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Review Article

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Dental Stem Cells: Repair, Differentiation, and Regeneration.

Michel Goldberg*

INSERM UMR-S 1124 and Université Paris Descartes, Sorbonne, Paris Cité, Cellules souches, Signalisation et Prions 75006 Paris, France, 45 rue des Saints Pères, Paris 75006, France

***Corresponding Author:** Michel Goldberg, Professor Emeritus, Faculté des Sciences Fondamentales et Biomédicales des Saints Pères. Université René Descartes, Paris Cité & INSERM UMR-S 1124, France.

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Abstract

Stem cells have the capacity to self-renew. They display clonogenic, proliferation capability, and the ability for multi-lineage differentiation related to their "steaminess". They are positive for surface markers such as CD29, CD44, CD 105, CD73, CD90 and Sca-1. Bone and bone-marrow-like (BMSCs) cells may be implicated in embryonic tissue repair and/or renewal. Adult stem cells display therapeutic potential. The dental pulp stem cells (DPSCs) family includes pulp stem cells (PSCs), exfoliated deciduous teeth (SHED), apical papilla (SCAP), periodontal ligament (PDLSC), and dental follicle (DFPSC). Alizarin red or Von Kossa staining are associated with the initial matrix mineralization. The MSC niches constitute a specialized microenvironment. The perivascular and perineural sheath regions have osteogenic potential. They may contribute to regenerate a wounded dental tissue, despite there is no evidence that BMSCs give rise to functional odontoblasts and generate dentin.

Keywords: *Stem cells; Self-renewal; Surface markers; Steaminess; Multi-lineage differentiation; Mesenchymal stem cells; Exfoliated deciduous teeth; Apical papilla; dental follicle; Periodontal ligament; Niches; Tissue repair; Initial mineralization; Functional odontoblasts; Regeneration*

Introduction

I- Biological properties of mesenchymal stem cells

Five different types of stem cells have been identified. They retain the capacity to self-renew, they display clonogenic proliferation and have the ability for multilineage differentiation. They are capable of restoring a tissue and they are implicated in regenerative medicine. They display colony-forming capability. They are highly positive for different panels of surface markers. Mesenchymal stem cells (MSCs) are virtually found in all adult tissues. MSC (mesenchymal stromal cells rather than stem cells) are plastic adherent. They display high expression for CD 29, CD44, CD105, CD73, CD90, Sca-1 and HLA I, characteristics of hematopoietic stem cells. However, they lack CD45, CD34, CD14, CD71, CD79 α.

Two properties characterize stem cells:

Self-renewal: A stem cell goes through numerous cycles of cell division while maintaining an undifferentiated state. They divide into one mother stem cell, whereas the daughter cell underwent multi-lineage differentiation.

Potency: Differentiation occurs when two daughter cells are identical. They undergo mitosis and divide into specialized cells identical to the original. Stem cells are totipotent or pluripotent. Potency specifies the differentiation potential into different cell types.

Stem cells differentiate into osteoblasts/odontoblasts, adipocytes, and chondroblasts, and neurogenic cells *in vitro.* They have been successfully isolated from a variety of human dental tissues such as alveolar mesenchymal stem cells, dental follicle progenitor, dental pulp, gingiva-derived, periodontal ligament, apical papilla (SCAP), exfoliated deciduous teeth (SHED) and tooth germ progenitor cells (TGPCs). They include more than 200 different types of cells. These early isolated cells demonstrated alkaline phosphatase reactivity, partial cytodifferentiation and contribute to the formation of mineralized nodules. Dental pulp stem cells reveal clonogenic population. They display multilineage differentiation.

Dental tissues originated from ectomesenchyme. They are neural crest derived cells that express nestin, a neural cell marker (Arthur., *et al.* 2008). Less than 1% of the cells have true stem cell-like properties. The side population of DPSCs expresses alkaline phosphatase (ALP). They were far less in side-population compared to the main population.

Characteristics of DPSCs

They are negative for CD14, CD34, CD45, MyoD, neurofilament, and type II collagen, peroxisomal proliferator-activated receptor gamma 2. They are positively stained for CD29 and CD44, stem cell factors and CD117 markers, associated with the endothelium, the case for CD106, CD146, smooth muscle (α-smooth muscle actin), bone (alkaline phosphatase, type I collagen, osteonectin, osteopontin, and osteocalcin), and fibroblasts (type III collagen and fibroblast growth factor). They are also positive for CD73, CD90, and Stro-1. The expression of embryonic stem cell markers such as Oct-4, Sox2, and c-Myc play key-factor in reprogramming pluripotency markers.

Origins and niches of DPSCs: The niche is responsible for maintaining and controlling a quiescent stem cell population, capable of responding according to host requirements. In rat dental pulp, 0.40% of the pulp cells in young rats and 0.11% in aged rats comprised side-population cells (Kerkis., *et al.*) When porcine dental pulp cells with stem/progenitor cell were further subfractionated into CD31 − ; CD146 − and CD31 + ; CD146 − cells, distinct properties were found. The CD31 −; CD146 − cells expressed higher levels of stem cell markers compared to the CD31 +; CD146 − population. In addition, they had strong vasculogenic potential both via direct differentiation and through paracrine-mediated mechanisms.

II- Implication of dental stem cells in repair, terminal differentiation and regeneration

Being encased in a hard structure, the dental pulp stem cells include a series of 5 different stem/progenitor cells that have been isolated and characterized: dental pulp stem cells (DPSCs) and post-natal populations. They have mesenchymal stem-cell-like bone marrow (BMSCs) qualities (Friedenstein., *et al*. 1976; 1978. Caplan, 1991; Prockop, 1997; Pittenger., *et al*. 1999; Gronthos., *et al*. 2000, 2003, Bianco., *et al*. 2001)). They include various lineages of cells, such as osteogenic, chondrogenic, adipogenic (Huang., *et al*. 2009). Inflammation is playing a role in regulating angiogenic responses and wound healing processes such as reparative dentinogenesis.

1-Dental pulp stem cells (DPSCs) – Stem cells may be isolated from the pulp tissue of extracted human third molars (Gronthos., *et al*. 2000, 2003; D'Aquino et al., 2008). Dental pulp stem cells display clonogenic, rapid and easy access to the collection site, with highly efficient extraction, and extensive differentiation ability. CD44 positively labeled DPSC-1 and DPSC-2 bone marrow stem cells (Kerkis., *et al*. 2006). Integrin β1 is also positive, and α-SM actin. Type I and Type III collagens, osteocalcin, osteonectin, alkaline phosphatase and FGF-2 are positively detected. Bone marrow stromal cells (BMSCs) are precursors of osteoblasts/odontoblasts. Interaction with biomaterials facilitates tissue reconstruction. They generate a dentin-like structure, lined by human odontoblast-like cells surrounding a pulp-like tissue. They have a long lifespan. They build Havers channels with appropriate vascularization.

2- Stem cells from human exfoliated deciduous teeth (SHED) – Stem cells from human exfoliated deciduous teeth (SHED) are mesenchymal cells present within exfoliated deciduous tooth pulp tissue that can differentiate into a broad range of different cell types. Immature (Miura., et al. 2003). DPSCs express the embryonic cell markers Oct4, Nanog, stage-specific embryonic antigens (SSEA-3, SSEA-4) and tumor recognition antigens (TRA-1-60 and TRA-1-81). SHEDs represent a population of postnatal stem cells capable of extensive proliferation and multipotential differentiation. Deciduous teeth may be a resource of stem cells to repair damaged tooth structures. They induce bone

regeneration, and treat neural tissue injury and/or degenerative diseases. Studies provide a description of a stem-cell population supporting further studies aiming to determine the efficacy of using SHED in cellular-based therapies.

3- Stem cells from apical papilla (SCAP) – Human immature dental pulp cells (IDPSC) constitute a sub-population displaying regenerative capacity (Sonoyama., *et al*. 2006, 2008; Huang.,*et al*. 2008). They include mesenchymal stem cells (MSCs). They were also obtained in vitro from these cells, including endodermal and ectodermal lineages. A new population of mesenchymal stem cells located in the apical papilla of immature teeth has been discovered, termed stem cells from the apical papilla (SCAP). These stem cells are the source of odontoblasts that are responsible for the formation of root dentin.

4- Dental follicle progenitors cells (DFPCs) – Dental follicle stem cells are implicated in tissue engineering (Honda., *et al.* 2010). The dental follicle is a loose mesenchymal tissue surrounding the developing tooth germ that participates in the formation of periodontal progenitor cells. The term "tissue engineering" (Langer & Vacanti, 1993) was accepted. Dental bone marrow- derived MSC follicle cells (DFCs) were evaluated by immunocytochemistry, using embryonic stem cells markers (OCT4 and SOX2), mesenchymal stem cells (MSCs) markers (Notch1, STRO-1, CD44, HLA-ABC, CD90), neural stem cells markers (nestin and β-III-tubulin), neural crest stem cells (NCSCs) markers (p75 and HNK1) and a glial cells marker (GFAP)(Lopes de Lima., *et al*. 2017).

5- Periodontal ligament stem cells (PDLSCs) – Periodontal ligament stem cells are present in the perivascular space of the periodontium. They are responsible for the regeneration of periodontal components, including the periodontal ligament, alveolar bone, and cementum. These precursors have the potential for unlimited or prolonged self-renewal.

Embryonic and adult stem cells : involvement in repair

Embryonic stem cells are the cells of the inner mass of a blastocyte, at an early stage embryo. They reaches this stage at 4-5 days post-fertilization, and consists of 50-150 cells. Formed by four layers: ectoderm, neuroectoderm, endoderm, pluripotent stem cell lines are derived directly from early mouse embryos (Smith A. 2001). They are issued from the implantation in the uterus until the end of the second month of gestation. The blastocyte is formed and cultured approximately 5 days after fertilization. The oocyte fertilized is totipotent. Successive divisions generate the morula with 32-64 totipotent cells. Once the gastrula established, embryonic stem cells may be maintained in permanent culture, and transported between laboratories. In bone marrow, there is roughly one stem cell per 100 000 cells. Such cell displays restricted differentiation potential and poor growth. They are limiting the applicability of embryonic stem cells to tissue engineering.

Stem cells have the capacity of self-renewal and differentiation (Bishop., *et al*. 2002). Dental pulp stem cells play role in tissue engineering. They play role and opportunities for translational research (El Backly & Marei, 2017).

Characteristics of ESCs: They may be maintained for several passages at undifferentiated state, maintaining a normal chromosomal composition. Surface markers, such as CD9, CD24, alkaline phosphatase and genes involved in pluripotency including Oct-4, Rex-1, SOX-2, Nanog, LIN28, Thy-1, and SSEA-3 and -4 have been identified. A human embryonic stem cell is also defined by the expression of several transcription factors and cell surface proteins. The transcription factors ensure the suppression of genes that lead to differentiation.

Adult stem cells do not display the characteristic morphology or surface markers allowing to distinguish between SC and mature cells. CD34- or CD133-positive, CD-38 and lineage-negative populations contains other cell types and hematopoietic stem cells, also present in "negative" populations. Niches are composed by other cells, extracellular matrix and signaling factors displaying therapeutic potential (Chagastelles & Nardi, 2011). We note that iPSCs and ESCs have many similar properties, such as pluripotency and differentiation potential. The high proliferative characteristics of multipotent bone marrow stromal cells (BMSCs) and their potential leads these cells to develop into osteoblasts, chondrocytes, adipocytes, in muscle and neural tissues. They maintain their ability to differentiate into multiple stromal cell lineages.

Clonogenic and highly proliferative cells were disaggregated enzymatically. They were termed DPSCs and compared to bone marrow cells (BMSCs). Cells express similar mineralized matrix proteins, such as dentin matrix protein 1, fibronectin, different collagen type, alkaline phosphatase, osteonectin, osteopontin, bone sialoprotein, and osteocalcin. Postnatal dental pulp contains cells that are capable of regenerating a tissue, properties that effectively

define them as stem cells. The existence of stem cells in the adult organism and postulation of their biological niche is substantiated (Baksh., *et al*. 2004).

A protocol has been proposed for identifying the subpopulations of dental pulp pluripotent-like stem cells (DPPSC). These cells are SSEA4+, OCT3/4+, NANOG+, SOX2+, LIN28+, CD13+, CD105+, CD34-, CD45-, CD90+, CD29+, CD73+, STRO1+ and CD146-. They show genetic stability in vitro. DPPSCs can differentiate in vitro into tissues that have similar characteristics to mesoderm, endoderm and ectoderm layers.

Post-natal bone marrow stromal stem cells are composed of two systems forming distinct lineages: the hematopoietic tissue proper and the associated supporting stroma. The two are separated in distinct stem cells cooperating functionally. Mitogenic factors stimulate the proliferation of CFU-F. They include platelet-derived growth factor, epidermal growth factor (EGF), basic fibroblast-growth factor, transforming growth factor-β and insulin-like growth-factor. Some colonies form nodules identified by alizarin red or von Kossa staining. Others cells accumulate fat, identified by oil red O staining, and some colonies form cartilage as identified by alcian blue staining.

The role of niches in adult stem cells in dental repair and regeneration

The MSC niche was proposed as a specialized micro-environment needed to retain their « steamness ». Endosteal niche maintain HSB quiescence over the long term, whereas the perivascular niche maintains HSB proliferation and mediate circulation (Baksh., et al. 2004). The DPSC niche was identified by antibodies against STRO-1, CD146, and pericyte-associated antigen (3G5). It was found in the perivascular and perineural sheath regions. These STRO-1+/ CD146+ DPSC staining of the perivascular region with small clusters of cells in the extravascular regions. The potential mechanisms are the capacity of MSCs to down-modulate immune reactions executed by T-, dendritic NK and B-cells. Some reports showed that DPSCs have osteogenic potential and may form bone-like structure *in vitro* and *in vivo* (Laino., *et al*. 2005, 2006; d'Aquino., *et al*. 2007). However, there has been no evidence demonstrating that BMSCs can give rise to functional odontoblasts and generate dentin.

Conflicts of interest : The author declares no conflict of interest

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