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# Adult Mesenchymal Stem Cells Explored in the Dental Field

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**Abstract** During the last decade it was realized that stem cell-based therapies hold an enormous therapeutic potential, improving the life of patients with conditions ranging from neurodegenerative and traumatic diseases to regenerative medicine requiring replacement of complex structures such as bones and teeth. Based on their ability to regenerate and/or repair damaged tissue and eventually restore organ function, multiple types of stem/progenitor cells have been discovered. In the field of periodontal regeneration and tooth engineering, several types of adult multipotent mesenchymal stem cells from various sources are currently being investigated. These include the bone marrow stromal stem cells (BMSSCs), adipose-derived stromal cells (ADSCs), dental pulp stem cells (DPSCs), dental follicle stem cells (DFSCs), stem cells from human exfoliated deciduous teeth (SHEDs), stem cells from the apical papilla (SCAP), periodontal ligament stem cells (PDLSCs), alveolar bone proper-derived stem cells, and gingival stem cells.

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The potential of these different MSCs as precursors for regenerative purposes in the dental field is discussed in this chapter.

**Keywords** Adipose-derived stromal cell · Alveolar bone proper-derived stem cell · Bone marrow stromal stem cell · Dental follicle stem cell · Dental pulp stem cell · Gingival stem cell · Mesenchymal stem cell · Periodontal ligament stem cell · Periodontium · Stem cells from human exfoliated deciduous tooth · Stem cells from the apical papilla

## Contents



## 1 Introduction

Over the last decade, various medical disciplines have begun to explore the possible applications of stem cells and tissue engineering in the fields of repair and regeneration of damaged/injured tissues of the human body. The defined long-term goal is to make regenerative medicine take its place in clinical practice as an important future therapeutic modality.

Stem cells are capable of self-renewal through mitosis and they can give rise to cells that have the potential to differentiate into specialized cell types. Embryonic stem cells (ESCs) are pluripotent and can differentiate into almost every cell type of the human body. However, due to ethical and legal issues the use of ESCs is controversial, thus restricting their application for regenerative purposes in the clinic. Unlike ESCs, adult stem cells have the potential to be used for the treatment of various diseases. They have several advantages over ESCs: (i) their use is less problematic because they can be retrieved without destroying an embryo (ii) they reside in almost all tissues of the human body including dental tissues, and

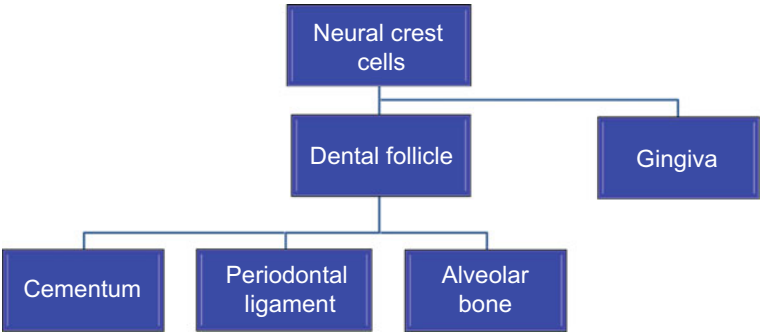


**Fig. 1** Developmental origin of the dental tissues

(iii) their use in an autologous setting circumvents any problems with rejection by the host immune system.

Adult stem cells, also known as mesenchymal stromal cells, mesenchymal stem cells, or multipotent stromal cells (MSCs) are a heterogeneous subset of pluripotent stromal cells that can be isolated from many different adult tissues and demonstrate the potential to give rise to cells of various lineages [13]. These cell populations do not develop to sizable proportions under normal culture conditions but their isolation and expansion requires enriched specific culture media under special inductive culture conditions.

Morphologically, MSCs may be either large and flat or elongated and fibroblastlike. This is not a defining or distinguishing feature of these cells. Their identification is based on the positive expression of specific surface markers (CD44, CD73, CD90, CD105, CD106, STRO-1) and the absence of expression of hematopoietic cell surface markers (CD34, CD45, CD11a, CD19) and HLA-DR, as well as on their ability of self-renewal and multipotency. Human MSCs display plastic adherence under standard culture conditions and can form colonies (i.e., they are clonogenic). Their multipotent nature is evident from the ability to differentiate along various lineages including those for osteoblasts, adipocytes, myelosupportive stroma, chondrocytes, and neuronal cells in response to specific stimuli [13].

The neural crest cells, a transient, migratory, multipotent cell population in vertebrates, participated in the embryonic development of most dental tissues including the gingiva, the dental follicle, the periodontal ligament, and the alveolar bone (Fig. 1). Several adult cell populations with stem cell properties have recently been isolated and partially characterized from these tissues. The intention of this review is to give an overview of the stem cell types investigated in the dental field including their tissue sources, properties, differentiation potential, and comparative assessment of their advantages for tissue engineering.

## 2 Types of Adult Stem Cells Explored in the Dental Field

### *2.1 Bone Marrow Stromal Stem Cells (BMSSCs)*

In addition to hematopoietic progenitors or stem cells (HSCs), the bone marrow contains bone marrow stromal stem cells (BMSSCs) that give rise to nonhematopoietic tissues. BMSSCs are bone marrow cell populations that were the first mesenchymal stem cells to be isolated exploiting their property to adhere to tissue culture plastics [18].

BMSSCs have been isolated and characterized from the extra [18, 76, 6, 42, 69] as well as the intra- oral [1, 27, 51] bone marrow. They are cable of forming colony-forming unit-fibroblasts (CFU-Fs) in vitro [18] and express Oct-4, Nanog, STRO-1, CD73, CD90, CD105, CD146 and are negative for CD14, CD34, CD45 and HLA-DR [19, 13, 74, 4, 24]. They are capable of differentiation into multiple mesenchymal lineages including osteoblasts, adipocytes, chondrocytes, muscle cells, tenocytes, or nerve cells [6, 69, 49, 39, 10, 42, 80, 18].

BMSSCs cultures usually encompass a mixture of fibroblasts, osteoblasts, adipocyte progenitors and reported range of up to 4-19% stem cells [66]. The majority of attempts to engineer teeth initially employed purified BMSSCs cell populations [65]. Indeed, bone as well as soft tissues could be formed from heterogeneous populations. Ohazama and colleagues [61], were able to generate tooth-like structures after transferring whole transplants from bone marrow-derived cells into the renal capsule. Moreover, they amalgamated embryonic oral epithelium with three types of stem cells, namely neural stem cells, ESCs, and adult bone marrow-derived cells. They transferred the combination into adult jaw and renal capsules and observed formation of tooth-like structures and bone. A study conducted by Li and coworkers [45] yielded similar results, demonstrating that the combination of oral epithelial cells from rat embryos with BMSSCs resulted in the expression of a variety odontogenic genes such as Pax-9, dentine sialophosphoprotein (DSPP), and dentine matrix protein 1 (DMP1) and histologically produced tooth-like structures.

In the field of periodontal regeneration BMSSCs have shown great promise. The auto-transplantation of BMSSCs in an animal study resulted in almost complete regeneration of periodontal defects in only four weeks. Histologically, the presence of cementum, periodontal ligament (PDL), and alveolar bone was confirmed [35]. Therefore, BMSSCs represent a competitive MSC source for the regenerative treatment of periodontal diseases, despite showing a major limitation in their application, having a strongly age-dependent differentiation capability which considerably decreases with increasing donor age [33].

## 2.2 Adipose-derived Stromal Cells (ADSCs)

Adipose tissues represent a readily available source of multipotent post-natal stem cells first described in 2001 [91]. Adipose-derived stromal cells (ADSCs) are characterized by stable proliferation doubling kinetics in vitro [65]. The good accessibility and tissue abundance is clearly an advantage of ADSCs. ADSCs can be obtained via minimally invasive methods, including the increasingly popular cosmetic liposuction procedure, and in larger quantities than BMSSCs, making their utilization as a stem cell source very attractive [91].

In accordance with the criteria for multipotent stromal cells defined by Dominici et al. [13], ADSCs exhibited a multilineage differentiation potential into osteogenic, chondrogenic, and adipogenic directions in vitro [46] and were able to form osteoid matrix [28] and bone [34] in vivo. ADSCs further strongly expressed multiple important bone marker proteins including alkaline phosphatase (ALP), type I collagen, osteopontin, and osteocalcin [83].

In 2008 Jing and co-workers found that ADSCs could be differentiated into the odontogenic lineage and might represent a promising alternative for seeding cells for tooth regeneration to replace lost teeth in elderly patients [33].

In the field of tooth tissue engineering, a recent study further demonstrated that incubating primary cultures of human ADSCs in a dental-inducing medium and subsequently culturing the aggregates in three-dimensional conditions can transdifferentiate the cells to produce a specific three-dimensional organization and phenotype resembling a dental bud in vitro [16].

## 2.3 Dental Pulp Stem Cells (DPSCs)

It is well known that upon pulpal injury, reparative/tertiary dentine forms as a protective barrier for the pulpal chamber [59]. This natural regenerative aptitude of the dentin/pulp complex points to the possibility that dental pulp may contain stem cells or progenitors responsible for its regeneration/repair [65].

Dental pulp stem cells (DPSCs) were first identified by Gronthos et al. [23] who showed that DPSCs from CFU-F and could produce dentine-pulplike structures. DPSCs when compared to BMSSCs cultured under the same conditions showed a higher proliferation rate which could be attributed to their strong expression of cyclin-dependent kinase 6 [74].

The expression by these cells of a range of perivascular cell markers including STRO-1, CD146/MUC-18, VCAM-1, and α-smooth muscle actin pointed to the fact that DPSCs are located in the perivascular niches within the dentin/pulp complex and represent a heterogeneous population of MSCs [23, 74].

DPSCs possess a self-renewal capability and multilineage differentiation potential into chondrocytes, adipocytes, odontoblasts, and neural-like cells under appropriate induction conditions [21, 31, 29]. DPSCs loaded on a hydroxyapatite/

tricalcium phosphate (HA/TCP)-scaffold formed bone after transplantation in immunocompromised mice. In addition, it was revealed that even after two years of storage, DPSCs were still able to differentiate into pre-osteoblasts and form woven bone, while preserving their cellular integrity [64, 63]. A recent study showed that the Coculture of dental pulp stem cells with endothelial cells enhances osteo-/odontogenic and angiogenic potential in vitro with greater ALP activity, greater amount of calcification, higher expression of ALP, BSP, and DSPP genes and stabilized vessel-like structures formed by endothelial cells [12]. A further study demonstarted that DPSCs derived from maxillary premolar in combination with anorganic scaffolds could regenerate experimentally-created periodontal defects [54].

Yet, in a contrasting study by Zhang and colleagues, DPSCs seeded onto three-dimensional spongeous collagen, fibrous titanium mesh, and porous ceramic scaffolds, and implanted in nude mice for six or twelve weeks did not form the expected dentine-pulplike complex but differentiated into tissues that resembled connective tissue [89].

## *2.4 Dental Follicle Stem Cells (DFSCs)*

The dental follicle is a mesenchymal component that surrounds the tooth germ during development in its socket prior to eruption [65] and from which cementum, PDL, and alveolar bone arises through complex interactions [87]. Dental follicle stem cells (DFSCs) were initially isolated from follicles of human impacted third molars scheduled for extraction. They were shown to express the stem cell markers STRO-1, Notch-1, and nestin [55, 56]. DFSC cell lines were found to be heterogeneous and to consist of three main lineages: a highly undifferentiated, periodontal ligament type lineage, a cementoblastic, and an osteoblastic lineage [48].

DFSCs, similar to other MSCs, demonstrated a multilineage differentiation ability into osteoblasts/cementoblasts [83, 36], adipocytes, and neurons [36, 57, 86, 9] as well as PDL-like tissue [87].

Compared to DPSCs, DFSCs showed a faster proliferation rate (as was evidenced by a higher number of population doublings), a greater percentage of cells expressing the surface marker STRO-1, and an increased capacity for in vivo dentine regeneration. However, DFSCs exhibit telomerase activity, a characteristic feature of ESCs [77, 78, 85]. Telomerase is an enzyme that adds DNA sequence TTAGGG to the 5' end in the telomere regions of the chromosomes. Normally the telomere region in each chromosome is shortened with every replication cycle (mitosis). Due to the action of telomerase in some cells expressing it, including ESCs and cancer cells, this region is not significantly shortened during mitosis and aging of the chromosomes is hindered, which principally confers immortality to the cells. Whether this expression is an advantage or may pose a potential risk for malignant tumor formation similar to the situation in ESCs in tissue engineering still needs to be extensively investigated.

## 2.5 Stem Cells from Human Exfoliated Deciduous Teeth (SHEDs)

Stem cells from human exfoliated deciduous teeth (SHEDs) were identified in freshly exfoliated deciduous teeth containing living pulp remnants by Miura and colleagues. They linger alive inside the dental pulp for a very short time after tooth exfoliation during which they can be harvested, representing an interesting and easily accessible stem cell source.

SHEDs show major advantages over other types of MSCs including a higher proliferation rate compared to DPSCs and BMSSCs, (SHED > DPSCs > BMMSCs) [30], a similar multilineage differentiation capacity to other MSCs with the ability to differentiate into neurons, adipocytes, osteoblasts, and odontoblasts, in addition to easier accessibility with little or no morbidity [40, 53].

SHEDs express CD146/MUC18 and STRO-1 similar to other MSCs [74] and a variety of osteoblastic and odontoblastic markers including Runx2, ALP, matrix phosphoglycoprotein, bone sialoprotein (BSP), and DSPP. They further exhibit the embryonic stem cell markers Nanog, Oct4, stage-specific embryonic antigens (SSEA-3, SSEA-4), and tumor recognition antigens (TRA-1-60 and TRA-1-81) [37].

SHEDs show adipogenic, neurogenic, myogenic as well as chondrogenic differentiation potential similar to other stem cell populations [37, 53]. Regarding their osteogenic potential, Miura et al. [37] stated that SHEDs could not be differentiated directly into osteoblasts, but had distinctive osteoinductive abilities, inducing new bone formation by recruiting host osteogenic cells. In contrast, Cordeiro and co-workers showed that when SHEDs were seeded in poly-*L*-lactide acid (PLLA)-scaffolds and transplanted into the subcutaneous tissue of immunodeficient mice, they differentiated into odontoblast like cells and into blood vessels that anastomosed with the host vasculature forming a continuous vascular supply to the newly implanted construct. These studies show that SHEDs might be promising source of stem cells for tooth structure repair and bone regeneration [65].

## 2.6 Stem cells from the apical papilla (SCAP)

Stem cells from the apical papilla (SCAP) were first described in 2008 [78]. Compared to DPSCs and BMMSCs, SCAP showed similar osteo/dentinogenic with lower adipogenic differentiation potential. SCAP further expressed a higher proliferation rate and mineralization potential compared to DPSCs [2]. Similar to other stem cell populations, SCAP expressed STRO-1 and CD146, were positive for CD34 and negative for CD45 as well as showed multiple dentinogenic markers including ALP, bone sialophosphoprotein, osteocalcin [2], and the growth factors TGFbetaRI and FGFR1 [78]. Compared to DPSCs, SCAP express lower levels of DSP, matrix extracellular phosphoglycoprotein (MEPE), transforming growth factor $\beta$ receptor II (TGF$\beta$RII), FGFR3, Flt-1 (VEGF receptor 1), Flg (FGFR1), and melanoma-

associated glycoprotein (MUC18) [30]. Upon stimulation with a neurogenic medium, SCAP expressed neurogenic markers as nestin and neurofilament M [78].

## 2.7 Periodontal Ligament Stem Cells (PDLSCs)

The periodontium, one of the highly specialized and complex connective tissues of the human body, is derived from the dental follicle and the neural crest cells [65]. The PDL harbors a heterogeneous population of progenitor cells [44, 58], which are thought to be responsible for maintaining tissue homeostasis and to play a crucial role in periodontal regeneration [5]. A study by Seo and colleagues initially identified and characterized human PDL-derived stem cells from extracted teeth as periodontal ligament stem cells (PDLSCs).

PDLSCs exhibited an approximately 30 % higher number of population doublings compared to BMSSCs. It appeared that PDLSCs retain this high growth potential beyond 100 population doublings before they become senescent, compared to approximately 50 population doublings for BMSSCs [3]. In addition, PDLSCs showed a higher frequency of fibroblastic colony-forming units (aggregates of 50 cells or more) than that noted for BMSSCs (170 for PDLSCs and 14 for BMSSCs per $10^5$ cells plated; [72].

PDLSCs express the stem cell markers STRO-1 and CD146/MUC18 [72, 84] entailing a perivascular origin similar to all MSCs. A proportion of PDLSCs also co-expressed α-smooth muscle actin (similar to DPSCs), the pericyte-associated antigen 3G5, and were negative for the hematopoietic markers CD14, CD45, and CD34 [3]. PDLSCs express mature mineralized tissue markers such as ALP, type I and III collagens, osteonectin, osteopontin, osteocalcin, and BSP [22, 32, 72, 75] and high levels of scleraxis, a tendon-specific transcription factor associated with tendon cells [60, 72]. PDLSCs are multipotent, possessing the ability to differentiate into adipocytes, cementoblast like cells, osteoblasts, and collagen-forming cells [72].

Multiple studies on PDLSCs confirmed their aptitude for tissue regeneration and periodontal repair [38, 46, 72]. In the study by Seo et al. [72], human PDLSCs were loaded onto a HA/TCP-scaffold and subcutaneously implanted into immunocompromised mice, resulting in a cementum and PDL-like structure being produced. Orciani and colleagues demonstrated that osteogenically differentiated cells were marked by an increase in $Ca^{2+}$ and nitric oxide production and that the implantation of PDLSCs together with a nitric oxide donor could be a promising regimen for periodontal regeneration [62]. When PDLSCs were transplanted into surgically created periodontal defects, these cells were reported to integrate into the PDL, connect to the surrounding alveolar bone and cementum via Sharpey's fibers and regenerated the experimental defects [11, 47, 72].

This characteristic feature of PDLSCs to produce cementum and PDL-like tissue [72], in contrast to the dentine or pulplike structure and lamellar bone and

marrowlike structure generated by DPSCs and BMSSCs, respectively [23, 25, 42], verified that PDLSCs embody a distinctive MSC population [9].

Recently, Park and co-workers successfully isolated and characterized human PDLSCs from healthy (hPDLSCs) and inflamed (ihPDLSCs) PDL tissues and evaluated their regenerative potential. Both ihPDLSCs and hPDLSCs were successfully differentiated under an osteogenic/cementogenic and adipogenic microenvironment. The proliferative potential did not differ between healthy hPDLSCs and ihPDLSCs.

## *2.8 Alveolar Bone proper-derived Stem Cells*

The alveolar bone proper similar to the PDL is embryonically derived from the dental follicle. Recently, a scheme for the minimally invasive isolation of alveolar bone margin-derived stem cells was introduced [14]. The isolated cells showed plastic adherence and colony formation, and expressed the surface markers CD73, CD90, CD105, STRO-1, and CD146/MUC18, while lacking the expression of the hematopoietic markers CD14, CD34, and CD45. The cells could be differentiated into osteoblastic, adipocytic, and chondroblastic lineages and demonstrated a high expression of ALP, type I, III, and V collagens. The isolation scheme of alveolar bone margin-derived stem cells described in this study constituted a conservative alternative to many previously described isolation techniques for adult stem/progenitor cells from the dental pulp or periodontal ligament [20, 72, 77, 81] as well as the intra- [1, 27, 51] and extra-oral [41, 43] bone marrow. Further studies are needed to verify the regenerative potential of these cells as well as to compare them with other stem cell populations.

## *2.9 Gingival Stem Cells*

Representing a key component of the periodontium, one of the gingiva's eminent characteristics is its remarkable regenerative and wound healing capacity with a rapid reconstitution of tissue architecture, with little evidence of scarring [7]. The multiple functions of gingival fibroblasts, their diversity in responsiveness to growth factors and in the ability to produce specific extracellular matrix proteins during healing, demonstrated that gingival connective tissue fibroblasts constitute a heterogeneous population of cells [26, 67, 68, 70, 71]. This also entails the existence of a population of stem/progenitor cells that give rise to these heterogeneous cell populations. Because the lamina propria of oral mucosa including alveolar mucosa, gingival, and palatal mucosa originates from the embryonic neural crest this may suggest that a primitive population is retained in the adult human gingiva. Recent studies reported on the isolation of progenitors from the oral soft tissue, such as the rugae and incisive papillae of the palate [82], the maxillary tuberosity [52], the oral mucosa [50], and the attached gingiva [17, 79].

The isolated gingival stem cells expressed CD73, CD90, and CD105 and lacked expression of CD14, CD34, and CD45. They demonstrated a multilineage differentiation capacity into adipogenic, osteogenic, and chondrogenic lineages [52]. The immunomodulatory properties of gingival margin-derived stem cells were exploited experimentally in the therapy of inflammatory destructive diseases including arthritis and colitis through inhibiting the proliferation of T-lymphocytes and promoting the proliferation of regulatory T cells [89]. A recent study further demonstrated a remarkable periodontal regenerative potential of these cells in conjunction with collagen and demineralized bovine cancellous bone matrices [15]. Human gingiva is one of the most convenient tissues for biopsy and is considered an ideal source for stem cell isolation. The major advantage of this stem cell source appears to be the ability to obtain a large quantity without the need to sacrifice a tooth irreversibly to obtain its pulp, periodontal ligament, or dental follicle.

## 3 Concluding Remarks

Dental stem cells offer several advantages and promising facets over other types of stem cells, for example, a high proliferation rate, easy accessibility, and a relative ease of differentiation induction into distinct cell lineages. There is still much to learn about the nature, basic biology, and developmental potency of dental stem/progenitor cells. However, the perspectives for their exploitation in dental tissue regeneration are far-reaching. It is to be hoped that a better understanding of their biology will result in significant benefits for the management of dental diseases in patients.

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