# Stem Cells from Dental Tissue for Regenerative Dentistry and Medicine

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Patricia H.L. Pranke, Luciano Casagrande, and Simone Bonato Luisi

## Introduction

Tissue engineering is a multidisciplinary science that aims to develop biological substitutes for tissues and organs in order to restore their functions in cases of injuries and deformities. In recent years, dentistry has explored the potential of tissue engineering through basic and translational research in order to be able to repair oral tissue damaged by biological substitutes. This new science is based on the balanced use of responsive cells, capable of differentiating in tissue of interest; biocompatible matrices, those that will support cell growth and mimic the extracellular matrix; and bioactive molecules responsible for morphogenetic signals. Currently, stem cell research has grown significantly, due to the recognition that therapies based on stem cells have the potential for treating patients under different conditions, such as bone tissue deficiency and ischemic heart disease, and even in severe conditions, such as Alzheimer's disease and leukemia. Current evidence has demonstrated that stem cells are found in certain niches and some tissues contain more stem cells than others. Since the discovery that dental tissue can become a source of mesenchymal stem cells, a vast field of research has opened and there are promising opportunities for regenerative therapies. Due to its favorable characteristics, such as low risk of tumor formation, sources of relatively easy access, and lower ethical issues related to their use, stem cells from dental tissue are being considered as promising for clinical use in both medical and dental applications. The areas, such as endodontics, periodontics, oral surgery, and dental implants, are increasingly exploring the potential of dental tissue engineering in search of better and more advanced treatment options for their patients. Thus, this chapter aims to introduce the basic concepts of tissue bioengineering in dentistry, focusing on the isolation methods and characteristics presented by stem cells from dental tissue as well as its potential use in cell-based therapy.

P.H.L. Pranke, PhD (✉)
Department of Analysis, Universidade Federal do Rio Grande do Sul, Av. Ipiranga 2752, 304G, Porto Alegre, Rio Grande do Sul 90610-000, Brazil
e-mail: patriciapranke@ufrgs.br

L. Casagrande, DDS, MS, PhD
Department of Surgery and Orthopedics – Pediatric Dentistry, Federal University of Rio Grande do Sul, Ramiro Barcelos, 2492, Porto Alegre, Rio Grande do Sul 90035-003, Brazil
e-mail: luciano.casagrande@ufrgs.br

S.B. Luisi, MD, PhD
Conservative Dentistry Department, School of Dentistry – Rio Grande do Sul Federal University, Ramiro Barcelos, 2492, Porto Alegre, Rio Grande do Sul 90035-003, Brazil
e-mail: simoneluisi@hotmail.com

## Sources of Mesenchymal Stem Cells in Dental Tissue

Stem cells are commonly defined as clonogenic nonspecialized cells that have the ability to divide continuously by means of self-renewal and generate progenitor cells that differentiate into several cell lines [1]. These characteristics make a distinction between stem cells and restricted progenitors (e.g., circulating endothelial cell progenitor cells) or differentiated cells, which have a more narrow developmental potential and a reduced ability to proliferate [2]. These cells can be classified according to their origin, such as embryonic, adult, and, most recently, induced pluripotent stem cells (iPSCs). The embryonic stem cells (ESCs) are found in the early stages of embryonic development [3]. The cells in the embryos until 3 days are considered totipotent, and the cells from the inner mass of the blastocyst are pluripotent cells. Despite the great regenerative potential of these cells, their isolation and use face ethical and legal barriers [4] which reduce their attractiveness within the regenerative therapies [5].

The ethical issues surrounding the use of embryos to obtain stem cells and the possibility of these cells undergoing transplant rejection in patients generated the idea of

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producing pluripotent cells from non-stem cells of the patient himself/herself, reducing the risk of transplant rejection. It has been demonstrated that somatic cells can be reprogrammed by transferring their nuclear contents into oocytes [6], or by fusion with ESCs [7, 8]. This possibility indicates that eggs and ESCs contain factors that confer totipotency or pluripotency. It was then assumed that these factors would also have a crucial role in the induction of pluripotency in somatic cells. This hypothesis was confirmed by using fibroblasts from mice, transfected with transcription factors responsible for the maintenance of the pluripotency to generate iPSCs [9].

Adult stem cells (ASCs) are stem cells found in several different formed tissues, including the bone marrow, blood, brain, cord blood, and many other organs, which give rise to different tissues. There are different types of ASCs with different properties. One of the most studied types of ASCs is the mesenchymal stem cells (MSCs). These cells are considered to be an attractive source of cells for regenerative therapies [10, 11] because they have unique plasticity when exposed to different environments. Furthermore, clonogenic cells are capable of self-renewal and differentiation into multiple lines [12]. The first MSCs to be isolated and characterized were from the bone marrow (BMMSCs) [13]. These cells showed that they have the potential to differentiate into osteoblasts, chondrocytes, adipocytes, and myelosupportive fibrous stroma [10]. MSCs were isolated from various tissues, such as the brain, skin, hair follicle, skeletal muscle, and pancreas [14–16].

In 2000, MSCs were isolated from the pulp tissue of permanent teeth [17]. This discovery opened up a wide range of possibilities for the application of regenerative therapies based on the use of stem cells in oral tissue engineering. So far, five types of human stem cells of dental origin have been isolated and characterized: stem cells from the dental pulp (dental pulp stem cells – DPSCs), stem cells from the exfoliated deciduous teeth (stem cells from human exfoliated deciduous teeth – SHED), stem cells of the periodontal ligament (periodontal ligament stem cells – PDLSCs), stem cells from the apical papilla (stem cells from apical papilla – SCAP), and progenitor cells of the dental follicle (dental follicle progenitor cells – DFPCs). These populations of dental stem cells share characteristics common to other populations of mesenchymal stem cells [10].

The plasticity of stem cells is assessed by their capacity for self-renewal and proliferation and differentiation in many different directions. The comparison of the potential of proliferation, differentiation, and osteoinductive capacity of BMSCs, DPSCs, and SHED showed a superior cell proliferation in favor of the pulp and in particular of the primary teeth [18]. The great proliferative capacity and differentiation of stem cells from pulp tissue of primary teeth have raised interest from many areas. Recent evidence suggests the possible usefulness of dental pulp stem cells in the treatment of diseases and disorders such as muscular dystrophy, bone defects, and large jaw calvaria. It also aids in the repair of lesions of the cornea and shares similar characteristics of the liver cells. The dental pulp stem cells' plasticity will be commented on afterwards [19, 20].

## The Role of a Microenvironment for Pulp Stem Cell Differentiation: Growth Factors and Cytokines Determining the Stem Cells' Fate

Growth factors and bioactive molecules are morphogenic proteins or factors which bind to specific cell membrane receptors and trigger a variety of signaling pathways that coordinate in an orderly manner all cellular functions. Several growth factors are used to control the activity of the stem cells by increasing the rate of proliferation, inducing differentiation into another cell types, or stimulating the cells to synthesize and secrete a mineralized matrix. Most of these factors operate at very low concentrations although they are sufficient to induce changes in gene expression [21].

These molecules play a critical role during embryonic development by determining the fate of the stem cells and regulating the development of all organs and tissues. The same factors that modulate the growth of embryogenic tissue can also be used therapeutically to guide the differentiation of stem cells to various destinations and coordinate the cellular processes that ultimately result in the generation of a new organ or tissue. More specifically, there are many similarities between the factors involved in tooth formation and the molecules that regulate the formation of reactionary dentine [22].

The dentine matrix contains growth factors and cytokines (members of TGF-b superfamily, BMP, FGF, IGF, VEGF, Hedgehog, Wnt, and other genes and transcription factors) that are sequestered during dentinogenesis [23, 24]. Following physiological stimulation or injury, such as caries, trauma, and operative procedures, these molecules are released by acids with other extracellular matrix components [25]. Interestingly, when these molecules are released from the dentine, they are fully capable of inducing cellular responses, such as those which lead to the generation of reactionary dentine [26–29]. The arrangement of the tubular dentine facilitates the movement of growth factors released by etching agents, carious lesions, or pulp-capping materials from the dentine matrix to the pulp.

Even after its complete formation, the dentine-pulp complex retains the ability to respond to different stimuli [29–32]. Dentinal repair occurs through the activity of specialized cells, called odontoblasts, which are thought to be maintained by an undefined precursor population associated with pulp tissue [29].

Under injury of medium intensity, such as superficial dentin carious lesion, trauma, and restorative procedures, the odontoblasts increase their secretory activity, producing mineralized matrix. However, this activity is directly related to the maintenance of a viable population of cells near the injury site,

resulting in the deposition of mineral matrix and the growing distance between the carious lesion and pulp tissue [33].

With the progression of carious lesions, the layer of odontoblasts underlying carious lesions succumbs, and with prevailing favorable conditions of pulp vitality, a new cell population is recruited to the region and begins to produce mineralized tissue [32]. These events involve the recruitment of dental pulp stem cells (DPSCs) and their differentiation into functional odontoblasts, which secrete in mineralized matrix [26, 34].

Studies on mechanisms underlying the differentiation of dental pulp stem cells are critical for the understanding of the biology of dental tissue engineering. Recent research has produced important evidence concerning the role of bioactive molecules that are present in the dentine and which induce differentiation of pulp stem cells into odontoblasts [35]. It was observed that when SHED were seeded onto a polymeric-based scaffold made inside the pulp chamber of a dentine disk treated with EDTA, the cells expressed odontoblast differentiation markers, such as DMP-1, DSPP, and MEPE. In contrast, when SHED were seeded onto scaffolds surrounded by a deproteinized dentine disk (long-term treatment of sodium hypochlorite), no evidence of differentiation was found. This finding also demonstrated that that dentine-derived BMP-2 is required to induce the differentiation of SHED into odontoblasts.

It has been shown that stem cells from human exfoliated deciduous teeth can also differentiate into functional odontoblasts and endothelium [36]. When SHED were seeded onto tooth slice/scaffolds and implanted subcutaneously into immunodeficient mice, they differentiated into functional odontoblasts which generated the tubular dentine, as determined by tetracycline staining and confocal microscopy. These cells also differentiated into vascular endothelial cells, as determined by beta-galactosidase staining of LacZ-tagged SHED. The vascular endothelial growth factor (VEGF) induced SHED to express endothelium markers (VEGFR2, CD31, and VE-cadherin) and to organize the cells in vitro into capillary-like sprouts. Collectively, these experiments demonstrate that SHED can differentiate into endothelial cells that produce capillaries and odontoblasts capable of generating the tubular dentine, suggesting that the dentine matrix microenvironment plays a role in the fate of stem cells.

These basic areas of research involving cellular and molecular biology will provide guidelines for future translational experiments, targeting the regeneration of oral structures through the differentiation of stem cells of dental origin.

## Scaffolds for Dental Pulp Stem Cells

The eukaryotic cells require interactions with their microenvironment to survive, proliferate, and maintain their activity. In tissue physiology, it is the extracellular matrix proteins which mainly form this "three-dimensional" environment. In tissue engineering, these "3D" structures are initially supplied to the cells through the use of biocompatible and biodegradable matrices, known as scaffolds [37]. They provide a favorable microenvironment for adhesion, migration, cell growth, and differentiation and allow the transport of nutrients, oxygen, and waste products. Therefore, the scaffolds are considered a critical component in tissue engineering [31, 38].

Depending on the purpose of application, the scaffolds can be synthetic or organic, biodegradable, or permanent [38, 39]. Matrices made of synthetic polymers allow the manipulation of their physicochemical properties, such as rate of degradation, pore size, and mechanical strength. Synthetic polymers most common in tissue engineering are the poly-L-lactic acid (PLLA), polyglycolic acid (PGA), and their copolymers such as poly-lactic-co-glycolic acid (PLGA). These scaffolds are biodegradable and biocompatible and allow cell growth and differentiation, making them highly suitable for applications in tissue engineering [40–42]. The rate of degradation can be controlled by the ratio of PLLA/PGA used in the manufacture of such matrices. Notably, it is important that the rate of degradation of the scaffold is compatible with the rate of formation of the tissue. In other words, the matrix must be programmed to ensure the structural integrity of the cells used in tissue engineering until the newly formed tissue can become self-sustaining [43].

One of the earliest successful examples of dental tissue engineering was the use of copolymers (PGA/PLLA and PLGA), which allowed the formation of complex tooth structures with characteristics similar to natural tooth crowns [42] Furthermore, the PLLA base scaffolds have been extensively used in the study of pulp tissue engineering [35, 36, 44]. These PLLA matrices are prepared inside the pulp chamber of tooth slices from extracted third molars. The dental pulp stem cells are seeded within the matrix/dentine slice and transplanted into the subcutaneous space of immunodeficient mice [35, 36, 45, 46].

Studies have shown that 21–28 days after transplantation, the dental pulp stem cells from the permanent (DPSCs) and deciduous (SHED) teeth seeded onto this model (polymeric matrix/dentine disk) produced tissue with morphological characteristics resembling those of natural human teeth pulp [45]. From the point of view of translational research, the matrices designed for dental pulp tissue engineering should be based on injectable materials. The purpose of these injectable scaffolds would allow stem cell transplantation to the fullest extent of the root canal and pulp chamber. An excellent example of this approach was recently described by Galler and colleagues [47]. In this case, the production of Self-assembling Multidomain Peptide hydrogels was characterized as biocompatible and injectable. Interestingly, with the addition of a specific cleavage site of matrix metalloproteinase-2 (MMP-2) and dendrimer cell adhesion (RGD), survival and cell motility have been improved in these hydrogels.

In summary, the development and characterization of these scaffolds are rapidly becoming extremely attractive in the new field of dental materials, and it is, therefore, an emerging area which will certainly play a critical role in translating the laboratory results for implementation approach-based cellular therapies and tissue engineering in dental treatment.

## Blood Vessels and Tooth Tissue Regeneration

The establishment and maintenance of a vascular supplement is an essential requirement for the natural development of tissue. Vasculogenesis is defined as the formation of new blood vessels from differentiating endothelial cells derived from progenitor cells. The temporal and spatial regulation of vasculogenesis is required for embryogenesis, since the loss of a single allele of the gene causes embryonic death [48, 49].

Recently, it was observed that the stem cells of the pulp of the primary teeth have the potential to differentiate into vascular endothelial cells through a process similar to that of vasculogenesis. It has been shown that SHED differentiate into functional endothelial cells as they transport blood through the newly formed capillaries [36]. These surprising findings suggest that in the future the stem cell source of the dental pulp may be useful in the treatment of severe ischemic conditions such as myocardial infarction and cerebral ischemia.

Conceptually, angiogenesis is the process of the formation of new blood vessels from preexisting vasculature, making it therefore intrinsically different from the process of vasculogenesis. While vasculogenesis is extremely important in the early stages of embryonic development, angiogenesis allows remodeling of vascular networks, assuming an important role in postnatal physiological responses (e.g., in wound healing) and in pathological conditions.

In the context of the dental pulp, it is recognized that the events of healing of conservative treatments (direct pulp capping and pulpotomy) are orchestrated by a response regulated by angiogenesis. During the physiological process of healing, the sites of injured cells release chemotactic factors that contribute to the organization of the inflammatory process [50, 51].

Notably, the local cells release angiogenic factors that quickly organize an intense pro-angiogenic response, which allows for the influx of inflammatory cells and delivers oxygen and nutrients that are needed to maintain the high metabolic demands of cells actively engaged in tissue repair [50]. In dental pulp, a study showed that endothelial injury is involved in the recruitment of odontoblasts in the injury site [52].

Several bioactive molecules participate in inflammatory events and neo-formation of capillaries. Among them, vascular endothelial growth factor (VEGF) is considered the most important regulator of vasculogenesis and angiogenesis in physiological and pathological conditions [53, 54]. VEGF induces endothelial cells to form capillary structures, when cultured in collagen gel [55]. In vivo, VEGF increases permeability and induces potent pro-angiogenic responses [56, 57]. In addition, VEGF plays a critical role in the regulation of angiogenesis by increasing the survival of endothelial cells [55].

It has been observed that VEGF induces angiogenesis and improves survival of dental pulp cells of human tooth slices transplanted into the subcutaneous space of immunodeficient mice [58]. It has also been observed that VEGF induces the differentiation of SHED endothelial cells [36]. Iohara and colleagues [59] demonstrated that stem cells from porcine pulp increased blood flow in areas of experimental ischemia by the secretion of pro-angiogenic factors (VEGF), inducing an angiogenic response by the endothelial cells. It was also demonstrated that stem cell pulp under conditions of hypoxia, as occurs in some cases of dental trauma, induces pro-angiogenic responses directly related to the expression of VEGF by these cells [60]. Collectively, this data suggests that a local increase in bioavailability of VEGF is highly beneficial for the regeneration of complex dentine-pulp.

As mentioned earlier, the dentine matrix containing VEGF probably contributes to the angiogenic response mediated by dentine extracts [61]. Recently, the possibility of incorporating VEGF in scaffolds has been explored for use in dental tissue engineering. However, the biggest challenge yet for tissue regeneration is to ensure the rapid establishment of efficient anastomoses of blood vessels, facilitating the survival of transplanted cells and providing the flow of oxygen and nutrients needed to maintain the high metabolic demands of cells involved in tissue regeneration.

## Isolation and Characterization of Stem Cells from Tooth Structures

To comprehend the stem cell characteristics of dental tissue, it is necessary to understand the developmental process of teeth. Tooth development is initiated by the interaction of mesenchyme and oral epithelium. However, teeth are derived from the oral ectoderm and neural crest-derived mesenchyme and are composed of six sequential steps, such as thickening, bud, cap, bell, secretory, and eruption.

The pulp is formed from the dental papilla; cementoblasts and periodontal tissues are formed from the peripheral dental follicle. The mature tooth is composed of the enamel, dentine, pulp, and periodontal ligament tissue. Deciduous teeth begin to erupt in humans at an average of 6 months after birth and the deciduous dentition is complete at 3 years of age. After 6 years of age, the dentition is already mixed with the presence of the first permanent teeth but yet with all the maintenance of the deciduous teeth. However, with the progression of facial development, all the deciduous teeth are substituted by the permanent successors [62, 63]. The last permanent tooth to erupt is the third molar. This generally occurs between the ages of 16 and 20.

The first type of dental stem cell was isolated from the human pulp tissue in 2000 from normal human impacted third molars. The dental pulp stem cells (DPSCs) were primarily derived from the pulp tissue of human impacted third

molars [17]. However, these cells were also isolated from supernumerary [64] and natal teeth [65]. Extracted human third molars represent an easily accessible, often discarded tissue. In 2003, stem cells from exfoliated deciduous teeth (SHED) were isolated and characterized (normal exfoliated human deciduous incisors) [18].

Subsequently, three more types of dental mesenchymal stem cell populations were isolated and characterized. In 2004, periodontal ligament stem cells (PDLSCs) were isolated from normal human impacted third molars [66]. The stem cells from the apical papilla (SCAP) were extracted from normal human impacted third molars [67, 68]. In 2005, Morsczeck and colleagues isolated precursor cells from human dental follicles of normal impacted third molars, called dental follicle progenitor cells (DFPCs), or dental sac, which is an ectomesenchymal tissue surrounding the developing tooth germ [69].

In 2006, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy [70] established minimal criteria to define human MSCs, which include the following: (1) capacity of adhesion to plastic when maintained in standard culture conditions; (2) expression of CD105, CD73, and CD90 and lack of expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR surface molecules; and (3) differentiation into osteoblasts, adipocytes, and chondroblasts in vitro.

Table 15.1 summarizes the methods of isolation, the in vivo and in vitro characterization, and the plasticity of the five types of dental stem cells.

**Table 15.1** Human dental stem/progenitor cells (*hDS/PC*) from dental tissue: isolation, in vitro and in vivo characterization, and plasticity

| hDS/PC from dental tissues | Isolation | In vitro characterization | In vivo characterization | Plasticity |
|---|---|---|---|---|
| DPSCs | Enzymatic digestion of pulp tissue after separating the crown from the root of normal human impacted third molars [17] | Cells showed clonogenic capacity and are highly proliferative and capable of regenerating tissue [17] | DPSCs were capable of differentiating into odontoblasts and adipocytes and expressing nestin and GFAP. They formed dentine-pulp-like complex after in vivo transplantation [71] | Osteo-/dentinogenic [68] and neurogenic differentiation [72] |
| SHED | Enzymatic digestion of pulp tissue after separating the remnant crown from normal exfoliated human deciduous incisors [18] | High proliferation rate and number of population doublings [18]. Cell morphology similar to fibroblasts and high viability of the cells in first passage. More than 90 % of cultured cells positive for MSC markers [73] | Dentinogenic and neurogenic differentiation in vivo [18] | Adipogenic [18, 73], neurogenic [18], chondrogenic, and osteogenic differentiation in vitro [73] |
| SCAP | Enzymatic digestion of the apical papilla after separation from the surface of the immature roots from normal human impacted third molars [67, 68] | Showed fibroblast-like morphology [74] positive for STRO-1, higher telomerase activity than DPSCs from the same tooth, and improved migration capacity in a scratch assay, compared to DPSCs from the same tooth [67] | Cells were positive for STRO-1 and CD146 markers [73] | Osteo-/dentinogenic [68], odontogenic, and adipogenic differentiation were obtained from SCAP cultures [74] |
| PDLSCs | Enzymatic digestion of periodontal ligament after gently separated from the surface of the root from normal human impacted third molars [66] | The ability of PDL-derived cells to form adherent clonogenic cell clusters of fibroblast-like cells, similar to those recorded for different mesenchymal stem cell populations. PDLSCs showed clonogenic capacity, highly proliferative cells, capable of regenerating cementum/PDL-like tissue, properties that effectively define them as stem cells [66] | Express MSC markers. When transplanted into immunocompromised rodents, PDLSCs showed the capacity to generate a cementum/PDL-like structure and contribute to periodontal tissue repair [66]. Ex vivo-expanded ovine PDLSCs exhibited a high proliferation rate in vitro and expressed a phenotype consistent with human-derived PDLSCs [75] | Capacity to develop into cementoblast-like cells, adipocytes in vitro, and cementum/PDL-like tissue in vivo. Contain a subpopulation of cells capable of differentiating into cementoblasts/cementocytes and collagen-forming cells in vivo [66], as well as cells with potential to differentiate into osteoblasts, chondrocytes, and adipocytes, in vitro [76] |
| DFPCs | Explant cultures or enzymatic digestion of attached dental follicles after separation from normal human impacted third molars [69] | These fibroblast-like, colony-forming, and plastic-adherent cells expressed putative stem cell markers Notch 1 and nestin [69] | Human DFPCs are capable of differentiating into periodontal tissues in vivo, expressing bone sialoprotein and osteocalcin after transplantation in immunocompromised mice, but without any sign of cementum or bone formation [69] | Dental follicle derived from human third molar teeth represents a rational source for precursor cells for PDL-FB, cementoblasts, and osteoblasts for alveolar bone [69] |

*DPSCs* dental pulp stem cells, *SHED* stem cells from exfoliated deciduous teeth, *SCAP* stem cells from apical papilla, *PDLSCs* periodontal ligament stem cells, and *DFPCs* dental follicle progenitor cells

## Regenerative Strategies and Clinical Use of Stem Cells from Dental Tissue

### Bone Regeneration

Stem cells represent an easy and natural alternative to repair/regenerate damaged tissue, such as bone. This is essential especially when bone loss subsequent to degenerative or traumatic diseases cannot be amended through conventional therapies. Dental pulp stem/progenitor cells (DPCs) have been used for bone tissue engineering. d'Aquino and colleagues [77], in a clinical study, demonstrated that a DPC/collagen sponge biocomplex can completely restore human mandible bone defects and indicated that this cell population could be used for the repair and/or regeneration of tissue and organs. According to the authors, autologous DPCs are a new tool for bone tissue engineering.

DPSCs can also be used to improve the osteointegration of dental implants. According to Mangano and colleagues [78], titanium surfaces with stem cells from human dental pulp were capable of obtaining osteoblast differentiation of DPSCs and producing an appreciable amount of bone morphogenetic proteins as well as vascular endothelial growth factor and specific bone proteins. Therefore, complete osteointegration is obtained.

Different types of dental MSCs can be used in tissue engineering/regeneration protocols as an approachable stem cell source for osteo-/odontogenic differentiation and biomineralization that could be further applied for stem cell-based clinical therapies. According to Bakopoulou and colleagues [79], DPSCs and SCAP, both of which are types of MSCs, display an active potential for cellular migration, organization, and mineralization, producing 3D mineralized structures. These structures progressively expressed differentiation markers, including DSPP, BSP, OCN, and ALP, having the characteristics of osteodentine. The SCAP, however, show a significantly higher proliferation rate and mineralization potential, which might be of significance for their use in bone/dental tissue engineering.

The use of silk fibroin scaffolds combined with human stem cells, such as DPSCs and amniotic fluid stem cells (hAFSCs), has been successfully applied to repair critical-size cranial bone defects in immunocompromised rats. According to Riccio and colleagues [80], the progenitor cell association with an appropriate scaffold represents an optimal tool of bone regeneration and tissue engineering applications. It is a promising approach for the reconstruction of large human skeletal defects in craniofacial surgery.

### Lesions of the Cornea

Gomes and colleagues [81] carried out a transplantation of tissue-engineered human undifferentiated immature dental pulp stem cell (hIDPSC) sheets in an animal model. This study was shown to be a valid alternative for ocular surface reconstruction in cases of bilateral total limbal stem cell deficiency and provides a new perspective in the field with important clinical implications.

### Neurological Disease

Adult human DPSCs provide a readily accessible source of exogenous stem/precursor cells, which have the potential for use in cell therapeutic paradigms to treat neurological disease [71]. DPSCs reside within the perivascular niche of dental pulp and are thought to originate from migrating cranial neural crest (CNC) cells. During embryonic development, CNC cells differentiate into a wide variety of cell types, including neurons of the peripheral nervous system. Previously, it was demonstrated that DPSCs derived from adult human third molar teeth differentiate into cell types reminiscent of CNC embryonic ontology. According to Arthur and colleagues [71], DPSCs, when exposed to the appropriate environmental cues, would differentiate into functionally active neurons. The study demonstrated that ex vivo-expanded human adult DPSCs responded to neuronal inductive conditions both in vitro and in vivo. Human adult DPSCs acquired a neuronal morphology and expressed neuronal-specific markers at both the gene and protein levels. Culture-expanded DPSCs also exhibited the capacity to produce a sodium current consistent with functional neuronal cells when exposed to neuronal inductive media.

According to Völner and colleagues [82], human dental follicle cells (or the DFPCs) displayed characteristics of neural progenitor cells, and they are a promising alternative for new cell therapy approaches. The authors demonstrated the differentiation of these cells into neuron-like cells after a two-step strategy for neuronal differentiation, showing that these cells were neural precursors without potential for glial cell differentiation.

According to Sakai and colleagues [83], tooth-derived stem cells (SHED and DPSCs) may be an excellent and practical cellular resource for the treatment of spinal cord injury (SCI). Their study showed that human dental pulp stem cells exhibited neuroregenerative activities, such as the following: (a) they inhibited the SCI-induced apoptosis of neurons, astrocytes, and oligodendrocytes, which improved the preservation of neuronal filaments and myelin sheaths; (b) they promoted the regeneration of transected axons by directly inhibiting multiple axon growth inhibitors, including chondroitin sulfate proteoglycan and myelin-associated glycoprotein, via paracrine mechanisms; and (c) they replaced lost cells by differentiating into mature oligodendrocytes under the extreme conditions of SCI.

It has been shown that DPSCs are responsive to the surrounding microenvironment, surviving, migrating, and

differentiating accordingly into the appropriate cell types within the avian host neural tissue. These observations confirm the specificity of the neuronal differentiation response by adult human DPSCs as previously reported for SHED. Furthermore, it has been suggested that DPSCs and SHED are appropriate candidates for further evaluation as stem cell therapy-based treatments using animal models representative of neurological diseases and injury [71].

### Hepatocytes

The study of Ishkitiev and colleagues [19] successfully showed the differentiation of dental pulp cells into hepatocyte-like cells, demonstrating that the stem/progenitor cells of the dental pulp have a hepatic potential and can be an important cell source for liver cell therapy.

The MSCs from the permanent and deciduous teeth pulp, in culture, produce specific hepatic proteins. These cells also have the potential for performing specific functions for hepatocytes, including the ability to store the production of glycogen and urea. The use of these cells for hepatic diseases could signal a new approach for treatment of patients in autologous use, in the near future.

## Diabetes

It was demonstrated that DPSCs derived from deciduous teeth could be differentiated into pancreatic cell lineage and offer an unconventional and non-controversial source of human tissue that could be used for autologous stem cell therapy in diabetes without risk of rejection. The methods that have been developed could be transferred from bench to bedside for the treatment of children with type 1 diabetes. It has been suggested that banking of dental pulp stem from deciduous teeth should be considered for those patients at risk of developing maturity-onset type 2 diabetes [84].

### Vasculogenesis as a Potential Treatment for Ischemic Disease

Using a study model of nude rats, DPSCs were able to repair infarcted myocardium. This capacity was associated with an increase in the number of vessels and a reduction in infarct size, probably because of the ability of the cells to secrete proangiogenic and antiapoptotic factors. The degree of cardiac repair observed was similar to that obtained with MSCs from the bone marrow. Therefore, this study extends the knowledge of DPSCs' therapeutical properties and provides a new source of stem cells for the treatment of ischemic diseases [85].

**Conclusion**

Human dental tissue is showing to be a very interesting source of stem or progenitor cells with the potential of differentiating into different cell types. Another advantage is the fact that it is a noninvasive and disposable source of cells. Although it is a source with a very low number of cells, the strong capacity of these cells to proliferate and their high plasticity is a focus of interest for their use in cellular therapy and regenerative medicine for research or clinical proposals. It should be noted that the association of these cells with scaffolds has also been studied as a strategy to regenerate bone or tooth loss after trauma or disease. Besides bone and tooth regeneration, these cells could also be an innovative choice for treating other diseases, focusing on regenerative medicine.

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