulp has opened new paths for considering more conservative therapies<sup>[6]</sup>. Nevertheless, the formal demonstration that pulpal resident stem cells are actually the reparative dentin-forming cells recruited in response to injury is still lacking. The hypothesis that a subset of stem cells carried by the vasculature replenishes the pulp after lesion can not be totally excluded. In the pulp, as in most tissues, the size of the pool of stem cells is very small (< 1%) and little is known about their anatomical sites within the pulp<sup>[12]</sup>. Moreover, the responsiveness of the pulp provides a dynamic system for tissue repair that may imply migration of stem cells from their resting places to the site of injury. Undifferentiated mesenchymal/mesectodermal cells present in the stroma, perivascular cells such as Rouget's pericytes or fibroblasts have all been proposed as potential progenitors mediating pulp repair after destruction of the odontoblasts and the Hoehl's sub-odontoblastic cell layer<sup>[13]</sup>. Advances in imaging technology and identification of stem cell markers are still needed to visualize stem/precursor cells *in situ*.

## WHERE ARE THE DENTAL PULP STEM CELLS NICHES?

Stem cells are rare cells that are uniquely capable of both reproducing themselves and generating the differentiated cell types that are needed to carry out specialized functions. Stem cell behaviour is regulated by inputs from their local environment often referred as the "stem cell niche". Niches are defined as specific anatomic locations that provide structural support, trophic support, topographical informations and the appropriate physiological cues to control the maintenance, quiescence, self-renewal, recruitment towards differentiation and long-term regenerative capacity of stem cells. Hallmarks of a niche may

include: the stem cell itself, stromal supporting cells that interact directly with the stem cells *via* secreted factors and cell surface molecules, extracellular matrix (ECM) that provides structure and mechanical signals, neuronal inputs and vascular network that carry systemic signals and represent a conduit for recruitment of inflammatory and circulating cells into the niche. In teeth, as in the adult blood system, multiple niches may exist and specific markers allowing the definitive identification of stem cells within the pulp are lacking.

Some data suggest that pericytes could differentiate into osteoblast-like cells, so odontogenic stem cells may reside in a perivascular niche<sup>[14]</sup>. In this context, it is interesting to mention that many haematopoietic stem cells (HSCs) and neuronal stem cells (NSCs) are localized close to the vascular network; this could be important to communicate “insult” and expose stem cells to signals activating their recruitment. Besides, alterations in ECM components and matrix elasticity related to damage or ageing may also provide mechanical signals that could have a profound impact on stem cell activity<sup>[15]</sup>. Thus, it appears that the distribution of tissue stem cells is not random and, within the dental pulp, there are potentially several distinct niches of stem/progenitor cells. Nevertheless, still little information is available regarding their topological organization and the inputs that recruit osteo-odontogenic stem cells to form reparative dentin<sup>[3]</sup>. In contrast to other tissues known to have a constant regeneration potential, such as intestine and bone marrow, dental pulp stem cells will react to form reparative dentin only after injury. This implies that signals ensure their survival and prevent their differentiation while maintaining their responsiveness following pulp damage. Whether an endogenous pool of stem cells associated with supportive stromal cells are mobilized at the site of injury and/or whether attraction of migrating stem cell is necessary to repopulate a niche and expand precursor cells at the appropriate site for dentin repair is unknown. In addition, the alteration of the dentin mineralized matrix promotes the release of bio-active molecules including high concentrations of  $\text{Ca}^{2+}$  which locally may also contribute to stem cells proliferation and differentiation in the post-injury pulpal environment.

## DENTAL STEM CELLS-DIFFERENT TYPES, DIFFERENT LOCATIONS

In addition to DPSCs which were derived from the pulp of human permanent third molar, other stem cell populations have been isolated from exfoliated deciduous teeth (SHED), periodontal ligament (PDLs), apical papilla (SCAP) and dental follicle (DFSCs). As for DPSCs, further studies are still needed to evaluate their potential of differentiation and their regenerative activity.

SHED, isolated from the pulp of human deciduous teeth appear distinct from DPSCs, having a higher proliferative rate and distinct gene expression profiles. SHED have osteoinductive capacity *in vivo*<sup>[16]</sup>. They can survive

in mouse brain, expressing neural markers and possible application of SHED was even considered in alleviating Parkinson's disease<sup>[17]</sup>. As odontoblasts, they have a neural crest origin which may sustain their ability to adapt in a neuronal environment. Since children lose 20 deciduous teeth, SHED may be potentially used as an autologous stem cell source for dental pulp engineering once the children become adult. The commercial banking of these cells is becoming widespread but whether SHED maintain their stem cell properties after long-term storage (cryopreservation for more than 10 years) have not been assessed.

PDLSCs which derived from human periodontal ligament (PDL), a connective tissue between the cementum and the inner wall of the alveolar bone socket, represent a population of stem cells capable to differentiate in cementoblast-like cells and type 1 collagen-forming cells. Interestingly, transplantation of human PDLSCs in the periodontal defect of immunocompromised mice, promotes the formation of a periodontal -like tissue, suggesting that PDLs may be a potential tool for alveolar bone repair<sup>[18]</sup>.

SCAP are derived from the apical part of the papilla of growing tooth roots<sup>[19,20]</sup>. It is important to note that the apical papilla tissue is present while the root apex is still open, before tooth eruption. *In vitro*, SCAP have been shown to exhibit dentinogenic and adipogenic properties, they also express neuronal markers. In clinical practice, they are easily accessible from extracted wisdom molars which develop later in life. Whether SCAP may represent a source of autologous stem cells for tooth repair remains an open question.

DFSCs derived from the dental follicle, a fibrous ectomesenchymal tissue that surrounds the developing tooth germ during the crown formation and disappears during the early stages of root development<sup>[21,22]</sup>. This tissue will form the periodontium, *i.e.*, cementum, periodontal ligament and alveolar bone. Thus, DFSCs may correspond to a heterogeneous cell populations with different type of stem cells including cementoblasts, osteoblasts, stromal cells. In the adults, DFSCs can be easily accessible in impacted wisdom tooth during crown formation but not later (Table 1).

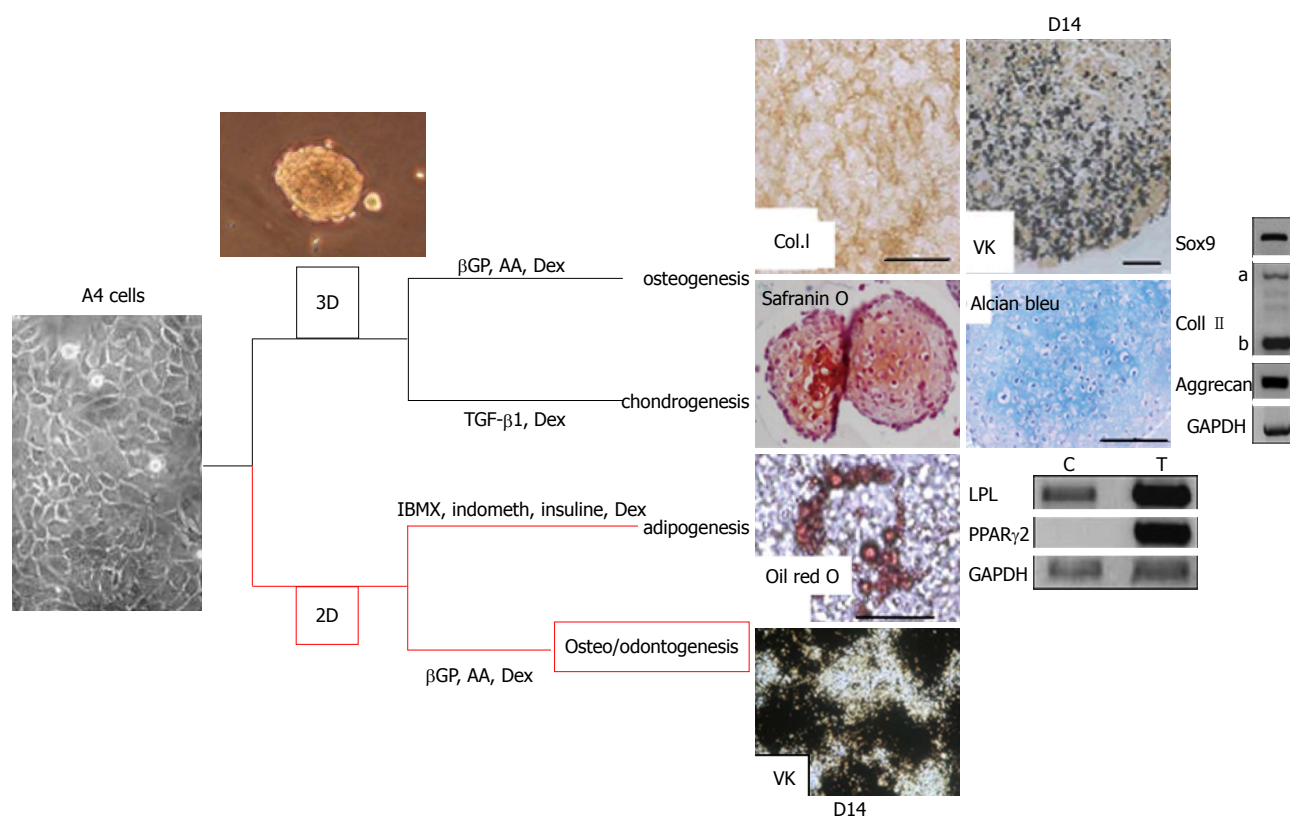
Finally, since 2009, several publications describe new populations of mesenchymal stem cells isolated from the human oral mucosa and gingiva (Zhang *et al.*, 2012). Their differentiation and therapeutic potentials remain to be determined.

## PERSPECTIVES AND OPEN QUESTIONS

Dental stem cells research is still confronted with the lack of precise knowledge related to the location and identity of the cells participating to reparative dentin formation. To this end, our laboratory developed the strategy and established stem cell lines from embryonic pulp of transgenic mouse<sup>[23]</sup> and Figure 1. One of the clones has the capacity after implantation in a rat molar, and in the ab-

**Table 1** Types of dental pulp stem cells and their properties

Type of stem cells after birth	Dental stem cell properties	Signaling inputs for reparative dentin formation
Stem cells permanently present in the adult tooth:	Self-renewal	Tooth injury may promotes stem cell recruitment by:
Dental pulp stem cell	Ability to enter in mitosis in response to appropriate signals and to differentiate towards odonto/osteogenic cells	Local secreted factors:
Periodontal ligament stem cells	Long-term survival and maintenance of reparative capacity	bioactive extracellular matrix molecules
Apical papilla stem cells	Distinct subpopulations expressing markers of mesenchymal stem cells of the bone marrow (STRO-1, CD44, CD 106, 3G5, CD146, CD90, Sca-1...)	Ca <sup>2+</sup> release
Stem cells present in deciduous tooth:		Mechanical inputs: changes in matrix elasticity
Exfoliated deciduous teeth stem cells		Diffusible signals emanating from stromal, inflammatory, circulating... cells
Stem cells present during crown formation:		
Dental follicle stem cells		



**Figure 1** The A4 cells cultured in 2D or 3D, differently supplemented, give rise to different cell phenotypes, and consequently promote osteogenesis, chondrogenesis, adipogenesis or dentinogenesis. GAPDH: Glyceraldehyde phosphate dehydrogenase; TGF- $\beta$ 1: Transforming growth factor  $\beta$ 1.

sence of any carrier or biomolecule, to promote efficient dentin repair<sup>[24,25]</sup>. Clonal cell lines derived from the dental sphere may represent valuable tool to answer several questions that are of fundamental importance to stem cell biology and clinical applications: Where are localized the presumptive stem cells niches? What are the markers allowing to visualize resident or migrating stem cells *in situ*? Which signals and molecular pathways sustain stem cells recruitment within the pulp and parodontium upon injury? By combining *in vitro* and *in vivo* experimental approaches, the answers to these questions will lead to a better understanding of stem cells potential for tooth repair and pave the way to develop new stem cell-based therapies.

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