Stem Cell Biology and Regenerative Medicine

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Dental Stem Cells

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Preface

Stem cells are a class of undifferentiated master cells that have robust self-renewal kinetic and differentiation potential into many specialized cell types in the body.

Stem cell research has been a field of great clinical interest with immense possibilities of using the stem cells to replace, restore, or enhance the biological function of damaged tissues and organs due to accidents, diseases, and/or developmental defects.

Recent studies have demonstrated that mesenchymal stem cells (MSCs) are found in various tissues in an adult organism. MSCs derived from teeth and supporting tissues, called dental stem cells (DSCs), have been mainly characterized into five different cell types including dental pulp stem cells (DPSCs), dental follicle stem cells (DFSCs), periodontal ligament stem cells (PDLSCs), stem cells from human exfoliated deciduous teeth (SHEDs), and stem cells from the apical papilla (SCAPs).

The knowledge of stem cell technology is moving extremely fast in both dental and medical fields. Advances in DSC characterization, standardization, and validation of stem cell therapies and applications have been leading to the development of novel therapeutic strategies.

Several investigators, especially those who have made significant contribution to the field of DSC research, have been invited to create this book. With the help of their intense and substantive efforts, this book reviews different aspects, challenges, and gaps of basic and applied dental stem cell research, cell-based therapies in regenerative medicine concentrating on the application and clinical use, and recent developments in cell programming and tissue engineering. This review will be useful to students, teachers, clinicians, and scientists, who are interested or working in the fields of biology and medical sciences related to dental stem cell therapy and related practices.

Istanbul, Turkey — Fikrettin Şahin

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Chapter 1
Dental and Craniofacial Tissue Stem Cells: Sources and Tissue Engineering Applications

Paul R. Cooper

Abbreviations

ADSCs	Adipose stromal/stem cells
BMP	Bone morphogenetic protein
BMMSCs	Bone marrow stromal cells
DFSCs	Dental follicle stem cells
DSCs	Dental stem cells
DPSCs	Dental pulp stem cells
EGF	Epidermal growth factor
DMP1	Dentin matrix protein 1
DSPP	Dentin sialophosphoprotein
ESC	Embryonic stem cell
FBS	Fetal bovine serum
ECM	Extracellular matrix
FGF	Fibroblast growth factor
GMP	Good manufacturing practice
GMSCs	Gingiva-derived MSCs
HERS	Hertwig's epithelial root sheath
HS	Human serum
IEE	Inner enamel epithelium
iPSC	Induced pluripotent stem cell
OEE	Outer enamel epithelium
OESCs	Oral epithelial progenitor/stem cells
PDL	Periodontal ligament

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PDLSCs	Periodontal ligament stem cells
PSCs	Periosteum-derived stem cells
SCAPs	Stem cells from apical papilla
SGSCs	Salivary gland-derived stem cells
SHEDs	Stem cells from human exfoliated deciduous teeth
Shh	Sonic hedgehog
SR	Stellate reticulum
TGF-β	Transforming growth factor-β
TGPCs	Tooth germ progenitor cells
TMJ	Temporomandibular joint
VEGF	Vascular endothelial growth factor

1.1 Introduction

Stem cells are present in many tissues throughout the body and at the different developmental stages of the organism. They are reported to reside in specific areas within each tissue, in a so called "stem cell niche". They are also frequently described as being located within close proximity to the vasculature, *i.e.* in a perivascular niche [1–3], and this anatomical localisation may facilitate their rapid mobilisation to sites of injury [4]. Stem cells have been characterised based on their abilities to self-renew, along with their multi-lineage differentiation capabilities which enable complex tissue regeneration [5]. They have varying degrees of potency ranging from totipotent, pluripotent, multipotent through to unipotent. Totipotent stem cells are derived from the zygote, and can form embryonic and extra-embryonic tissues, including the ability to generate the placenta [6]. Pluripotent stem cells include embryonic stem cells (ESCs), and are derived from the inner cell mass of the developing blastocyst. Notably, ESCs can differentiate into the three main germ layers of the organism including the endoderm, mesoderm and ectoderm. Postnatal/adult stem cells are regarded as being multipotent and include populations of hematopoietic and mesenchymal stem cells (MSCs). They are capable of differentiating toward several germ layer lineages giving rise to cell types which are necessary for natural organ and tissue turn-over and repair. In addition, along with these naturally present stem cell types, induced pluripotent stem cells (iPSCs) have been generated within laboratory settings by transcriptional reprogramming of somatic cells. Notably, sources of these somatic cells have included ones of oral and dental origin. iPSCs are reprogrammed to an embryonic-like state and hence are pluripotent and can differentiate into cells of all three germ layers [7, 8].

The dental and craniofacial tissues are known to be a rich source of MSCs which are relatively easily accessible for dentists. Stem cell populations which have been identified and characterised within these tissues include dental pulp stem cells (DPSCs) [9], stem cells from the apical papilla (SCAPs) [10–12], dental follicle

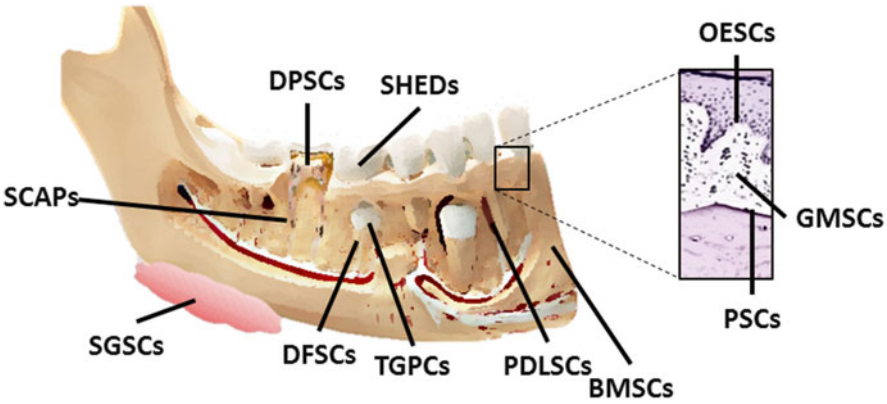

Fig. 1.1 The locations of developmental and postnatal stem cell populations in the dental and craniofacial region indicating sources for isolation from the mandible and teeth. The *insert* (*to the right*) shows the histology of the overlying masticatory mucosa (including oral epithelium, submucosa and bone tissue) and indicates the locations of the stem cell populations within it. Further details on all the stem cell populations shown are provided in the main text body. Abbreviations used are: BMMSCs—bone marrow-derived mesenchymal stem cells (MSCs) from mandible (also maxilla); DPSCs—dental pulp stem cells; SHEDs—stem cells from human exfoliated deciduous teeth; PDLSCs—periodontal ligament stem cells; DFSCs—dental follicle stem cells; TGPCs—tooth germ progenitor cells; SCAPs—stem cells from the apical papilla; OESCs—oral epithelial progenitor/stem cells; GMSCs—gingiva-derived MSCs; PSCs—periosteum-derived stem cells; SGSCs—salivary gland-derived stem cells

precursor cells (DFSCs) [13–16], periodontal ligament stem cells (PDLSCs) [17, 18], stem cells from human exfoliated deciduous teeth (SHEDs) [19] and tooth germ progenitor cells (TGPCs) [20]. Furthermore, the presence of other, perhaps as yet less well characterised stem cell types within the orofacial region have been reported including oral epithelial progenitor/stem cells (OESCs) [21], gingiva-derived MSCs (GMSCs) [22, 23], periosteum-derived stem cells (PSCs) [24] and salivary gland-derived stem cells (SGSCs) [25–27]. In addition, well characterised MSCs which are not exclusive to the oral and craniofacial tissues, include bone marrow-derived MSCs (BMMSCs) [28], which can be harvested from maxilla and mandibular bone, as well as adipose tissue-derived stem cells (ADSCs) [29]. These stem cell populations and their isolation and application will be discussed in greater detail in the following sections. Figure 1.1 pictorially shows the dental and craniofacial locations of these stem cell groups.

The oral and dental stem cell (DSC) populations are defined as MSCs according to the minimal criteria proposed by the International Society for Cellular Therapy (ISCT) in 2006 [30]. The criteria defining them, which are tissue independent, include their ability to adhere to standard tissue cultureware along with their expression profile of Cluster of Differentiation (CD) and other markers. According to the ISCT, MSCs should express CD105, CD73 and CD90 but lack expression of CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR cell surface molecules.

More recently, the expression of other cell surface markers for human MSCs, including CD271 and MSC antigen-1, have been reported [31, 32]. The presence of (or lack of) combinations of these markers are not only used to define stem cell populations but are also used for their isolation, although across species, this may not be entirely reproducible. Further defining criteria from the ISCT state that MSCs must be capable of differentiating into osteogenic, adipogenic and chondrogenic lineages in vitro [33].

The harvesting of MSCs from postnatal dental, craniofacial and other tissues is not always straightforward and this can be hampered by these cells being present at relatively low frequencies within tissues, *i.e.* <1 % of the total cell population. The simplest approach for isolating postnatal MSCs utilises their ability to adhere to cultureware which was initially demonstrated for BMMSCs [34]. This approach has also been used for craniofacial and dental MSCs, and generates a heterogeneous population of cells which exhibit the MSC-like properties of clonogenicity and a high proliferative capacity [9, 19]. However, frequently reported in the literature is the increasing use of fluorescence-activated cell sorting (FACs) and magnetic activated cell sorting (MACs) approaches [35]. These methods enable the isolation of cells from dissociated tissue which are positive and/or negative for many of the defining markers previously described. For DPSC isolation, several studies have applied positive selection for a range of different markers including STRO-1, CD105, c-kit, CD34 and low-affinity nerve-growth-factor receptor (LNGFR) with negative selection for CD31 and CD146 [36–40]. These studies indicate that the dental pulp likely contains several different MSC populations/niches, and this is also probably true for other dental and craniofacial tissues. It should, however, be noted that selection of MSCs using STRO-1, CD146 and pericyte-associated antigen also supports the premise that perivascular niches exist in a variety of tissues throughout the body including those from the dental and craniofacial regions [9, 11, 19, 41].

Recent work has also built upon the cultureware adhesion approach initially reported for BMMSC isolation with studies now demonstrating that several MSC-types can be derived via selective adhesion to cultureware surfaces coated with extracellular matrix (ECM) derived molecules. This potentially biomimetic approach may be based on the in vitro recapitulation of the niche environment whereby MSCs in vivo are maintained in a quiescent state by the ECM until released and activated during tissue disease or trauma. This MSC selection technique has been shown to be successfully applied using ECM-derived proteins such as fibronectin, type I collagen, type II collagen, vitronectin, laminin and poly-L-lysine [42–45].

It is also notable that isolated cells may not always be of a pure population and may be somewhat heterogeneous in nature, subsequently representing various differentiation states. It remains unclear, and is under considerable debate, as to whether a pure population of cells is indeed needed for therapeutic application, as within tissues stem cells interact with a variety of other cell types to enable repair. Further confounding this issue is the fact that MSCs are derived from different donors, *e.g.* age range and sexes, and isolated cells may subsequently respond differently in vitro and in vivo [28]. Current research, therefore, aims to identify the

most appropriate isolation conditions which will enable predictable clinical application and outcomes.

Over the coming years within the dental field, stem cells combined with tissue engineering strategies are expected to provide novel therapeutic approaches to regenerate teeth or tooth component tissue and for repair of defects in periodontal tissues and alveolar bone. Specific oral tissues and organs which are already being targeted for regenerative medicine strategies include the salivary glands, tongue, craniofacial skeletal muscles, and component structures of the temporomandibular joint. The properties and characteristics of craniofacial and dentally relevant MSCs are subsequently discussed below as is dental tissue development, tissue engineering and clinical application progress.

1.2 Dental Tissue Development and Repair

In general, the development of many organs requires heterologous cell and tissue interactions. For tooth development these interactions occur between the ectodermally-derived enamel organ epithelium and cranial neural crest–derived ectomesenchyme. These epithelial-mesenchymal interactions also underpin the development and morphogenesis of many other human organs including hair, mammary gland and salivary glands. Significant work over recent years has shown that complex growth and transcription factor signalling are critical to coordinate these cellular events [46]. Gene and protein expression profiles are tightly regulated throughout all stages of tooth development, and the signalling networks generated are similar to those found in the development of other organs. Notably, it is these networks which are reactivated during many repair and regeneration events later on in life. Indeed, recent studies have now made significant in-roads into the characterisation of these intracellular signalling cascades essentially for coordinating tooth development [47].

The initiation stage of tooth development is characterized by the formation of the dental lamina and this occurs at around the fifth week of human gestation [10th embryonic day (ED 10) of mouse development]. During this stage, a variety of cellular and molecular events occur which determine tooth type, position and orientation within the developing jaws. Subsequently, the dental epithelium begins to proliferate to give rise to a narrow horseshoe-like ribbon of cells termed the dental lamina, and their morphology reflects the future position of the dental arches. Embryonic epithelial thickenings (ectodermal/dental placodes) of the dental lamina subsequently develop which are the first morphological indications of teeth and precede the local appearance of an ectodermal organ. Many growth factors and signalling molecules such as fibroblast growth factors (FGFs), Paired box's (PAXs), WNTs, sonic hedgehog (SHH), msh homeobox's (MSXs), distal-less homeobox's (DLXs) and bone morphogenetic proteins (BMPs) are the main regulators of this process which provide the relevant positional information for dental placode development [48, 49].

The dental epithelium continues to proliferate and begins to invaginate into the ectomesenchyme, and forms tooth buds with the dental placodes continuing to secrete potent signalling molecules [50–52]. Subsequently, at 20 locations in the human dental lamina, at around weeks 7–9 of human gestation and mouse (ED 11–11.5), the epithelial cells begin to proliferate and intrude into the mesenchyme to give rise to an early bud stage structure. The ectomesenchymal cells proliferate and accumulate around each epithelial bud, and the innermost cells of the epithelial develops a star-like morphology with the onset of synthesis and secretion of glycosaminoglycans. This structure becomes hydrated resulting in the cells becoming more widely distributed with this internal area of the tooth bud now containing the stellate reticulum and the intermediate layer. During the bud stage of tooth development, the odontogenic potential no longer resides with the epithelium but is driven by the ectomesenchyme [53].

The tooth bud becomes transformed into a cap-like structure by differential proliferation and infolding of the epithelium. The local mesenchymal cells begin to secrete a range of ECM molecules, such as tenascin and syndecan, which bind to, and increase the local concentrations of growth factors. The inductive signalling results in differential multiplication of the epithelial layer with concomitant transformation of the tooth bud into a pyramid-like structure with the dental lamina at its tip which marks the future site of the tooth crown. Evidence indicates that BMP4 is key to the mesenchymal signalling that induces transition from bud to cap stage due to its regulation of several key transcription factors. Subsequently, an epithelial mass, the enamel knot, within the central base of this structure develops, and this reportedly acts as a transient organizer of the morphogenetic signalling for adjacent cells via its expression of FGFs. The enamel knot is removed via apoptosis at the end of the cap stage and is entirely lost by the time of the bell stage [54–56]. The epithelium expands and folds inside the core of the bud in an anterior to posterior manner and the whole structure begins to resemble an upturned cap. The inner enamel epithelium (IEE) is found internally within the cap while the outer structure is covered by the outer enamel epithelium (OEE). Between the IEE and OEE sheets are vacuolised cells of the stellate reticulum and an intermediate cell layer which is referred to as the enamel or dental organ. The condensed mesenchymal tissue within the IEE and between the cervical loop (outer rim of the entire structure) is the dental papilla which develops into the future dental pulp tissue. The condensed mesenchyme surrounding the dental papilla and dental organ is the dental follicle which gives rise to the cementoblasts, osteoblasts and fibroblasts of the periodontal ligament [57].

Cup position and height are tooth- and species-specific; therefore, correct spacing and size are accurately regulated in multicuspid teeth via primary and secondary enamel knots. Indeed, secondary knot formation marks the onset of the bell stage of tooth development and the IEE continues infolding according to the organising signals that they express. The IEE subsequently displaces the stellate reticulum, and the structure acquires the form of a bell. At this point, the dental mesenchyme does not appear to be undergoing cell proliferation, and the enamel organ is separated from the dental papilla, with the tooth cusps starting to form and the crown height

increasing. Crown morphogenesis and cytodifferentiation occur during the bell stage with the cells differentiating in situ to give the crown its final shape [58–61]. Subsequently, the mesenchymal cells bordering the dental papilla are attached to the basement membrane of the IEE, and they take on a polarised columnar form and differentiate into the odontoblasts which secrete the predentine. Immediately following the deposition of the predentine the basement membrane breaks down and subsequent signalling leads to cells of the IEE, which are in contact with the predentine, differentiating into polarised columnar ameloblasts which begin their synthesis of enamel. Mineralization occurs and converts the predentine to dentine, and further secretion of predentine results in the odontoblasts receding from the dentino-enamel junction. The odontoblasts leave cellular processes within dentinal tubules as they traverse towards the pulp core. The two hard tissues of the tooth matrix, the enamel and dentine, are characterised by their apposition of hydroxyapatite crystal. Notably, the basal cells of the intermediate layer support the process of enamel formation and following tooth eruption transform into the junctional epithelium. The dental lamina disintegrates, and the pulp and enamel organ are encased in a condensed mesenchyme, which constitutes the dental follicle which ultimately gives rise to cementoblasts, osteoblasts and fibroblasts [62, 63].

A multitude of genes have been identified as being active during tooth development and morphogenesis which indicate the complexity of the process. Our increased understanding of these molecular and cellular events is necessary to underpin the development of future stem cell-based therapies for bio-tooth engineering.

1.2.1 Dentinogenesis

Whilst primary dentinogenesis occurs at a rate of ~4 μm/day during tooth development, namely secondary dentinogenesis continues to occur at ~0.4 μm/day following tooth root formation throughout the life of the tooth. Tertiary dentinogenesis refers to the process of repair and regeneration in the dentine–pulp complex which represents a natural wound healing response. Following relatively mild dental injury, such as during early stage dental caries, primary odontoblasts are reactivated to secrete a reactionary dentine which is tubular and continuous with the primary and secondary dentin. However, in response to injury of a greater intensity, *e.g.* a rapidly progressing carious lesion, the primary odontoblasts die beneath the lesion. Subsequently, if conditions are appropriately conducive, *e.g.* caries is arrested; the stem/progenitor cells within the pulp are signalled to home to the site of injury and differentiate into odontoblast-like cells. These cells deposit a tertiary reparative dentine matrix resulting clinically in dentine bridge formation walling off the dental injury. Clearly, the relative complexity of these two tertiary dentinogenic processes differ with reactionary dentinogenesis somewhat more simply requiring only the up-regulation of existing odontoblast activity, whereas reparative dentinogenesis involves recruitment, differentiation, as well as up-regulation of dentine synthetic

and secretory activity. It is understood that tertiary dentine deposition rates somewhat recapitulate those of development and are also reported to be ~4 μm/day. Notably, tertiary dentinogenic events are understood to be signalled by released bioactive molecules similar to those present during tooth development which were initially sequestrated within the dentine during its formation [64–66]. Indeed, an array of molecules are bound within dentine and are known to be released from their inactive state by carious bacterial acids and restorative materials, such as calcium hydroxide, and are known to stimulate dentine bridge formation. At the stem cell level, released dentine matrix components may stimulate cell proliferation and expansion, recruitment to the site of injury, differentiation into odontoblast-like cells and the up-regulation of synthetic and secretory activity. Indeed, prime candidate signalling molecules for stimulating these events come from the BMP and transforming growth factor (TGF)-β superfamilies with TGF-β1 alone being shown to stimulate many of these processes in vitro and in animal models. However, it is likely that synergistic signalling due to many of the bioactive molecules released from the dentine ECM are potent regulators of DSC repair processes in vivo (reviewed in [67, 68]). Notably, however, while it is generally assumed regenerative processes utilises tissue resident cell sources, a mouse parabiosis model has recently demonstrated that progenitor cells can be derived externally to the pulp [69]. The source and properties of stem cells involved in repair and regenerative responses are discussed in Section 1.3.

1.3 Stem Cell Populations

1.3.1 BMMSCs

Originally in 1970, Friedenstein et al. [34] reported the isolation of adherent colony forming cells from bone marrow, and demonstrated their ability to differentiate toward various mesenchymal tissue lineages. In 1999, Pittenger et al. [70] characterized human BMMSCs from the iliac crest, and showed that they could be expanded in culture, and were able to differentiate down osteogenic, adipogenic and chondrogenic lineages. More recent work has gone on to demonstrate BMMSCs also have the capacity to differentiate into non-typical mesenchymal lineages such as ones involved in neurological repair [71]. Perhaps predictably BMMSCs most robustly form bone in vitro and in vivo, indicating their utility in bone regenerative therapy which is frequently exploited clinically in oral and dental procedures. While BMMSCs are generally isolated from bone marrow aspirates derived from the iliac crest during a relatively invasive and painful surgery, they can also be isolated from the maxilla and mandible. These orofacially-derived BMMSCs, derived from cranial neural crest cells, are subsequently likely more applicable for dental treatments although their safe expansion in numbers is required prior to use in therapeutic procedures [72–74].

1.3.2 Adipose Tissue-Derived Stem Cells (ADSCs)

ADSCs can be relatively abundantly harvested via lipectomy or from lipoaspirates from many sites within the adult human body including craniofacial regions. Notably, their harvest generally results in low donor-site morbidity, and the tissue isolated is regarded as clinical waste as liposuction is routinely performed during cosmetic surgery, *e.g.* cheek and chin reshaping. While intrinsically ADSCs exhibit some differences compared with BMMSCs, ADSCs appear to exhibit good mineralised tissue lineage responses, and therefore have potential for use in bone and tooth tissue repair including applications in osseointegration [29]. For dental structures, ADSC transplantation has been used to regenerate pulp tissue and whole teeth containing dentine, with periodontal ligament and alveolar bone attachments in animal models [75–78]. Further work characterising the application of ADSCs for bone, tooth and periodontal tissue regeneration should result in the development of robust protocols which utilise waste fat tissue for clinical application.

1.3.3 Dental Tissue Stem Cells

1.3.3.1 Postnatal Dental Tissue-Derived Stem Cells

A clonogenic and highly proliferative DPSC population exhibiting phenotypic characteristics similar to those of BMMSCs were initially isolated by enzymatic disaggregation of adult dental pulp. Only a few years later, SHEDs were isolated, which were also shown to exhibit the stem cell properties of self-renewal and multi-lineage differentiation potential. In animal studies, DPSCs and SHEDs have demonstrated the ability to generate a mature dentine–pulp-like structure. Further studies using SHEDs have shown that they can induce bone-like matrix formation which may relate to processes that occur in deciduous tooth roots, whereby resorption occurs concurrently with new bone formation. Notably, DPSCs and SHEDs have significant clinical application potential for autologous regenerative treatment approaches as both can be derived from what is regarded as clinical waste tissue. Indeed, DPSCs can be obtained from teeth extracted for orthodontic reasons, whilst SHEDs are harvestable from primary teeth which are naturally exfoliated (reviewed in [79]). Interestingly, up until recently, it was believed that due to the reciprocal interactions which occur between the embryonic oral epithelium and neural crest-derived mesenchyme during tooth morphogenesis, the stem cells from the tooth were derived from a neural crest origin. However, Kaukua et al. [80] recently demonstrated that a significant population of MSCs involved in development, self-renewal and repair of teeth are derived from peripheral nerve-associated glia. While this study was performed in a murine incisor model system, which may limit its relevance to humans, it does, however, indicate our continued need to better understand both tooth development and regeneration events.

The periodontal ligament provides another source of postnatal MSCs in the form of PDLSCs which can also be isolated from extracted waste teeth. Perhaps not surprisingly due to their localisation, PDLSCs have been demonstrated to be able to regenerate several periodontal tissues including cementum, periodontal ligament and alveolar bone in animal studies. However, recent work has indicated that the local derivation of the PDLSCs may significantly influence their differentiation capabilities as PDLSCs from the alveolar bone surface exhibited superior alveolar bone regeneration properties compared with PDLSCs from the root surface [17, 18, 81].

1.3.3.2 Stem Cells Derived from Developing Dental Tissue

Within the developing dental tissues of the dental follicle, including the dental mesenchyme and apical papilla, MSC-like cell populations have been identified. The dental follicle, also termed the dental sac, contains the developing tooth and within it, DFSCs with the ability to regenerate several periodontal tissue types are found [13–16]. At the late bell stage of tooth development, stem cells derived from the dental mesenchyme of the third molar tooth germ have also been identified and these are termed as TGPCs [82]. These isolated MSC-like cells demonstrated a high proliferative capacity along with the requisite capability to differentiate in vitro into the three germ layer lineages. SCAPs [11, 12] have also been identified in developing tooth roots. In comparison with DPSCs, SCAPs have demonstrated increased proliferation rates and enhanced regenerative capabilities for dentine-pulp complex tissue in animal model studies. Furthermore, as these cells exhibit a developmentally immature phenotype and can be isolated from the clinical waste postnatal or adult tissue of extracted wisdom teeth, they could provide a valuable source of autologous stem cells for future regenerative therapies.

1.3.3.3 Oral Mucosal and Periosteum-Derived Stem Cells

The oral mucosa comprises stratified squamous epithelium composed of oral keratinocytes and an underlying connective tissue. The connective tissue consists of a well vascularised lamina propria and a submucosa which can contain minor salivary glands, adipose tissue, neuronal structures and lymphatics. Within the oral mucosa two different types of human postnatal stem cells have been identified; OESCs and GMSCs [21–23]. OESCs are reportedly relatively small oral keratinocytes (<40 mm in diameter) and while being unipotent, they can regenerate oral mucosal tissue ex vivo which may have clinical utility for intra-oral grafts.

GMSCs are reported in the gingival lamina propria which attaches directly to the periosteum of the underlying bone [21]. In addition, a neural crest stem cell-like population has also been isolated from the adult human gingival lamina propria which are termed oral mucosa stem cells (OMSCs) [22]. The relative clinical ease by which relatively high numbers of both GMSCs and OMSCs could be isolated makes these cells promising candidates for use in future clinical therapies.

The periosteum of bone comprises two distinct layers; the outer layer which contains mainly fibroblasts and elastic fibres, while the inner layer contains MSCs along with other progenitor cell populations. Periosteum-derived cells may have preferential application for bone regeneration and subsequently may have application in craniofacial therapies [83–85]. Indeed, locally derived periosteum cells may have particular application for bone repair in procedures such as periosteal flap surgery in conjunction with implant placement along with use in large defect repair procedures [86–88].

1.3.3.4 Salivary Gland-Derived Stem Cells

Salivary glands develop from the endoderm and when mature comprise of acinar and ductal epithelial cells with exocrine function. While the existence of salivary gland stem cells have been proposed following in vivo studies, stem cells that give rise to the entirety of the epithelial cell types present within the gland have yet to be identified [25, 27]. MSC-like cells from human salivary glands have, however, been reported based on their expression of embryonic and postnatal stem cell markers along with their ability to differentiate toward adipogenic, osteogenic and chondrogenic lineages [89–91]. Stem cells isolated from this tissue may have particular application for use in the rescue of dysfunctional gland activity in particular in head and neck irradiated cancer patients who exhibit salivary gland dysfunction [92].

1.3.3.5 Induced Pluripotent Stem Cells (iPSCs)

The possibility of reprogramming somatic cells to an early embryonic development stage by introducing the four transcriptional factors, Oct3/4, Sox2, Klf4 and c-Myc, was initially reported by Takahashi and Yamanaka [93]. Originally, normal mouse adult skin fibroblasts were used and the resultant reprogrammed cells were termed as iPSCs. A year later, this work was replicated using human skin cells which subsequently indicated the potential to generate patient-specific cells with ESC-like characteristics [7, 94]. Indeed, animal studies have demonstrated iPSCs can generate all the tissues and organs of the body. Notably, it has been shown that iPSCs can be derived from many cell types derived from oral and dental tissues which can be relatively easily harvested by dentists. Interestingly, many of these cells have exhibited relative high reprogramming efficiencies which may be explicable as oral and dental MSCs already express relatively high levels of endogenous multipotent transcription factors [82, 95–99]. In the future, the use of oral and dental waste tissue may, therefore, provide an ideal cell source for use in iPSC technology in particular for the regeneration of autologous craniofacial soft and hard tissue structures. Indeed, recent work utilising iPSCs in a mouse model using enamel matrix derived molecules demonstrated increased periodontal tissue regeneration, while in vitro work has demonstrated iPSC application for biotooth-engineering of ameloblast- and odontoblast-like cells [100–102].

Notably, there remain drawbacks with the use of iPSC technology. Much is still to be learned as to how to optimise their generation and reprogramming efficiency as well as in controlling their differentiate fate. A major concern also lies with the risk of tumour formation by iPSCs following clinical implantation. Such a concern arises due to the use of the c-Myc oncogene as a reprogramming factor along with the use of the retroviral insertion system for gene transfer. Recent research, however, may have resolved these issues by using alternative genes for reprogramming along with the application of small reprogramming molecules. Indeed, the use of non-viral components such as proteins, microRNAs, synthetic mRNAs and episomal plasmids is being pioneered. A further clinical concern also arises due to delivery of residual undifferentiated iPSCs remaining amongst the differentiated target cell population. These cells may proliferate uncontrollably and generate teratomas at the site of implantation. To overcome this issue the use of selective ablation approaches to remove teratomas via suicide genes and chemotherapy, as well as the use of antibody-based cell sorting approaches to remove teratoma-forming cells, are being developed [7, 103–113].

1.4 Scaffolds and Morphogens for Stem Cell Tissue Engineering

1.4.1 Scaffolds

For dental and oral tissue engineering strategies, along with stem cells, suitable biomimetic scaffolds and appropriate morphogens/growth factors are required [114]. Clinically, for periodontal tissue repair, material-based guided tissue regeneration (GTR) approaches have been developed. Subsequently, biocompatible or bioinert scaffolds are used to enable connective tissue and bone regeneration from local tissue MSC populations [115–118]. Alveolar bone augmentation approaches, such as guided bone regeneration (GBR), utilise bioactive materials, such as calcium phosphate (CaP)-based biomaterials and collagen-based grafts. While these materials are bioactive and osteoconductive, they are not osteoinductive; hence, scaffolds are being developed, which incorporate bone formation promoting growth factors [119–122].

Fibrous silk protein (fibroin) biomaterial scaffolds are also being developed for their use in tooth and bone repair. These scaffolds can be generated and harvested from silkworms and spiders, and can exhibit properties of controllable porosity, surface roughness and stiffness. They can be further functionally modified to mimic the natural ECM environment to facilitate stem cell recolonization, differentiation and tissue regeneration for therapeutic applications [123–125].

Recent studies have demonstrated the utility of hydrogel scaffolds for tooth tissue engineering applications and their promise is likely based on them exhibiting similar biomechanical properties to pulp tissue. The seeding of pulp derived cells on

collagen scaffolds with subsequent animal implantation has demonstrated the formation of dental tissue structures [126, 127]. Furthermore, DPSCs encapsulated in collagen hydrogels have been shown to differentiate and deposit a mineralised ECM in the presence of natural tissue morphogens [40, 128]. Others have generated pulp-like tissue in vivo following the seeding of SHEDs and human endothelial cells on biodegradable poly-L-lactic acid hydrogel scaffolds [129]. A peptide-amphiphile hydrogel scaffold containing bioactive osteogenic supplements has also been shown to promote differentiation of encapsulated SHED and DPSCs [130]. While challenges still remain, the development of the most appropriate scaffolds which optimise stem cell responses for clinical application is progressing at a rapid rate.

1.4.2 Role of Growth Factors and Morphogens for Tissue Regeneration

Our understanding of the molecules involved in signalling tissue development and repair will underpin the generation of novel naturally inspired clinical therapies. Current knowledge of this molecular signalling is advancing with the tooth's hard and soft tissue ECM being shown to provide both biochemical and biomechanical regulatory cues. Indeed, comparable with repair processes in other tissues, the regulation of dental tissue regeneration involves signalling derived from its ECM with inherent growth factors known to coordinate recruitment, proliferation and differentiation of MSC populations [65, 68, 131, 132].

In the periodontal tissues, the application of platelet rich plasma (PRP) enables the delivery of a cocktail of potent growth factors and morphogens. Indeed currently, there is significant interest in the use of PRP in combination with bone grafts and/or stem cells to enable more predictable periodontal regeneration [133]. Enamel matrix derivatives (EMDs), obtained from porcine tooth buds, also contain a complex cocktail of growth factors and can also stimulate periodontal tissue regenerative events. Indeed both PRP and EMD are morphogenically complex and have been shown to include BMP-2, platelet derived growth factor (PDGF)-BB and FGF-2, amongst others [134–136]. These molecules likely act synergistically on MSCs. However, the action of individual growth factors has been exploited with BMP-2 being used in absorbable collagen sponge scaffolds to induce bone formation for sinus and alveolar ridge augmentation therapies. Both PDGF-BB and FGF-2 in combination with CaP or hydrogel scaffolds have also shown some clinically efficacious potential based on their ability to stimulate vascular responses which underpin many MSC-based repair mechanisms [137–145].

The indirect application of MSCs due to their release of growth factor with paracrine effects has also recently been highlighted. These secretomes contain a multitude of bioactive molecules such as insulin-like growth factor (IGF)-1 and vascular endothelial growth factor (VEGF) which promote many tissue repair mechanisms [146]. Notably, DPSC secretomes exhibit significant neurogenic repair

activity as well as being able to immunomodulate T-cell, B-cell, natural killer cell, and dendritic cell function [147, 148]. Further work is still required to better characterise the active components of the secretomes to determine optimal concentrations for targeted tissue repair and regeneration application.

1.5 Stem Cell Applications for Dental and Craniofacial Tissue Regeneration

The use of stem cells for regenerative medicine/dentistry is progressing and currently, the use of adult/postnatal stem cells exhibits the most realistic clinical opportunity. Regeneration of bone and periodontal tissues using MSCs has received considerable attention with several studies already reporting clinical application. Clearly, stem cells used in dental tissue engineering should be; (i) relatively easily isolated, (ii) straightforward to deliver in a reproducible and clinically simple procedure, (iii) clear of any patient safety issues, and (iv) ultimately differentiate into and regenerate the target tissue or organ.

BMMSCs and ADSCs, in particular those derived from the orofacial region, may provide an appropriate source for craniofacial tissue repair. Other dental and craniofacial tissue-derived MSCs may be more appropriate for regenerating dental mesenchyme-derived hard and soft tissues, including those of the dentine, pulp and supporting periodontal tissues. The application of MSCs for complete repair of complex oral organs, such as teeth and salivary glands, which also require cells to differentiate down epithelial lineages may however be challenging. Pluripotent embryonic stem cells may, therefore, have utility in these cases; however, medical and ethical issues associate with their application and the use of iPSCs still require further technical and safety advancements before they can be applied. For all stem cell sources, their downstream processing following isolation still remains an issue for the clinician who would also require onsite specialist equipment and expertise to enable their purification and expansion.

1.5.1 Tooth and Tooth Component Tissue Regeneration

Ultimately, it is aimed that a lost tooth will be replaced by a fully functional bioengineered one; however, current studies indicate that tooth component tissue, such as root and crown dentine are more realistically clinically achievable. Recent work using animal models has shown that complex root/periodontal structures can be regenerated using PDLSCs and SCAPs in conjunction with hydroxyapatite scaffolds [11, 149]. The structures regenerated provided suitable abutments for prosthetic devices enabling the support of an artificial crown with dental functionality. Clearly, future work in this area may enable development of the underpinning technology necessary for human application.

The regeneration of an entire tooth structure is now appearing feasible in the future based on animal studies utilising several different MSC sources. For tooth bioengineering, the generation of embryonic tooth primordia has been commonly used. Initial studies have transplanted pelleted dissociated porcine tooth buds in the omentum of athymic rats which resulted in the generation of complex tooth structures which comprised a pulp chamber, dentine, putative Hertwig's Epithelial Root Sheath (HERS) and an enamel organ. Transplantation of dissociated rat and mouse tooth buds have also resulted in the development of similar tooth structures. Notably, as is described previously, tooth development requires the reciprocal interactions between embryonic oral epithelial cells and neural-crest derived mesenchyme. Subsequently, recent work has attempted to determine if mouse-derived ESCs, neural stem cells and BMMSCs can appropriately respond to mouse embryonic oral epithelium derived cells. Data indicated that odontogenic differentiation was most apparent in explants containing BMMSCs although other cell types demonstrated some potentiality [100, 102, 150–154]. Work conducted by Volponi et al. [155] has demonstrated tooth tissue regeneration following transplantation of human adult gingival epithelial cells combined with mouse embryonic tooth mesenchyme cells in kidney capsules. The tooth structures generated at six weeks of transplantation contained vascularized pulp-like tissue and signs of root development including the presence of ameloblast-like cells and epithelial rests of Malassez.

Significantly, a murine model has recently demonstrated that, following the transplantation into the alveolar bone of a bioengineered tooth germ, reconstituted in vitro in a collagen hydrogel scaffold using epithelial and mesenchymal progenitor/stem cells, a functioning tooth was formed. Notably, the in vitro step used recapitulated the developmental events necessary for complex tooth tissue generation and the subsequent bioengineered tooth, which when erupted and occluded, exhibited appropriate mineralised tissue properties [151]. Furthermore, the pulpal tissue was appropriately innervated and relevantly serviced by a blood supply. The generation of fully functional tooth units in animal models which have utilised MSC and iPSC sources support the concept that bioengineered structures may one day be routinely generated for patients. Clearly, significant work is still required to bring this to fruition and to provide clinically relevant alternatives for patients who require dental implants.

1.5.2 Regeneration of Other Complex Craniofacial Tissues and Organs

The regeneration of salivary gland function is important in particular for head and neck oncology patients who have undergone surgery and/or radiotherapy. Recent mouse model studies using ADSCs, BMMSCs and primitive salivary gland stem cells have shown that this may one day be clinical feasible. [89, 92, 156, 157]. The temporomandibular joint (TMJ) disc or condyle can become damaged due to disease such as arthritis or through trauma. MSCs in conjunction with hydrogels and ultrasound approaches have been used successfully to reconstruct condylar defects

in animal model systems [158–160]. The regeneration of tongue tissue is also important to many patients. Several animal model systems using MSCs and relevant scaffold systems has now shown tongue tissue repair is possible [161–163]. Overall studies are now showing that for many complex tissue and organ systems within the oro-craniofacial region, bioengineering approaches may one day become a clinical reality for patient treatment.

1.6 Stem Cell Storage and Processing

While growing evidence demonstrates that dental and oral tissues provide a rich source of MSCs, their use in regenerative therapies may be limited due to the requirement to isolate tissue at the time of need, *e.g.* tooth extraction. The banking of DSCs or tissues obtained from deciduous and wisdom teeth may, therefore, provide a practical approach for future stem-cell-based regenerative therapies. Recently, in several countries worldwide stem cell and tissue banks in the dental field have been developed, *e.g.*, Advanced Center for Tissue Engineering Ltd., Tokyo, Japan (http://www.acte-group.com/); Teeth Bank Co., Ltd., Hiroshima, Japan (http://www.teethbank.jp/); Store-A-ToothTM, Lexington, USA (http://www.store-a-tooth.com/); BioEDEN, Austin, USA (http://www.bioeden.com/) and Stemade Biotech Pvt. Ltd., Mumbai, India (http://www.stemade.com/) (reviewed in [164]). These banking approaches routinely utilise cryopreservation which aims to enable the long term storage of viable stem cells from tissues such as the PDL, pulp, apical papilla and whole tooth tissue. Subsequently, it is envisaged that the stem cells will be retrieved in the future from this cryopreservation and applied in autologous regenerative therapies for the patient. Much work, however, is still needed to determine the utility of these biobanks, their longevity, and value for money and the MSC processing procedures required.

Currently, it is not entirely clear as to how long term cryopreservation affects MSC viability and phenotype [165]. Therefore, alternative storage approaches are being developed which may be beneficial. Indeed, recent studies have shown that MSCs encapsulated in hydrogels may provide a means to decrease archiving costs while maintaining MSC phenotype and properties. Furthermore, the potential of tissue engineered product vitrification has also been investigated with studies using bone constructs consisting of a hydroxyapatite scaffold-cell complexes demonstrating higher cell survival rates compared with conventional freezing approaches [166, 167]. Further studies of these emerging biobanking approaches are clearly needed.

MSC handling and ex vivo expansion will be required for clinical application due to the relatively low number of stem cells, <0.1 % of all cell types, present within tissues. To achieve this, Good manufacturing practice (GMP)-compliant environments have been developed and are reported to generate clinical-grade MSCs from several tissue-types, *e.g.* adipose and bone marrow [168]. Currently, there are minimal published reports evident on GMP-handling and processing for dental MSC-types. It is proposed that standard GMP procedures should be more routinely applied

across institutes, as previous work has demonstrated significant MSC heterogeneity which may be due to donor or operator variability. Indeed, data has indicated donor age may be one key source of this heterogeneity with some studies showing that proliferative potential and differentiation capability decrease in an age-related manner both in vivo and in vitro, *i.e.* during culture passage [169–171]. Indeed, our work using DPSCs has also shown that with higher passages, MSC properties, such as proliferation rates and differentiation capabilities, diminish [172]. To overcome these issues, others have supplemented prolonged in vitro cultures with growth factors to maintain MSC-like properties [173]. Further optimisation of laboratory procedures may minimise culture differences, and novel techniques which involve spheroid culturing in the presence of growth factors or the use of relevant hypoxic conditions [174], which better mimic the MSC niche in vivo, may be exploited.

Cell culture requires several kinds of supportive factors including animal-derived reagents, such as fetal bovine serum (FBS), and variation in lots have been cited as a further source of MSC heterogeneity. There are also safety concerns relating to the use of animal- derived reagents for human MSC expansion due to possible risk of contaminations such as prions, viruses, and zoonosis, along with the potential for host immunological reactions against xenogeneic proteins. Subsequently, autologous human serum (HS) has been proposed for clinical applications as a replacement for FBS. There exist major drawbacks, however, with using autologous HS due to the need for its harvest in sufficient volumes at the time of need potentially from patients who may already be compromised with regards to their health. Alternatively, the utility and safety of other approaches; such as the use of pooled human platelet lysates, has been proposed [175–179]. To overcome these culture-related issues, the development of well-defined serum-free media which can support the growth of MSCs is being explored. While chemically defined media may include some animal or human serum, efforts are being made to generate a more xeno-free culture media which would circumnavigate safety issues and improve clinical grade cell culture consistency [180–183]. However, while initial studies support the potential GMP application of serum-free media, more work, examining a wider range of MSC-types, is still required. Furthermore, along with the standardisation of laboratory protocols, procedures for aspiration, including harvesting site locations, should also be undertaken as consistently as possible.

GMP-compliant MSC processing requires multiple complex steps which can include surgical tissue dissection, cell dissociation, dispersion, expansion and collection. An expensive clean room is also required along with experienced and specialist technical staff. Work is generally labour-intensive and the complexity of the process can result in human error and culture contamination. Subsequently, the use of automated cell processing approaches has been explored. However, while such an approach would eliminate human error, the risk of contamination from operators, and reduce variability within the procedure, robotic approaches are extremely costly [184–186]. The development and miniaturisation of benchtop devices and processes through collaborations between biologists, mechanical and computer engineers may enable automated processes to be more broadly accessible within clinical practices in the future.

Safety issues are a major concern prior to clinical application of bioengineering approaches and regulation of use of cell and cell-based products differs from country to country. Safety tests of the cultured cells need to demonstrate that they are free from infectious agent contamination and tumour formation ability. Indeed, cultures should be free from bacteria, fungi, mycoplasma, viruses (*e.g.* hepatitis B, hepatitis C, and human T-lymphotropic virus), and endotoxin levels should also be monitored [187]. While tumour formation is a common risk for both autologous and allogeneic cell therapies, the transplantation of MSCs is considered a relatively safe procedure. However, long-term culture is known to increase the chances of cellular transformation, therefore, karyotypic analyses and transplantation of the MSCs in immuno-deficient animals should be routinely used to assess cancer risk [188].

For more global standardisation, characterisation and clinical application, it may be necessary to safely transport MSCs between sites. The carriage of the MSCs provides further risks to viability and phenotype, potentially due to needs to maintain cells at constant carbon dioxide tension, temperatures and air pressure. There is also a clear need for appropriate biological safety regulation and associated accompanying documentation for clinical materials which can increase administration processes, time and costs. Minimising the impact of transportation is, therefore, critical for the clinical and commercial application of cell therapies and devices, and procedures which enable this are being developed. It is proposed that variations in temperature during transport might be the most important risk to cell viability and that the optimal temperature for carriage may depend on cell type. Hydrogel technology is currently providing a novel means (as previously discussed) to better maintain MSC phenotype over a longer term at relatively low (<37 °C) and wider temperature ranges. This approach may be advantageous to ensure consistency in cell therapies between distant sites.

1.7 Concluding Remarks

While promising data have already been generated in vitro and in preclinical studies using animals, research remains ongoing to ensure that there is a significant and sound knowledge-base prior to clinical translation. In order for this translation to be realised, collaborative work and appropriate communication and dissemination between researchers, clinicians, industry and healthcare workers worldwide need to remain ongoing. Indeed, while considerable advancements have already been made over recent decades, it is imperative that attempts to translate basic science findings are not made too soon as this may generate risk for the patient. Therefore, appropriate restraint within the scientific and clinical communities is essential, and subsequent steps should be approached with caution as the patient's safety is of prime importance. Towards this goal, research governance and peer review processes need to be firmly in place. It is also important to determine which patient groups would best benefit from translation of stem cell science advances rather than incentives for application being driven due to any financial or industrial gain.

Indeed, in terms of dental patient need, it is important to consider whether novel bio-inspired techniques offer significant advantage over those currently applied. Such a risk-reward strategy may also help drive which approaches to prioritise for more rapid clinical fruition. For example, in regenerative endodontics, loss of pulp vitality in the child or younger adult in which full root formation is also not complete may provide a more realistic and successful application of pulp tissue bioengineering approaches which offers a long term patient benefit. Conversely, tooth bioengineering approaches within significantly older patients may be more challenging, less likely to succeed and therefore, current treatment approaches may be more appropriate. Indeed, it will be essential, as is currently the case, for a complete consideration of the patient's condition including age, need, diet and lifestyle, to be undertaken before any clinical work is performed which utilise MSC-based therapies.

In order to ensure coherent and comprehensive advancements within dentistry, we should also aim to learn from parallel areas of tissue engineering which are well advanced for repair at other sites of the body. Indeed in the US, stem cell research has recently become one of the pillars of the health programme and the US Military are significantly investing (>$250-million) in this area for soldier rehabilitation. It is, therefore, envisaged that ongoing and increased activity will lead to advances that will benefit dental medicine and enable clinicians to deliver novel therapies as part of their routine practice. Within the clinical setting, as has already been discussed, concomitantly advancements in related technologies will need to occur such that following tissue isolation, its processing and preparation happen as rapidly and routinely as possible. It is, therefore, envisaged that intelligent automatic and robotic systems will be necessary to help the clinician undertake these tasks and standardise the processes involved. Along with this is the need for the continued education of the dental team in areas of stem cell biology, biomaterials, and novel clinical procedures and equipment in order to ensure benefits are realised for patients.

References

1. Crisan M, Yap S, Casteilla L et al (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell 3:301–313
2. Da Silva ML, Chagastelles PC, Nardi NB (2006) Mesenchymal stem cells reside in virtually all post-natal organs and tissues. J Cell Sci 119:2204–2213
3. Kiel MJ, Morrison SJ (2008) Uncertainty in the niches that maintain haematopoietic stem cells. Nat Rev Immunol 8:290–301
4. Caplan AI (2008) All MSCs are pericytes? Cell Stem Cell 3:229–230
5. Slack JM (2008) Origin of stem cells in organogenesis. Science 322:1498–1501
6. Lakshmipathy U, Verfaillie C (2005) Stem cell plasticity. Blood Rev 19:29–38
7. Takahashi K, Tanabe K, Ohnuki M et al (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131:861–872
8. Takahashi K, Okita K, Nakagawa M et al (2007) Induction of pluripotent stem cells from fibroblast cultures. Nat Protoc 2:3081–3089
9. Gronthos S, Mankani M, Brahim J et al (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97:13625–13630

10. Huang GT, Sonoyama W, Liu Y et al (2008) The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod 34:645–651
11. Sonoyama W, Liu Y, Fang D et al (2006) Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One 1, e79
12. Sonoyama W, Liu Y, Yamaza T et al (2008) Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 34:166–171
13. Honda MJ, Imaizumi M, Tsuchiya S et al (2010) Dental follicle stem cells and tissue engineering. J Oral Sci 52:541–552
14. Morsczeck C, Gotz W, Schierholz J et al (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 24:155–165
15. Park BW, Kang EJ, Byun JH et al (2012) In vitro and in vivo osteogenesis of human mesenchymal stem cells derived from skin, bone marrow and dental follicle tissues. Differentiation 83:249–259
16. Yao S, Pan F, Prpic V et al (2008) Differentiation of stem cells in the dental follicle. J Dent Res 87:767–871
17. Seo BM, Miura M, Gronthos S et al (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364:149–155
18. Seo BM, Miura M, Sonoyama W et al (2005) Recovery of stem cells from cryopreserved periodontal ligament. J Dent Res 84:907–912
19. Miura M, Gronthos S, Zhao M et al (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100:5807–5812
20. Ikeda E, Yagi K, Kojima M et al (2008) Multipotent cells from the human third molar: feasibility of cell-based therapy for liver disease. Differentiation 76:495–505
21. Izumi K, Inoki K, Fujimori Y et al (2009) Pharmacological retention of oral mucosa progenitor/stem cells. J Dent Res 88:1113–1118
22. Marynka-Kalmani K, Treves S, Yafee M et al (2010) The lamina propria of adult human oral mucosa harbors a novel stem cell population. Stem Cells 28:984–995
23. Zhang Q, Shi S, Liu Y et al (2009) Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 183:7787–7798
24. Lim SM, Choi YS, Shin HC et al (2005) Isolation of human periosteum-derived progenitor cells using immunophenotypes for chondrogenesis. Biotechnol Lett 27:607–611
25. Denny PC, Denny PA (1999) Dynamics of parenchymal cell division, differentiation, and apoptosis in the young adult female mouse submandibular gland. Anat Rec 254:408–417
26. Kishi T, Takao T, Fujita K et al (2006) Clonal proliferation of multipotent stem/progenitor cells in the neonatal and adult salivary glands. Biochem Biophys Res Commun 340:544–552
27. Man YG, Ball WD, Marchetti L et al (2001) Contributions of intercalated duct cells to the normal parenchyma of submandibular glands of adult rats. Anat Rec 263:202–214
28. Derubeis AR, Cancedda R (2004) Bone marrow stromal cells (BMSCs) in bone engineering: limitations and recent advances. Ann Biomed Eng 32:160–165
29. Mizuno H, Tobita M, Uysal AC (2012) Concise review: adipose-derived stem cells as a novel tool for future regenerative medicine. Stem Cells 30:804–810
30. Horwitz EM, Le Blanc K, Dominici M et al (2005) Clarification of the nomenclature for MSC: the International Society for Cellular Therapy position statement. Cytotherapy 7:393–395
31. Battula VL, Treml S, Bareiss PM et al (2009) Isolation of functionally distinct mesenchymal stem cell subsets using antibodies against CD56, CD271, and mesenchymal stem cell antigen-1. Haematologica 94:173–184
32. Buhring HJ, Battula VL, Treml S et al (2007) Novel markers for the prospective isolation of human MSC. Ann NY Acad Sci 1106:262–271
33. Dominici M, Le Blanc K, Mueller I et al (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8:315–317

34. Friedenstein AJ, Chailakhjan RK, Lalykina KS (1970) The development of fibroblast colonies in monolayer cultures of guinea-pig bone marrow and spleen cells. Cell Tissue Kinet 3:393–403
35. Zannettino AC, Paton S, Kortesidis A et al (2007) Human multipotential mesenchymal/stromal stem cells are derived from a discrete subpopulation of STRO-1bright/CD34 /CD45(−)/glycophorin-A-bone marrow cells. Haematologica 92:1707–1708
36. Laino G, D'aquino R, Graziano A et al (2005) A new population of human adult dental pulp stem cells: a useful source of living autologous fibrous bone tissue (LAB). J Bone Miner Res 20:1394–1402
37. Nakashima M, Iohara K, Sugiyama M (2009) Human dental pulp stem cells with highly angiogenic and neurogenic potential for possible use in pulp regeneration. Cytokine Growth Factor Rev 20:435–440
38. Yang X, Van Den Dolder J, Walboomers XF et al (2007) The odontogenic potential of STRO-1 sorted rat dental pulp stem cells in vitro. J Tissue Eng Regen Med 1:66–73
39. Yang X, Zhang W, Van Den Dolder J et al (2007) Multilineage potential of STRO-1+ rat dental pulp cells in vitro. J Tissue Eng Regen Med 1:128–135
40. Zhang W, Walboomers XF, Van Kuppevelt TH et al (2006) The performance of human dental pulp stem cells on different three-dimensional scaffold materials. Biomaterials 27:5658–5668
41. Shi S, Gronthos S (2003) Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res 18:696–704
42. Carrion B, Huang CP, Ghajar CM et al (2010) Recreating the perivascular niche ex vivo using a microfluidic approach. Biotechnol Bioeng 107:1020–1028
43. Tsuchiya K, Chen G, Ushida T et al (2001) Effects of cell adhesion molecules on adhesion of chondrocytes, ligament cells and mesenchymal stem cells. Mater Sci Eng C 17:79–82
44. Waddington RJ, Youde SJ, Lee CP et al (2009) Isolation of distinct progenitor stem cell populations from dental pulp. Cells Tissues Organs 189:268–274
45. Zhu Q, Safavi KE, Spangberg LS (1998) Integrin expression in human dental pulp cells and their role in cell attachment on extracellular matrix proteins. J Endod 24:641–644
46. Thesleff I, Partanen AM, Vainio S (1991) Epithelial-mesenchymal interactions in tooth morphogenesis: the roles of extracellular matrix, growth factors, and cell surface receptors. J Craniofac Genet Dev Biol 11:229–237
47. Jussila M, Thesleff I (2012) Signaling networks regulating tooth organogenesis and regeneration, and the specification of dental mesenchymal and epithelial cell lineages. Cold Spring Harb Perspect Biol 4:a008425
48. Jung HS, Francis-West PH, Widelitz RB et al (1998) Local inhibitory action of BMPs and their relationships with activators in feather formation: implications for periodic patterning. Dev Biol 196:11–23
49. Sarkar L, Cobourne M, Naylor S et al (2000) Wnt/Shh interactions regulate ectodermal boundary formation during mammalian tooth development. Proc Natl Acad Sci U S A 97:4520–4524
50. Åberg T, Wang XP, Kim JH et al (2004) Runx2 mediates FGF signalling from epithelium to mesenchyme during tooth morphogenesis. Dev Biol 270:76–93
51. Chen Y, Zhang Y, Jiang TX et al (2000) Conservation of early odontogenic signaling pathways in Aves. Proc Natl Acad Sci U S A 97:10044–10049
52. Mostowska A, Kobielak A, Trzeciak WH (2003) Molecular basis of non-syndromic tooth agenesis: mutations of MSX1 and PAX9 reflect their role in patterning human dentition. Eur J Oral Sci 111:365–370
53. Mina M, Kollar EJ (1987) The induction of odontogenesis in non-odontogenic mesenchyme combined with early murine mandibular arch epithelium. Arch Oral Biol 32:123–127
54. Ferguson C, Tucker AS, Christensen L et al (1998) Activin is an essential early mesenchymal signal in tooth development that is required for patterning of the murine dentition. Genes Dev 12:2636–2649

55. Jernvall J, Åberg T, Kettunen P et al (1998) The life history of an embryonic signaling center: BMP4 induces p21 and is associated with apoptosis in the mouse tooth enamel knot. Development 125:161–169
56. Vaahtokari A, Åberg T, Jernvall J et al (1996) The enamel knot as a signaling centre in the developing mouse tooth. Mech Dev 54:39–43
57. Slavkin HC (1974) Embryonic tooth formation. A tool for developmental biology. Oral Sci Rev 4:7–136
58. Coin R, Lesot H, Vonesch JL et al (1999) Aspects of cell proliferation kinetics of the inner dental epithelium during mouse molar and incisor morphogenesis: a reappraisal of the role of the enamel knot area. Int J Dev Biol 43:261–267
59. Lyngstadaas SP, Møinichen CB, Risnes S (1998) Crown morphology, enamel distribution, and enamel structure in mouse molars. Anat Rec 250:268–280
60. Salazar-Ciudad I, Jernvall J (2002) A gene network model accounting for development and evolution of mammalian teeth. Proc Natl Acad Sci U S A 99:8116–8120
61. Slavkin HC (1991) Molecular determinants during dental morphogenesis and cytodifferentiation: a review. J Craniofac Genet Dev Biol 11:338–349
62. Goldberg M, Septier D, Lécolle S et al (1995) Dental mineralization. Int J Dev Biol 39:93–110
63. Marks SC Jr, Schroeder HE (1996) Tooth eruption: theories and facts. Anat Rec 245:374–393
64. Simon SR, Berdal A, Cooper PR et al (2011) Dentin-pulp complex regeneration: from lab to clinic. Adv Dent Res 23:340–345
65. Smith AJ, Cassidy N, Perry H et al (1995) Reactionary dentinogenesis. Int J Dev Biol 39:273–280
66. Smith AJ, Lumley PJ, Tomson PL et al (2008) Dental regeneration and materials: a partnership. Clin Oral Investig 12:103–108
67. Ferracane JL, Cooper PR, Smith AJ (2010) Can interaction of materials with the dentin-pulp complex contribute to dentin regeneration? Odontology 98:2–14
68. Smith AJ, Scheven BA, Takahashi Y et al (2012) Dentine as a bioactive extracellular matrix. Arch Oral Biol 57:109–121
69. Frozoni M, Zaia AA, Line SR et al (2012) Analysis of the contribution of nonresident progenitor cells and hematopoietic cells to reparative dentinogenesis using parabiosis model in mice. J Endod 38:1214–1219
70. Pittenger MF, Mackay AM, Beck SC et al (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147
71. Dezawa M, Kanno H, Hoshino M et al (2004) Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. J Clin Invest 113:1701–1710
72. Akintoye SO, Lam T, Shi S et al (2006) Skeletal site-specific characterization of orofacial and iliac crest human bone marrow stromal cells in same individuals. Bone 38:758–768
73. Chai Y, Jiang X, Ito Y et al (2000) Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development 127:1671–1679
74. Igarashi A, Segoshi K, Sakai Y et al (2007) Selection of common markers for bone marrow stromal cells from various bones using real-time RT-PCR: effects of passage number and donor age. Tissue Eng 13:2405–2417
75. Hung CN, Mar K, Chang HC et al (2011) A comparison between adipose tissue and dental pulp as sources of MSCs for tooth regeneration. Biomaterials 32:6995–7005
76. Ishizaka R, Iohara K, Murakami M et al (2012) Regeneration of dental pulp following pulpectomy by fractionated stem/progenitor cells from bone marrow and adipose tissue. Biomaterials 33:2109–2118
77. Tobita M, Uysal AC, Ogawa R et al (2008) Periodontal tissue regeneration with adipose-derived stem cells. Tissue Eng Part A 14:945–953
78. Wen X, Nie X, Zhang L et al (2011) Adipose tissue-deprived stem cells acquire cementoblast features treated with dental follicle cell conditioned medium containing dentin non-collagenous proteins in vitro. Biochem Biophys Res Commun 409:583–589

79. Gronthos S, Brahim J, Li W et al (2002) Stem cell properties of human dental pulp stem cells. J Dent Res 81:531–535
80. Kaukua N, Shahidi MK, Konstantinidou C et al (2014) Glial origin of mesenchymal stem cells in a tooth model system. Nature 513:551–554
81. Wang L, Shen H, Zheng W et al (2011) Characterization of stem cells from alveolar periodontal ligament. Tissue Eng Part A 17:1015–1026
82. Oda Y, Yoshimura Y, Ohnishi H et al (2010) Induction of pluripotent stem cells from human third molar mesenchymal stromal cells. J Biol Chem 285:29270–29278
83. De Bari C, Dell'Accio F, Vanlauwe J et al (2006) Mesenchymal multipotency of adult human periosteal cells demonstrated by single-cell lineage analysis. Arthritis Rheum 54:1209–1221
84. Sakaguchi Y, Sekiya I, Yagishita K et al (2005) Comparison of human stem cells derived from various mesenchymal tissues: superiority of synovium as a cell source. Arthritis Rheum 52:2521–2529
85. Wang Q, Huang C, Zeng F et al (2010) Activation of the Hh pathway in periosteum-derived mesenchymal stem cells induces bone formation in vivo: implication for postnatal bone repair. Am J Pathol 177:3100–3111
86. Nagata M, Hoshina H, Li M et al (2012) A clinical study of alveolar bone tissue engineering with cultured autogenous periosteal cells: coordinated activation of bone formation and resorption. Bone 50:1123–1129
87. Schmelzeisen R, Schimming R, Sittinger M (2003) Making bone: implant insertion into tissue-engineered bone for maxillary sinus floor augmentation – a preliminary report. J Craniomaxillofac Surg 31:34–39
88. Soltan M, Smiler D, Soltan C (2009) The inverted periosteal flap: a source of stem cells enhancing bone regeneration. Implant Dent 18:373–379
89. Lombaert IM, Brunsting JF, Wierenga PK et al (2008) Rescue of salivary gland function after stem cell transplantation in irradiated glands. PLoS One 3, e2063
90. Matsumoto S, Okumura K, Ogata A et al (2007) Isolation of tissue progenitor cells from duct-ligated salivary glands of swine. Cloning Stem Cells 9:176–190
91. Sato A, Okumura K, Matsumoto S et al (2007) Isolation, tissue localization, and cellular characterization of progenitors derived from adult human salivary glands. Cloning Stem Cells 9:191–205
92. Nanduri LS, Maimets M, Pringle SA et al (2011) Regeneration of irradiated salivary glands with stem cell marker expressing cells. Radiother Oncol 99:367–372
93. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126:663–676
94. Yu J, Vodyanik MA, Smuga-Otto K et al (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318:1917–1920
95. Egusa H, Okita K, Kayashima H et al (2010) Gingival fibroblasts as a promising source of induced pluripotent stem cells. PLoS One 5, e12743
96. Miyoshi K, Tsuji D, Kudoh K et al (2010) Generation of human induced pluripotent stem cells from oral mucosa. J Biosci Bioeng 110:345–350
97. Tamaoki N, Takahashi K, Tanaka T et al (2010) Dental pulp cells for induced pluripotent stem cell banking. J Dent Res 89:773–778
98. Wada N, Wang B, Lin NH et al (2011) Induced pluripotent stem cell lines derived from human gingival fibroblasts and periodontal ligament fibroblasts. J Periodontal Res 46:438–447
99. Yan X, Qin H, Qu C et al (2010) iPS cells reprogrammed from mesenchymal-like stem/progenitor cells of dental tissue origin. Stem Cells Dev 19:469–480
100. Arakaki M, Ishikawa M, Nakamura T et al (2012) Role of epithelial-stem cell interactions during dental cell differentiation. J Biol Chem 287:10590–10601
101. Duan X, Tu Q, Zhang J, Ye J et al (2011) Application of induced pluripotent stem (iPS) cells in periodontal tissue regeneration. J Cell Physiol 226:150–157

102. Otsu K, Kishigami R, Oikawa-Sasaki A et al (2011) Differentiation of induced pluripotent stem cells into dental mesenchymal cells. Stem Cells Dev 21:1156–1164
103. Aasen T, Raya A, Barrero MJ et al (2008) Efficient and rapid generation of induced pluripotent stem cells from human keratinocytes. Nat Biotechnol 26:1276–1284
104. Cao F, Drukker M, Lin S et al (2007) Molecular imaging of embryonic stem cell misbehavior and suicide gene ablation. Cloning Stem Cells 9:107–117
105. Huangfu D, Maehr R, Guo W et al (2008) Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds. Nat Biotechnol 26:795–797
106. Huangfu D, Osafune K, Maehr R et al (2008) Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. Nat Biotechnol 26:1269–1275
107. Kim D, Kim CH, Moon JI et al (2009) Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. Cell Stem Cell 4:472–476
108. Miyoshi N, Ishii H, Nagano H et al (2011) Reprogramming of mouse and human cells to pluripotency using mature microRNAs. Cell Stem Cell 8:633–638
109. Nakagawa M, Takizawa N, Narita M et al (2010) Promotion of direct reprogramming by transformation-deficient Myc. Proc Natl Acad Sci U S A 107:14152–14157
110. Okita K, Matsumura Y, Sato Y et al (2011) A more efficient method to generate integration-free human iPS cells. Nat Methods 8:409–412
111. Schuldiner M, Itskovitz-Eldor J, Benvenisty N (2003) Selective ablation of human embryonic stem cells expressing a suicide gene. Stem Cells 21:257–265
112. Tang C, Lee AS, Volkmer JP et al (2011) An antibody against SSEA-5 glycan on human pluripotent stem cells enables removal of teratoma-forming cells. Nat Biotechnol 29:829–834
113. Warren L, Manos PD, Ahfeldt T et al (2010) Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. Cell Stem Cell 7:618–630
114. Nakashima M, Akamine A (2005) The application of tissue engineering to regeneration of pulp and dentin in endodontics. J Endod 31:711–718
115. Gottlow J, Nyman S, Lindhe J et al (1986) New attachment formation in the human periodontium by guided tissue regeneration. Case reports. J Clin Periodontol 13:604–616
116. Karring T, Nyman S, Gottlow J et al (1993) Development of the biological concept of guided tissue regeneration – animal and human studies. Periodontology 2000(1):26–35
117. Le Geros RZ (2008) Calcium phosphate-based osteoinductive materials. Chem Rev 108:4742–4753
118. Nyman S, Gottlow J, Lindhe J et al (1987) New attachment formation by guided tissue regeneration. J Periodontal Res 22:252–254
119. Darby I (2011) Periodontal materials. Aust Dent J 56:107–118
120. Klinge B, Flemmig TF (2009) Tissue augmentation and esthetics (Working Group 3). Clin Oral Implants Res 20:166–170
121. Kubo T, Doi K, Hayashi K et al (2011) Comparative evaluation of bone regeneration using spherical and irregularly shaped granules of interconnected porous hydroxylapatite. A beagle dog study. J Prosthodont Res 55:104–109
122. Le Geros RZ (2002) Properties of osteoconductive biomaterials: calcium phosphates. Clin Orthop Relat Res 395:81–98
123. MacIntosh AC, Kearns VR, Crawford A et al (2008) Skeletal tissue engineering using silk biomaterials. J Tissue Eng Regen Med 2:71–80
124. Mandal BB, Grinberg A, Gil ES et al (2012) High-strength silk protein scaffolds for bone repair. Proc Natl Acad Sci U S A 109:7699–7704
125. Sundelacruz S, Kaplan DL (2009) Stem cell- and scaffold-based tissue engineering approaches to osteochondral regenerative medicine. Semin Cell Dev Biol 20:646–655
126. Honda MJ, Tsuchiya S, Sumita Y et al (2007) The sequential seeding of epithelial and mesenchymal cells for tissue-engineered tooth regeneration. Biomaterials 28:680–689
127. Sumita Y, Honda MJ, Ohara T et al (2006) Performance of collagen sponge as a 3-D scaffold for tooth-tissue engineering. Biomaterials 27:3238–3248

128. Almushayt A, Narayanan K, Zaki AE et al (2006) Dentin matrix protein 1 induces cytodifferentiation of dental pulp stem cells into odontoblasts. Gene Ther 13:611–620
129. Cordeiro MM, Dong Z, Kaneko T et al (2008) Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 34:962–969
130. Galler KM, Cavender A, Yuwono V et al (2008) Self-assembling peptide amphiphile nanofibers as a scaffold for dental stem cells. Tissue Eng Part A 14:2051–2058
131. Smith AJ, Tobias RS, Plant CG et al (1990) In vivo morphogenetic activity of dentine matrix proteins. J Biol Buccale 18:123–129
132. Smith AJ, Tobias RS, Cassidy N et al (1994) Odontoblast stimulation in ferrets by dentine matrix components. Arch Oral Biol 39:13–22
133. Intini G (2009) The use of platelet-rich plasma in bone reconstruction therapy. Biomaterials 30:4956–4966
134. Hammarstrom L (1997) Enamel matrix, cementum development and regeneration. J Clin Periodontol 24:658–668
135. Kawase T, Okuda K, Yoshie H et al (2000) Cytostatic action of enamel matrix derivative (EMDOGAIN) on human oral squamous cell carcinoma- derived SCC25 epithelial cells. J Periodontal Res 35:291–300
136. Sculean A, Schwarz F, Becker J et al (2007) The application of an enamel matrix protein derivative (Emdogain) in regenerative periodontal therapy: a review. Med Princ Pract 16:167–180
137. Andrae J, Gallini R, Betsholtz C (2008) Role of platelet-derived growth factors in physiology and medicine. Genes Dev 22:1276–1312
138. Javed F, Al-Askar M, Al-Rasheed A et al (2011) Significance of the platelet-derived growth factor in periodontal tissue regeneration. Arch Oral Biol 56:1476–1484
139. Kaigler D, Avila G, Wisner-Lynch L et al (2011) Platelet-derived growth factor applications in periodontal and peri-implant bone regeneration. Expert Opin Biol Ther 11:375–385
140. Kitamura M, Akamatsu M, Machigashira M et al (2011) FGF-2 stimulates periodontal regeneration: results of a multi-center randomized clinical trial. J Dent Res 90:35–40
141. Murakami S (2011) Periodontal tissue regeneration by signaling molecule(s): what role does basic fibroblast growth factor (FGF-2) have in periodontal therapy? Periodontology 2000(56):188–208
142. Ribatti D, Nico B, Crivellato E (2011) The role of pericytes in angiogenesis. Int J Dev Biol 55:261–268
143. Taba M Jr, Jin Q, Sugai JV et al (2005) Current concepts in periodontal bioengineering. Orthod Craniofac Res 8:292–302
144. Tsuji K, Bandyopadhyay A, Harfe BD et al (2006) BMP2 activity, although dispensable for bone formation, is required for the initiation of fracture healing. Nat Genet 38:1424–1429
145. Wikesjo UM, Qahash M, Huang YH et al (2009) Bone morphogenetic proteins for periodontal and alveolar indications; biological observations – clinical implications. Orthod Craniofac Res 12:263–270
146. El Omar R, Beroud J, Stoltz JF et al (2014) Umbilical cord mesenchymal stem cells: the new gold standard for mesenchymal stem cell-based therapies? Tissue Eng Part B Rev 20:523–544
147. Cooper PR, Holder MJ, Smith AJ (2014) Inflammation and regeneration in the dentin-pulp complex: a double-edged sword. J Endod 40:S46–S51
148. Mead B, Logan A, Berry M et al (2013) Intravitreally transplanted dental pulp stem cells promote neuroprotection and axon regeneration of retinal ganglion cells after optic nerve injury. Invest Ophthalmol Vis Sci 54:7544–7556
149. Yang B, Chen G, Li J, Zou Q et al (2012) Tooth root regeneration using dental follicle cell sheets in combination with a dentin matrix-based scaffold. Biomaterials 33:2449–2461
150. Duailibi MT, Duailibi SE, Young CS et al (2004) Bioengineered teeth from cultured rat tooth bud cells. J Dent Res 83:523–528
151. Ikeda E, Morita R, Nakao K et al (2009) Fully functional bioengineered tooth replacement as an organ replacement therapy. Proc Natl Acad Sci U S A 106:13475–13480

152. Ohazama A, Modino SA, Miletich I et al (2004) Stem-cell-based tissue engineering of murine teeth. J Dent Res 83:518–522
153. Oshima M, Mizuno M, Imamura A et al (2011) Functional tooth regeneration using a bioengineered tooth unit as a mature organ replacement regenerative therapy. PLoS One 6, e21531
154. Young CS, Abukawa H, Asrican R et al (2005) Tissue-engineered hybrid tooth and bone. Tissue Eng 11:1599–1610
155. Volponi A, Kawasaki M, Sharpe PT (2013) Adult human gingival epithelial cells as a source for whole-tooth bioengineering. J Dent Res 92:329–334
156. Kojima T, Kanemaru S, Hirano S et al (2011) Regeneration of radiation damaged salivary glands with adipose-derived stromal cells. Laryngoscope 121:1864–1869
157. Sumita Y, Liu Y, Khalili S et al (2011) Bone marrow derived cells rescue salivary gland function in mice with head and neck irradiation. Int J Biochem Cell Biol 43:80–87
158. Alhadlaq A, Mao JJ (2003) Tissue-engineered neogenesis of human-shaped mandibular condyle from rat mesenchymal stem cells. J Dent Res 82:951–956
159. Alhadlaq A, Elisseeff JH, Hong L et al (2004) Adult stem cell driven genesis of human-shaped articular condyle. Ann Biomed Eng 32:911–923
160. El-Bialy T, Uludag H, Jomha N et al (2010) In vivo ultrasound-assisted tissue-engineered mandibular condyle: a pilot study in rabbits. Tissue Eng Part C Methods 16:1315–1323
161. Bunaprasert T, Hadlock T, Marler J et al (2003) Tissue engineered muscle implantation for tongue reconstruction: a preliminary report. Laryngoscope 113:1792–1797
162. Egusa H, Kobayashi M, Matsumoto T et al (2013) Application of cyclic strain for accelerated skeletal myogenic differentiation of mouse bone marrow-derived mesenchymal stromal cells with cell alignment. Tissue Eng Part A 19:770–782
163. Luxameechanporn T, Hadlock T, Shyu J et al (2006) Successful myoblast transplantation in rat tongue reconstruction. Head Neck 28:517–524
164. Bansal R, Jain A (2015) Current overview on dental stem cells applications in regenerative dentistry. J Nat Sci Biol Med 6:29–34
165. Davies OG, Smith AJ, Cooper PR et al (2014) The effects of cryopreservation on cells isolated from adipose, bone marrow and dental pulp tissues. Cryobiology 69:342–347
166. Chen B, Wright B, Sahoo R et al (2013) A novel alternative to cryopreservation for the short-term storage of stem cells for use in cell therapy using alginate encapsulation. Tissue Eng Part C Methods 19:568–576
167. Liu BL, McGrath JJ (2005) Ice formation of vitrification solutions for cryopreservation of tissues. Conf Proc IEEE Eng Med Biol Soc 7:7501–7504
168. Fekete N, Rojewski MT, Fürst D et al (2012) GMP-compliant isolation and large-scale expansion of bone marrow-derived MSC. PLoS One 7, e43255
169. D'Ippolito G, Schiller PC, Ricordi C et al (1999) Agerelated osteogenic potential of mesenchymal stromal stem cells from human vertebral bone marrow. J Bone Miner Res 14:1115–1122
170. Phinney DG, Kopen G, Righter W et al (1999) Donor variation in the growth properties and osteogenic potential of human marrow stromal cells. J Cell Biochem 75:424–436
171. Zhou S, Greenberger JS, Epperly MW et al (2008) Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell 7:335–343
172. Patel M, Smith AJ, Sloan AJ et al (2009) Phenotype and behaviour of dental pulp cells during expansion culture. Arch Oral Biol 54:898–908
173. Stevens A, Zuliani T, Olejnik C et al (2008) Human dental pulp stem cells differentiate into neural crest derived melanocytes and have label-retaining and sphere-forming abilities. Stem Cells Dev 17:1175–1184
174. Lennon DP, Edmison JM, Caplan AI (2001) Cultivation of rat marrow derived mesenchymal stem cells in reduced oxygen tension: effects on in vitro and in vivo osteochondrogenesis. J Cell Physiol 187:345–355
175. Horwitz EM, Gordon PL, Koo WK et al (2002) Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. Proc Natl Acad Sci U S A 99:8932–8937

176. Kuznetsov SA, Mankani MH, Robey PG (2000) Effect of serum on human bone marrow stromal cells: ex vivo expansion and in vivo bone formation. Transplantation 70:1780–1787
177. Schallmoser K, Strunk D (2013) Generation of a pool of human platelet lysate and efficient use in cell culture. Methods Mol Biol 946:349–362
178. Stute N, Holtz K, Bubenheim M et al (2004) Autologous serum for isolation and expansion of human mesenchymal stem cells for clinical use. Exp Hematol 32:1212–1225
179. Yamada Y, Ueda M, Hibi H et al (2004) Translational research for injectable tissue-engineered bone regeneration using mesenchymal stem cells and platelet-rich plasma: from basic research to clinical case study. Cell Transplant 13:343–355
180. Agata H, Watanabe N, Ishii Y et al (2009) Feasibility and efficacy of bone tissue engineering using human bone marrow stromal cells cultivated in serum-free conditions. Biochem Biophys Res Commun 382:353–358
181. Chase LG, Lakshmipathy U, Solchaga LA et al (2010) A novel serum-free medium for the expansion of human mesenchymal stem cells. Stem Cell Res Ther 1:8
182. Chase LG, Yang S, Zachar V et al (2012) Development and characterization of a clinically compliant xeno-free culture medium in good manufacturing practice for human multipotent mesenchymal stem cells. Stem Cells Transl Med 1:750–758
183. Lindroos B, Boucher S, Chase L et al (2009) Serum-free, xeno-free culture media maintain the proliferation rate and multipotentiality of adipose stem cells in vitro. Cytotherapy 11:958–972
184. Kato R, Iejima D, Agata H et al (2010) A compact, automated cell culture system for clinical scale cell expansion from primary tissues. Tissue Eng Part C Methods 16:947–956
185. Kino-Oka M, Ogawa N, Umegaki R et al (2005) Bioreactor design for successive culture of anchorage-dependent cells operated in an automated manner. Tissue Eng 11:535–545
186. Koller MR, Manchel I, Maher RJ et al (1998) Clinical-scale human umbilical cord blood cell expansion in a novel automated perfusion culture system. Bone Marrow Transplant 21:653–663
187. Rayment EA, Williams DJ (2010) Concise review: mind the gap: challenges in characterizing and quantifying cell- and tissue-based therapies for clinical translation. Stem Cells 28:996–1004
188. Bernardo ME, Zaffaroni N, Novara F et al (2007) Human bone marrow derived mesenchymal stem cells do not undergo transformation after long-term in vitro culture and do not exhibit telomere maintenance mechanisms. Cancer Res 67:9142–9149

Chapter 2
Immunomodulatory Properties of Stem Cells Derived from Dental Tissues

Pakize Neslihan Taşlı, Safa Aydın, and Fikrettin Şahin

2.1 Mesenchymal Stem Cell (MSC)-Mediated Immunomodulation

MSCs are basically characterized by their potential to adhere to plastic surfaces in cell culture conditions, express cell surface markers of CD105, CD44, CD29, CD73, and CD90, but not express hematopoietic stem cell surface makers such as CD45, CD34, CD14, and differentiate into osteo-, adipo-, and chondro-genic cell lines [1]. Along with their high self-renewal and multi-lineage differentiation abilities, they possess an immune-suppressive activity, which make them promising candidates to be used in cell and even tissue mediated treatments of a various immune and inflammation dependent diseases.

MSCs can be easily obtained from various human tissues, preserved in culture conditions, and used in even histocompatibility antigen (HLA) un-matching conditions of transplantation patients. Though they are mainly isolated from bone marrow, they can also be obtained from fat tissue [2], cartilage [3], liver [4], amniotic fluid [5], tooth germ [6], hair follicle [7] and so on [8–10].

Several reports indicate that MSC could be used in vast amount immune disorders. In particular, researchers have found that systematic infusion of allogenic stem cells may offer a new suitable treatment for Graft versus host disease (GVHD) patients [11]. In addition, Prasad et al. described a stem cell derived agent to treat GVHD patients [12]. The ability of MSCs to stimulate activation, proliferation, and

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function of immune cells may take part on tissue healing and regeneration, which would also contribute to treatment of several immune disease including GVHD.

2.1.1 Interactions of MSCs with White Blood Cells

2.1.1.1 T Lymphocyte-Mediated Suppression

The basic role of T cells, one main component of the adaptive immunity which forms immunological memory, is to create specialized immune reactions to particular pathogens [13]. Interaction between MSCs and T cells is critical for the activity of immune system. Direct cell to cell contact of MSCs with $CD8^+$ (cytotoxic T cells) or $CD4^+$ (regulatory T cells) helper T cells moderates the expression of cytokines for signaling and inhibition of T cell activation [14, 15].

Recent studies have demonstrated that MSCs express Fas ligand (FasL) known as a death receptor which inhibit T cell migration [13], triggering stimulated T cell death via direct cell interaction [16]. Even though inhibition of T cell proliferation via MSCs action has partially known, suppressive effect of MSCs has not been elucidated in the manner of autologous or allogeneic respective [17, 18]. One possible action of MSCs is suppression of $CD8^+$ cytotoxic T cell proliferation, rather than a direct suppression of cytolysis [19, 20]. Furthermore, MSCs can enhance secretion of interferon gamma (IFNγ) and interleukin (IL)-17, while they induce T helper cells to produce IL-4 [21, 22].

It has been reported that MSCs can promote the proliferation of regulatory T cells and increase their modulatory ability directly or indirectly. When peripheral blood mononuclear cell (PBMCs) are triggered by mitogens, modulatory phenotype presenting $CD4^+$ $CD25^+$ T cells start to proliferate in the existence of MSCs. Inhibition of T cell proliferation by MSCs is triggered by allogeneic, mitogenic, or antigen specific stimuli [23].

Notably, scientists found that suppressed T cell proliferation via triggering apoptosis when T cells were stimulated by foreign mitogen, had no influence on latent T cells [23]. However, the proliferation of inactive and separating thymocytes was repressed via MSCs once treated in absence of systemic elements. These findings propose that MSCs can be capable of stimulating the endurance of T cells in a latent state. Furthermore, it has also been revealed that MSCs can repress T cell activation but not their toxicity by arresting T cells in G0/G1 cell cycle phase through suppression of cyclin D and increment of p27kip1 expression [24–26]. As a mechanism of action, it has been reported that MSC-mediated repression of T cell proliferation was not found to be because of soluble HLA-G5 isoform, but of the surface expression of HLA G1 [24].

In last decade, many studies using the heading ‘MSCs in Solid Organ Transplantation (SOT)’ were designed to understand what is unknown about the MSCs use in the setting of SOT, and how to progress best in clinical trials [27]. Although there is not any clinical studies evaluating the efficiency of MSCs against Solid Organ Allograft (SOA) rejection, a plentiful and increasing quantity of evi-

dences obtained from animal models propose that this methodology may be promising. Based on in vitro and in vivo animal models, a possible role of these cells on the prevention of acute tissue allograft rejection has been recommended [28–31].

General MSC-based treatment studies have proved that allogenic MSCs can diffuse into related tissue during SOT. Enhanced allograft persistence or abolition in the simultaneous suppression is few of the frequently reported conclusions. Although human clinical trials are not adequate, preclinical studies have shown that MSCs could be important therapeutic tools for organ transplantation approaches not just as they have the ability to modulate the host immunity in a way that may promote tolerance of the transplanted organ, but also their regeneration capability and trophic factor expression profiles may also help to lessen inflammatory responses to the allograft. One of the main aim of SOT studies is to inhibit T cell response against external antigen. In this line, MSCs can suppress the proliferation of cytotoxic T cells while enhancing the activities of helper and regulatory T cells. Some immunomodulation-related studies have proposed that these properties of MSCs may be the key reasons for their ability to prevent the allo-immune reactions in vivo [32]. More research must be carried out because lack of knowledge about MSCs-mediated immune suppression; nevertheless, the fewer toxicity and possible long term immunosuppression effect of MSCs make them a potentially striking therapeutic applicant with respect to outmoded T cell modulatory agents. Another advantage of MSCs use for the inhibition of SOA rejection is the infusion of these cells while SOT may hold the potential to endorse a state of cell chimerism and continuing tolerance of the transplanted organ via host immune system [33, 34].

2.1.1.2 B Lymphocyte-Mediated Suppression

Even though the main player of the immune suppression response is the T cells, B cells can also secret antibodies to regulate immune response, and they closely cooperate with T cells. The exact mechanism of MSCs on B cells still remains unknown. However, most of the immune suppression related studies have shown that MSCs can suppress B cell proliferation, differentiation, and cytokine production in in vitro co-culture assays and in vivo multiple sclerosis models [35–38]. In contrast, it has also been shown that MSCs increase B cell proliferation and trigger cytokine productions from B cells [38, 39].

MSCs can enhance the production of IgG from peripheral blood and spleen originated B cells, but they can also inhibit IgG secretion if a heavy primary stimulus is used to trigger B cells. One of the possible actions of B cell dependent MSCs-mediated suppression can be formed by differentiated B cell or the direct effect of the local stimulating signals. MSCs can inhibit B cell proliferation when B cells starts to secret activation signals to the culture medium in co-culture system, suggesting that MSCs need activation indicators derived from B cells to inhibit B cell stimulation. This cross talk between MSCs and B cells lead to inhibition of B cell proliferation. Some of the important factors secreted from MSCs are transforming growth factor (TGF)-β, hepatocyte growth factor (HGF), prostaglandin E2 (PGE2),

and indoleamine 2,3-dioxygenase (IDO), which conduct MSC-derived immunosuppressive action on B cells [40].

As a conclusion, important part of B cell stimulation is carried out via T cell dependent pathways; hence, the effect of MSCs on T cell activity may also indirectly inhibit B cell actions [37]. Furthermore, MSCs seem to display a direct stimulus on B cells by direct cell to cell interactions and via production of vital inducer signals [36, 40]. One other critical issue is that majority of these experiments have been made in in vitro conditions, not in vivo or ex vivo. Therefore, actual immunosuppression effect of DSCs on B lymphocytes in vivo still remains to be determined.

2.1.1.3 Dendritic Cell-Mediated Suppression

MSCs have also been proposed to be effective against monocytes, monocyte derivative dendritic cells, macrophages, natural killer cells and neutrophils. Several in vitro works have presented that MSCs can inhibit the evolution of monocytes into dendritic cells via suppressing the antigen presentation role of these cells [41, 42]. As recently suggested, dendritic cells are an important part of immune reaction in regards of immune response and tolerance, depending on the stimulation and maturation period and the cytokine environment at locations of inflammation [43]. The inhibitory effect of MSCs on dendritic cell differentiation, maturation and function could be via suppressing $CD14^+$ monocyte differentiation into matured dendritic cells and triggering the production of inducer molecules [44, 45]. In addition, MSCs can suppress the differentiation of allo-antigen stimulated monocytes toward mature dendritic cells [45]. Immature dendritic cells can induce cytokine production characterized by a reduced secretion of pro-inflammatory molecules including tumor necrosis factor alpha (TNFα), IFNγ and IL-12, and an amplified secretion of the anti-inflammatory molecule such as IL-10, when cultured in the presence of MSCs [44–47]. Similarly, as MSCs cultured with mature dendritic cells, they started to display decreased function of antigen-presenting, and down-regulated IL-12 production [45].

2.1.1.4 Natural Killer Cell (NKs)-Mediated Suppression

NK cells take crucial roles in intrinsic immune response, particularly in anti-tumor and anti-viral functions due to their cytotoxic activity, and they have the ability to produce large amount of pro-inflammatory molecules, including TNFα and IFNγ [48]. Impulsive cytotoxic function of NK cells especially target the cells displaying decreased HLA-I expression. Moreover, MSCs suppress IL-2 and IL-15 mediated NK cell proliferation, IFNγ secretion, and cytotoxic function of both latent and stimulated NK cells [20, 21, 49]. Recently, researches have demonstrated that cell-cell interaction is the most critical event in the MSC-mediated immunosuppression, whereas other cells were driven by soluble elements dependent on HLA-I characteristic. Donor cells isolated from HLA incompatible patients are much more vulnerable

to be lysed by triggered NK cells via secretion of high amount of IFNγ and TNFα ex vivo [50]. MSCs suppress the expression of NKp30 and NKG2D surface receptors which are take part in NK cell function and lysing of targeted cells [51].

Direct cell to cell interaction of MSCs with active immune cells leads inhibition or limitation of inflammatory responses and enhances the mitigating and anti-inflammatory pathways. Depending on activation type and expression profile resulted via danger signals, inhibition or limitation can be chosen by MSCs [52]. In addition, transition of MSCs into infected and damaged tissue might interfere to secondary lymphoid organs which are crucial for the immunity. They can also diffuse into the damaged tissue or organ to trigger healing process, resulting in functional tissue or organ regeneration and local immunity. While these studies enlarge our understanding about MSC-mediated immunosuppression, further studies must be strictly conducted to fully elucidate exact action of mechanism underlying these suppressive, regenerative, and anti-inflammatory functions [11, 14].

2.2 Immuno-Modulation of DSCs

Immunomodulation properties of MSCs make them outstanding candidates as immunosuppressive drugs for the prevention and treatment of various inflammatory and autoimmune diseases [38, 53]. Starting from the 2000, unique MSC sources have been obtained from several dental tissues, which exhibit remarkable tissue regenerative and immunosuppressive properties [54]. These dental tissue derived cells include dental pulp stem cells (DPSCs), periodontal ligament stem cells (PDLSCs), gingival mesenchymal stem cells (GMSCs), tooth germ stem cells (TGSCs), apical papilla stem cells (SCAPs), exfoliated deciduous teeth stem cells (SHEDs), and dental follicles stem cells (DFSCs) [55–57]. Other than having spectacular self-renewal property and multipotency, DSCs have powerful immunosuppressive functions comparable to other stem cell types, making them promising cell sources for MSC-mediated transplantation treatment [58].

2.2.1 DPSCs

DPSCs have great regeneration capacity which can create pulp and blood vessels containing fibrous tissue resembling human tooth like structure [55]. Studies exploring the immunosuppressive characteristics of DSCs showed that DPSCs could suppress the proliferation of activated T cells better than bone marrow MSCs (BMMSCs) [17, 57]. This remarkable immunosuppressive function of DPSCs could make them appropriate cell type for allogenic bone marrow replacement therapy [59]. In addition, proliferation of PBMCs could be inhibited by TGF-β secreted from DPSCs, indicating the immunosuppressive role of MSCs triggered by signaling molecules derived from stimulated DSCs [60].

DPSCs have been used in an important transplantation study to treat Duchenne Muscular Dystrophy (DMD) of dogs carrying congenitally golden retriever muscular dystrophy (GRMD). Researchers found that DPSCs displayed immune suppression in GRMD dogs and helped to relieve disease symptoms [61]. In addition, DPSCs has been shown to trigger T cell apoptosis in vitro, and improve inflammation dependent tissue injuries as given to a murine colitis model in vivo. siRNA mediated inhibition of FasL expression, a trans membrane protein, involves in induction of the Fas/ FasL apoptotic pathway in DPSCs, leading to partial suppression of T cell apoptosis in vitro, and reduction in therapeutic effects of DPSCs in mice colitis models in vivo. Moreover, it has been proposed that FasL expression levels could regulate duplication rate and differentiation capacity of DPSCs.

2.2.2 PDLSCs

Periodontal ligament (PDL) tissue is originated from neural crest, and it can be obtained from dental follicle. Mainly, PDL cells connect the cementum to the bone, and support the tooth organ in the alveolar socket [62, 63]. These cells could also arrange the tooth nutrient source, homeostasis, restoration and regeneration of injured tissues [64, 65].

Several comparative studies showed that both PDLSCs and BMMSCs demonstrated suppressive effects on the production of allogeneic and xenogeneic PBMCs by blocking the cell doubling via secretion of TGF-β, HGF, and IDO [66]. Another study exploring the immunosuppressive role of PDLSCs has indicated that the inflamed PDLSCs displayed considerably reduced suppressor effects on T cell activation compared to healthy cells. In vitro co-culture studies showed that stimulated PBMCs displayed significantly less activation of $CD4^{+}CD25^{+}Foxp3^{+}$ regulatory T cells, and IL-10 production when inflamed PDLSCs were used. Additionally, inhibition of Th17 differentiation and IL-17 secretion from inflamed PDLSCs was significantly less compared to healthy PDLSCs, proving inflamed PDL tissue serve less immunosuppressive stem cells [67].

2.2.3 TGSCs

TGSCs derived from third molar tooth germs of young adults are MSCs with high proliferation and differentiation capacity [68]. Besides their availability in adult body and having no ethical problems associated with embryonic stem cells, human TGSCs can be converted into osteogenic, adipogenic, myogenic, neurogenic, odontogenic [6, 69–71] and endothelial cell linages [72]. Human TGSCs were used as immunosuppressive agent in rat tooth socket. It has been reported that TGSCs not only suppressed the immunity but also they regenerated the tooth socket at early

dental organogenesis [73]. Exact mechanism of immunomodulatory properties of tooth germ stem cells are still unknown; hence, further studies are required in vitro and in vivo.

2.2.4 GMSCs

GMSCs were firstly isolated at 2009, and they have been shown to be easily obtained from waste materials of routine dental treatment. GMSCs exert stem cell-like characteristics and immunosuppressive properties similar to other DSCs [54, 74]. GMSCs have the ability to stimulate a strong inhibitory action against immunity cells by enhancing the IFNγ-dependent IDO, IL-10, cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS) synthesis [75].

Zhang et al. showed that when macrophages were co-cultured with GMSCs, they developed an anti-inflammatory M2 phenotype, which might enhance wound healing process [76]. Another study reported that systemic infusion of GMSCs intensely improved contact dermatitis as revealed by a reduced penetration of dental cells, $CD8^+$ T cells, Th17, and Mast Cells (MCs) [77]. Many in vivo transplantation studies have shown that stimulated GMSCs exerted stronger regeneration ability than non-stimulated healthy GMSCs, supporting the idea that GMSCs contribute to the pathogenesis of drug-stimulated gingival hyperplasia.

2.2.5 SCAPs

SCAP cells are obtained from cervical loop which contributes to tooth formation and pulp tissue development [78, 79]. SCAP cells showed a two-fold higher cell doubling rate compared to DPSCs. Moreover, SCAP cells can suppress T cell proliferation in an apoptosis independent way [57]. Further studies are necessary to explore the potential usage of SCAP cells as an immunosuppressive agent.

2.2.6 SHEDs

SHED cells are obtained from the coronal pulp of exfoliated deciduous teeth [56]. SHED cells exert MSC characteristics but high levels of alkaline phosphatase (ALP) were detected under osteogenic induction conditions. SHEDs have been shown to suppress Th17 function proposing that SHEDs could be used to treat systemic lupus erythematosus (SLE) in vivo [54].

Available data in the literature provides valuable information about MSC-regulated immune suppression. However, advanced in vivo and clinical studies should be completed to reveal multiple signals and complicated mechanisms controlling immune regulatory pathways.

2.3 Relation Between Immunosuppression and Stem Cell Expression Profiles

The discovery of DSCs in the pulp tissue by Gronthos et al. was a milestone for MSC research and expanded new horizons for the development of alternative treatment strategies [55]. MSCs residing in dental tissues are characterized by differentiation ability into many cell types such as adipocyte, osteocyte, chondrocyte and myocyte under appropriate culture conditions [80]. In addition to MSC-like behavior at culture conditions, DPSCs are positive for MSC surface markers such as CD29, CD73, CD105 and CD 90. It has been revealed that stage-specific embryonic antigen-4 (SSEA-4), a globo-series ganglioside, is also expressed in DPSC populations, and can be used to sort DPSCs [81]. Moreover, STRO-1, which is a cell surface protein expressed by BMMSCs, is also expressed by DSCs [82]. Although STRO-1 is used as a specific marker to characterize DSCs, some STRO-1^+ cells express typical hematopoietic stem cell markers such as CD117 and CD34 [83, 84].

Use of DSCs for regenerative medicine could be problematic in some situations. Inflammation in periapical and pulp tissues could restrict the regeneration potential of DSCs [85]. In the case of inflammation caused by bacterial infection, several immune cells such as macrophages, neutrophils, T and B lymphocytes take in charge as immune protector [86]. In this situation, MSCs express certain cell surface markers providing the interaction with the immune system to suppress the immunity [86]. The immune system and MSC interactions are well-known for BMMSCs which express receptors for plenty of cytokines such as IL-4, IL-6, TNFα, IFNγ, and growth factors including epidermal growth factor (EGF), TGF-β and bone morphogenetic proteins (BMPs) [87, 88]. Moreover, MSCs express vital molecules necessary for cell to cell interactions with immune and hematopoietic cells, particularly various adhesion molecules.

Although similar expression profile of DSCs with other well-studied MSCs is expected, currently, there is not a comprehensive research for the investigation of surface proteins of all types of DSCs. Some receptors for distinct mediators expressed by DSCs are TGF-β, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and insulin-like growth factor (IGF) [89–93].

MSCs can receive signals from the inflammatory environment via several receptors. They express some growth factors and cytokines such as IL-11, IL-8 and IL-6, and involved in the early maturation of T cells [87]. It was shown that MSCs modify cytokine release of distinct types of adaptive immune cells like T cells or suppress their proliferation [94–96]. Immunomodulatory potential of MSCs have been described for BMMSCs, and also have been confirmed for DPSCs. Responses of activated T cells are prohibited by DPSCs and SCAPs [97, 98]. It has also been reported that in the existence of DPSCs and PDLSCs, repression of PBMC proliferation has been observed [99]. Similar effects have also been demonstrated for gingival fibroblast [95].

MSCs derived from dental tissue express some critical receptors for inflammatory agents, which can be produced by injured cells and inflammatory cells.

They can also be released from dental tissues. Released cytokines and growth factors are able to decrease or increase the proliferation or differentiation potential of MSCs derived from dental tissues.

2.4 Clinical Applications

Clinical study is the process of experiments and trials, observational studies in medical and other types of research. The aim of a clinical study is to investigate the activity, safety and mechanism of action of an investigated product, drug or device. In this regard, MSCs are being currently used in clinical applications for the treatment/prevention of various disorders including immunity related disorders. In particular, human MSCs derived from adult donors are isolated and cultured in the laboratory conditions, and they have displayed the ability to find injured tissue, reduce and control the inflammation [100]. In addition, BMMSCs are evaluated for the treatment of GVHD [101] and their safety, tolerability and effectivity after liver or kidney transplantation are also being investigated in clinics [102]. Currently, there are no clinical studies investigating immunosuppression properties of DSCs. However, as DSCs have been proven to display comparable immunosuppression properties with other well-studies MSCs, it is worth to conduct clinical trials using different DCSs. For instance, DPSCs have exhibited 18 % higher suppression rate of T lymphocyte growth in comparison with BMMSCs, indicating possible superior immunosuppression potential of DSCs. In addition to their outstanding immunosuppressive activity, DSCs have several advantages which makes them promising candidates to be used in various clinical trials including;

- They are cost-effective, easily obtainable and do not need ethical and safety concerns as long as they are derived from regular orthodontic procedures [103].
- They can be easily cryopreserved, stored long-term, and combined with many structural materials [104].
- They have neuro-protective effects on dopaminergic neurons and motor neurons in spinal cord [105, 106].

2.5 Safety Issues

Absence of clinical studies investigating in vivo survival of MSCs after tissue or cell transplantation can bring serious doubts to usage of MSCs in human cases. Few human studies have indicated that long term side effects of MSCs are still unknown. Not just only immunosuppressive effect of DSCs against immunogenic cells, but also their anti-inflammatory role, tissue repairing function, and interaction between these actions must be studied before performing clinical studies. In addition, several questions including “is this immunogenic suppression mediated by MSCs stable for long time”, “do DSCs cause malignant transformation in the related or un-related

tissue or organs", or "do they result in any ectopic tissue transformation" should be answered. Until these questions will be resolved, usage of MSCs can remain doubtful in transplantation cases or treatment of immune-related disorders.

2.6 MSC Paradigm; Suppression or Modulation

Do MSCs only act as an immunosuppressive agent or do they have much more complicated modulatory function? Warning signals which are expressed from foreign microbial infection, trigger toll like receptor (TLR) production from immune cells. Expression of TLR from induced immune cells leads a great host response, leading homeostasis [107–109]. MSCs can recognize the warning signal from foreign agent to find the damaged tissues to exert their suppressive function. Recently, researchers founded that MSCs not only suppress immune cells but also they can suppress inflammation at related area [52]. TLR-3 expression causes production of immune suppressive signals secreted from MSCs, while stimulation of TLR-4 with lipopolysaccharide (LPS) causes production of pro-inflammatory factors [110]. This process can be shifted by specific TLR expression depending on reaction against foreign warning signal. TLR-3 expression leads to T cell derived immune suppression and increased deposition of fibronectin [111]. However, TLR-4 expression results in the suppression of inflammation related mediators and suppression of T cell suppression [111]. Exact mechanism of this dual role of MSCs in immune system needs to be evaluated by further in vivo and ex vivo analysis.

2.7 Conclusion

In short time after Gronthos et al. isolated MSCs derived from dental pulp tissue of permanent teeth, the immunomodulatory properties of DSCs have gained wide attraction from scientists. In this manner, many properties of DSCs including their remarkable immune suppression effects are explored. Researchers showed that DSCs are much more usable in the manner of accessibility, strong immune modulation capacity, low frequency of exposure to the inflammatory environment, and also, they have higher proliferation rate compared to other well-known MSCs [54, 60, 112].

Although there is not any systemic clinical study, pre-clinical and in vitro studies have proved that DSCs could serve a promising immune suppressive in human cases. This unique property of MSCs is not only important for tissue transplantation treatments but also for allogenic-based regenerative medicine and tissue engineering tools. As additional approaches such as pre-treatment of DSCs with cytokines including IFNγ, TNFα and IL-1β increase suppression effect and anti-inflammatory mediator secretion, stem cell culture and treatment should be optimized before treatment. Second important issue could be the time of application. It has been

proposed that although same MSC type have been used, one of them failed to treat GVDH in mice due to having different infusion time [113]. Such parameters should be optimized to increase success rate in MSC-based treatments. As all communications and interactions between DSCs and immune cells are not completely elucidated yet, further in vitro, in vivo, and ex vivo studies are highly warranted to fully clarify critical roles and the fundamental mechanisms of immunomodulatory features of DSCs to increase DSC use in clinics.

References

1. Brooke G, Cook M, Blair C, Han R, Heazlewood C, Jones B, Kambouris M, Kollar K, McTaggart S, Pelekanos R, Rice A, Rossetti T, Atkinson K (2007) Therapeutic applications of mesenchymal stromal cells. Semin Cell Dev Biol 18(6):846–858. doi:10.1016/j.semcdb.2007.09.012
2. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 7(2):211–228. doi:10.1089/107632701300062859
3. von der Mark K, Conrad G (1979) Cartilage cell differentiation: review. Clin Orthop Relat Res 139:185–205
4. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM (2001) Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 98(8):2396–2402
5. In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, Noort WA, Claas FH, Willemze R, Fibbe WE, Kanhai HH (2003) Amniotic fluid as a novel source of mesenchymal stem cells for therapeutic transplantation. Blood 102(4):1548–1549. doi:10.1182/blood-2003-04-1291
6. Tasli PN, Sahin F (2014) Effect of lactoferrin on odontogenic differentiation of stem cells derived from human 3rd molar tooth germ. Appl Biochem Biotechnol 174(6):2257–2266. doi:10.1007/s12010-014-1204-8
7. Rahmani W, Abbasi S, Hagner A, Raharjo E, Kumar R, Hotta A, Magness S, Metzger D, Biernaskie J (2014) Hair follicle dermal stem cells regenerate the dermal sheath, repopulate the dermal papilla, and modulate hair type. Dev Cell 31(5):543–558. doi:10.1016/j.devcel.2014.10.022
8. Benvenuto F, Ferrari S, Gerdoni E, Gualandi F, Frassoni F, Pistoia V, Mancardi G, Uccelli A (2007) Human mesenchymal stem cells promote survival of T cells in a quiescent state. Stem Cells 25(7):1753–1760. doi:10.1634/stemcells.2007-0068
9. Gucciardo L, Lories R, Ochsenbein-Kolble N, Done E, Zwijsen A, Deprest J (2009) Fetal mesenchymal stem cells: isolation, properties and potential use in perinatology and regenerative medicine. BJOG 116(2):166–172. doi:10.1111/j.1471-0528.2008.02005.x
10. Phinney DG, Prockop DJ (2007) Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair--current views. Stem Cells 25(11):2896–2902. doi:10.1634/stemcells.2007-0637
11. Le Blanc K, Tammik C, Rosendahl K, Zetterberg E, Ringden O (2003) HLA expression and immunologic properties of differentiated and undifferentiated mesenchymal stem cells. Exp Hematol 31(10):890–896
12. Prasad VK, Lucas KG, Kleiner GI, Talano JA, Jacobsohn D, Broadwater G, Monroy R, Kurtzberg J (2011) Efficacy and safety of ex vivo cultured adult human mesenchymal stem cells (Prochymal) in pediatric patients with severe refractory acute graft-versus-host disease in a compassionate use study. Biol Blood Marrow Transplant 17(4):534–541. doi:10.1016/j.bbmt.2010.04.014

13. Wang L, Zhao Y, Shi S (2012) Interplay between mesenchymal stem cells and lymphocytes: implications for immunotherapy and tissue regeneration. J Dent Res 91(11):1003–1010. doi:10.1177/0022034512460404
14. Nauta AJ, Fibbe WE (2007) Immunomodulatory properties of mesenchymal stromal cells. Blood 110(10):3499–3506. doi:10.1182/blood-2007-02-069716
15. Uccelli A, Moretta L, Pistoia V (2008) Mesenchymal stem cells in health and disease. Nat Rev Immunol 8(9):726–736. doi:10.1038/nri2395
16. Akiyama K, Chen C, Wang D, Xu X, Qu C, Yamaza T, Cai T, Chen W, Sun L, Shi S (2012) Mesenchymal-stem-cell-induced immunoregulation involves FAS-ligand-/FAS-mediated T cell apoptosis. Cell Stem Cell 10(5):544–555. doi:10.1016/j.stem.2012.03.007
17. Krampera M, Glennie S, Dyson J, Scott D, Laylor R, Simpson E, Dazzi F (2003) Bone marrow mesenchymal stem cells inhibit the response of naive and memory antigen-specific T cells to their cognate peptide. Blood 101(9):3722–3729. doi:10.1182/blood-2002-07-2104
18. Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O (2003) Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand J Immunol 57(1):11–20
19. Maccario R, Podesta M, Moretta A, Cometa A, Comoli P, Montagna D, Daudt L, Ibatici A, Piaggio G, Pozzi S, Frassoni F, Locatelli F (2005) Interaction of human mesenchymal stem cells with cells involved in alloantigen-specific immune response favors the differentiation of CD4+ T-cell subsets expressing a regulatory/suppressive phenotype. Haematologica 90(4): 516–525
20. Rasmusson I, Ringden O, Sundberg B, Le Blanc K (2003) Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. Transplantation 76(8):1208–1213. doi:10.1097/01.TP.0000082540.43730.80
21. Aggarwal S, Pittenger MF (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105(4):1815–1822. doi:10.1182/blood-2004-04-1559
22. Sun L, Akiyama K, Zhang H, Yamaza T, Hou Y, Zhao S, Xu T, Le A, Shi S (2009) Mesenchymal stem cell transplantation reverses multiorgan dysfunction in systemic lupus erythematosus mice and humans. Stem Cells 27(6):1421–1432. doi:10.1002/stem.68
23. Plumas J, Chaperot L, Richard MJ, Molens JP, Bensa JC, Favrot MC (2005) Mesenchymal stem cells induce apoptosis of activated T cells. Leukemia 19(9):1597–1604. doi:10.1038/sj.leu.2403871
24. Giuliani M, Fleury M, Vernochet A, Ketroussi F, Clay D, Azzarone B, Lataillade JJ, Durrbach A (2011) Long-lasting inhibitory effects of fetal liver mesenchymal stem cells on T-lymphocyte proliferation. PLoS One 6(5):e19988. doi:10.1371/journal.pone.0019988
25. Ramasamy R, Tong CK, Seow HF, Vidyadaran S, Dazzi F (2008) The immunosuppressive effects of human bone marrow-derived mesenchymal stem cells target T cell proliferation but not its effector function. Cell Immunol 251(2):131–136. doi:10.1016/j.cellimm.2008.04.009
26. Siegel G, Schafer R, Dazzi F (2009) The immunosuppressive properties of mesenchymal stem cells. Transplantation 87(9 Suppl):S45–49. doi:10.1097/TP.0b013e3181a285b0
27. Dahlke MH, Hoogduijn M, Eggenhofer E, Popp FC, Renner P, Slowik P, Rosenauer A, Piso P, Geissler EK, Lange C, Chabannes D, Mazzanti B, Bigenzahn S, Bertolino P, Kunter U, Introna M, Rambaldi A, Capelli C, Perico N, Casiraghi F, Noris M, Gotti E, Seifert M, Saccardi R, Verspaget HW, van Hoek B, Bartholomew A, Wekerle T, Volk HD, Remuzzi G, Deans R, Lazarus H, Schlitt HJ, Baan CC, Group MS (2009) Toward MSC in solid organ transplantation: 2008 position paper of the MISOT study group. Transplantation 88(5):614–619. doi:10.1097/TP.0b013e3181b4425a
28. Bartholomew A, Sturgeon C, Siatskas M, Ferrer K, McIntosh K, Patil S, Hardy W, Devine S, Ucker D, Deans R, Moseley A, Hoffman R (2002) Mesenchymal stem cells suppress lymphocyte proliferation in vitro and prolong skin graft survival in vivo. Exp Hematol 30(1):42–48
29. Crop MJ, Baan CC, Korevaar SS, Ijzermans JN, Alwayn IP, Weimar W, Hoogduijn MJ (2009) Donor-derived mesenchymal stem cells suppress alloreactivity of kidney transplant patients. Transplantation 87(6):896–906. doi:10.1097/TP.0b013e31819b3d72

30. Popp FC, Renner P, Eggenhofer E, Slowik P, Geissler EK, Piso P, Schlitt HJ, Dahlke MH (2009) Mesenchymal stem cells as immunomodulators after liver transplantation. Liver Transpl 15(10):1192–1198. doi:10.1002/lt.21862
31. Zhou HP, Yi DH, Yu SQ, Sun GC, Cui Q, Zhu HL, Liu JC, Zhang JZ, Wu TJ (2006) Administration of donor-derived mesenchymal stem cells can prolong the survival of rat cardiac allograft. Transplant Proc 38(9):3046–3051. doi:10.1016/j.transproceed.2006.10.002
32. Bartholomew A, Polchert D, Szilagyi E, Douglas GW, Kenyon N (2009) Mesenchymal stem cells in the induction of transplantation tolerance. Transplantation 87(9 Suppl):S55–57. doi:10.1097/TP.0b013e3181a287e6
33. Corry RJ, Chakrabarti PK, Shapiro R, Rao AS, Dvorchik I, Jordan ML, Scantlebury VP, Vivas CA, Fung JJ, Starzl TE (1999) Simultaneous administration of adjuvant donor bone marrow in pancreas transplant recipients. Ann Surg 230(3):372–379, discussion 379–381
34. Kawai T, Sachs DH, Sprangers B, Spitzer TR, Saidman SL, Zorn E, Tolkoff-Rubin N, Preffer F, Crisalli K, Gao B, Wong W, Morris H, LoCascio SA, Sayre P, Shonts B, Williams WW Jr, Smith RN, Colvin RB, Sykes M, Cosimi AB (2014) Long-term results in recipients of combined HLA-mismatched kidney and bone marrow transplantation without maintenance immunosuppression. Am J Transplant 14(7):1599–1611. doi:10.1111/ajt.12731
35. Asari S, Itakura S, Ferreri K, Liu CP, Kuroda Y, Kandeel F, Mullen Y (2009) Mesenchymal stem cells suppress B-cell terminal differentiation. Exp Hematol 37(5):604–615. doi:10.1016/j.exphem.2009.01.005
36. Augello A, Tasso R, Negrini SM, Amateis A, Indiveri F, Cancedda R, Pennesi G (2005) Bone marrow mesenchymal progenitor cells inhibit lymphocyte proliferation by activation of the programmed death 1 pathway. Eur J Immunol 35(5):1482–1490. doi:10.1002/eji.200425405
37. Gerdoni E, Gallo B, Casazza S, Musio S, Bonanni I, Pedemonte E, Mantegazza R, Frassoni F, Mancardi G, Pedotti R, Uccelli A (2007) Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. Ann Neurol 61(3):219–227. doi:10.1002/ana.21076
38. Rasmusson I, Le Blanc K, Sundberg B, Ringden O (2007) Mesenchymal stem cells stimulate antibody secretion in human B cells. Scand J Immunol 65(4):336–343. doi:10.1111/j.1365-3083.2007.01905.x
39. Traggiai E, Volpi S, Schena F, Gattorno M, Ferlito F, Moretta L, Martini A (2008) Bone marrow-derived mesenchymal stem cells induce both polyclonal expansion and differentiation of B cells isolated from healthy donors and systemic lupus erythematosus patients. Stem Cells 26(2):562–569. doi:10.1634/stemcells.2007-0528
40. Corcione A, Benvenuto F, Ferretti E, Giunti D, Cappiello V, Cazzanti F, Risso M, Gualandi F, Mancardi GL, Pistoia V, Uccelli A (2006) Human mesenchymal stem cells modulate B-cell functions. Blood 107(1):367–372. doi:10.1182/blood-2005-07-2657
41. Nauta AJ, Kruisselbrink AB, Lurvink E, Willemze R, Fibbe WE (2006) Mesenchymal stem cells inhibit generation and function of both CD34+−derived and monocyte-derived dendritic cells. J Immunol 177(4):2080–2087
42. Ramasamy R, Fazekasova H, Lam EW, Soeiro I, Lombardi G, Dazzi F (2007) Mesenchymal stem cells inhibit dendritic cell differentiation and function by preventing entry into the cell cycle. Transplantation 83(1):71–76. doi:10.1097/01.tp.0000244572.24780.54
43. Rutella S, Danese S, Leone G (2006) Tolerogenic dendritic cells: cytokine modulation comes of age. Blood 108(5):1435–1440. doi:10.1182/blood-2006-03-006403
44. Beyth S, Borovsky Z, Mevorach D, Liebergall M, Gazit Z, Aslan H, Galun E, Rachmilewitz J (2005) Human mesenchymal stem cells alter antigen-presenting cell maturation and induce T-cell unresponsiveness. Blood 105(5):2214–2219. doi:10.1182/blood-2004-07-2921
45. Jiang XX, Zhang Y, Liu B, Zhang SX, Wu Y, Yu XD, Mao N (2005) Human mesenchymal stem cells inhibit differentiation and function of monocyte-derived dendritic cells. Blood 105(10):4120–4126. doi:10.1182/blood-2004-02-0586
46. Nemeth K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, Mezey E (2009) Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogram-

ming of host macrophages to increase their interleukin-10 production. Nat Med 15(1):42–49. doi:10.1038/nm.1905
47. Spaggiari GM, Abdelrazik H, Becchetti F, Moretta L (2009) MSCs inhibit monocyte-derived DC maturation and function by selectively interfering with the generation of immature DCs: central role of MSC-derived prostaglandin E2. Blood 113(26):6576–6583. doi:10.1182/blood-2009-02-203943
48. Malhotra A, Shanker A (2011) NK cells: immune cross-talk and therapeutic implications. Immunotherapy 3(10):1143–1166. doi:10.2217/imt.11.102
49. Meisel R, Zibert A, Laryea M, Gobel U, Daubener W, Dilloo D (2004) Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. Blood 103(12):4619–4621. doi:10.1182/blood-2003-11-3909
50. Selmani Z, Naji A, Gaiffe E, Obert L, Tiberghien P, Rouas-Freiss N, Carosella ED, Deschaseaux F (2009) HLA-G is a crucial immunosuppressive molecule secreted by adult human mesenchymal stem cells. Transplantation 87(9 Suppl):S62–66. doi:10.1097/TP.0b013e3181a2a4b3
51. Spaggiari GM, Capobianco A, Abdelrazik H, Becchetti F, Mingari MC, Moretta L (2008) Mesenchymal stem cells inhibit natural killer-cell proliferation, cytotoxicity, and cytokine production: role of indoleamine 2,3-dioxygenase and prostaglandin E2. Blood 111(3):1327–1333. doi:10.1182/blood-2007-02-074997
52. West AP, Koblansky AA, Ghosh S (2006) Recognition and signaling by toll-like receptors. Annu Rev Cell Dev Biol 22:409–437. doi:10.1146/annurev.cellbio.21.122303.115827
53. Uccelli A, Moretta L, Pistoia V (2006) Immunoregulatory function of mesenchymal stem cells. Eur J Immunol 36(10):2566–2573. doi:10.1002/eji.200636416
54. Yamaza T, Kentaro A, Chen C, Liu Y, Shi Y, Gronthos S, Wang S, Shi S (2010) Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. Stem Cell Res Ther 1(1):5. doi:10.1186/scrt5
55. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97(25):13625–13630. doi:10.1073/pnas.240309797
56. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100(10):5807–5812. doi:10.1073/pnas.0937635100
57. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT (2008) Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 34(2):166–171. doi:10.1016/j.joen.2007.11.021
58. Tomar GB, Srivastava RK, Gupta N, Barhanpurkar AP, Pote ST, Jhaveri HM, Mishra GC, Wani MR (2010) Human gingiva-derived mesenchymal stem cells are superior to bone marrow-derived mesenchymal stem cells for cell therapy in regenerative medicine. Biochem Biophys Res Commun 393(3):377–383. doi:10.1016/j.bbrc.2010.01.126
59. Pierdomenico L, Bonsi L, Calvitti M, Rondelli D, Arpinati M, Chirumbolo G, Becchetti E, Marchionni C, Alviano F, Fossati V, Staffolani N, Franchina M, Grossi A, Bagnara GP (2005) Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. Transplantation 80(6):836–842
60. Wada N, Menicanin D, Shi S, Bartold PM, Gronthos S (2009) Immunomodulatory properties of human periodontal ligament stem cells. J Cell Physiol 219(3):667–676. doi:10.1002/jcp.21710
61. Kerkis I, Ambrosio CE, Kerkis A, Martins DS, Zucconi E, Fonseca SA, Cabral RM, Maranduba CM, Gaiad TP, Morini AC, Vieira NM, Brolio MP, Sant'Anna OA, Miglino MA, Zatz M (2008) Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic? J Transl Med 6:35. doi:10.1186/1479-5876-6-35
62. Lee S, An S, Kang TH, Kim KH, Chang NH, Kang S, Kwak CK, Park HS (2011) Comparison of mesenchymal-like stem/progenitor cells derived from supernumerary teeth with stem cells from human exfoliated deciduous teeth. Regen Med 6(6):689–699. doi:10.2217/rme.11.95
63. Mao JJ, Giannobile WV, Helms JA, Hollister SJ, Krebsbach PH, Longaker MT, Shi S (2006) Craniofacial tissue engineering by stem cells. J Dent Res 85(11):966–979

64. Bartold PM, McCulloch CA, Narayanan AS, Pitaru S (2000) Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. Periodontol 24:253–269
65. Pitaru S, Pritzki A, Bar-Kana I, Grosskopf A, Savion N, Narayanan AS (2002) Bone morphogenetic protein 2 induces the expression of cementum attachment protein in human periodontal ligament clones. Connect Tissue Res 43(2–3):257–264
66. Kim HS, Kim KH, Kim SH, Kim YS, Koo KT, Kim TI, Seol YJ, Ku Y, Rhyu IC, Chung CP, Lee YM (2010) Immunomodulatory effect of canine periodontal ligament stem cells on allogenic and xenogenic peripheral blood mononuclear cells. J Periodontal Implant Sci 40(6):265–270. doi:10.5051/jpis.2010.40.6.265
67. Liu D, Xu J, Liu O, Fan Z, Liu Y, Wang F, Ding G, Wei F, Zhang C, Wang S (2012) Mesenchymal stem cells derived from inflamed periodontal ligaments exhibit impaired immunomodulation. J Clin Periodontol 39(12):1174–1182. doi:10.1111/jcpe.12009
68. Yalvac ME, Ramazanoglu M, Rizvanov AA, Sahin F, Bayrak OF, Salli U, Palotas A, Kose GT (2010) Isolation and characterization of stem cells derived from human third molar tooth germs of young adults: implications in neo-vascularization, osteo-, adipo- and neurogenesis. Pharmacogenomics J 10(2):105–113. doi:10.1038/tpj.2009.40
69. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S (2002) Stem cell properties of human dental pulp stem cells. J Dent Res 81(8):531–535
70. Guven EP, Tasli PN, Yalvac ME, Sofiev N, Kayahan MB, Sahin F (2013) In vitro comparison of induction capacity and biomineralization ability of mineral trioxide aggregate and a bioceramic root canal sealer. Int Endod J 46(12):1173–1182. doi:10.1111/iej.12115
71. Guven EP, Tasli PN, Yalvac ME, Sofiev N, Kayahan MB, Sahin F (2013) In vitro comparison of induction capacity and biomineralization ability of mineral trioxide aggregate and a bioceramic root canal sealer. Int Endod J 46:1173–1182. doi:10.1111/iej.12115
72. Dogan A, Demirci S, Sahin F (2015) In vitro differentiation of human tooth germ stem cells into endothelial- and epithelial-like cells. Cell Biol Int 39(1):94–103. doi:10.1002/cbin.10357
73. Guzman-Uribe D, Estrada KN, Guillen Ade J, Perez SM, Ibanez RR (2012) Development of a three-dimensional tissue construct from dental human ectomesenchymal stem cells: in vitro and in vivo study. Open Dent J 6:226–234. doi:10.2174/1874210601206010226
74. Zhang Q, Shi S, Liu Y, Uyanne J, Shi Y, Shi S, Le AD (2009) Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 183(12):7787–7798. doi:10.4049/jimmunol.0902318
75. Ravanidis S, Bogie JF, Donders R, Craeye D, Mays RW, Deans R, Gijbels K, Bronckaers A, Stinissen P, Pinxteren J, Hellings N (2015) Neuroinflammatory signals enhance the immunomodulatory and neuroprotective properties of multipotent adult progenitor cells. Stem Cell Res Ther 6(1):176. doi:10.1186/s13287-015-0169-z
76. Zhang QZ, Su WR, Shi SH, Wilder-Smith P, Xiang AP, Wong A, Nguyen AL, Kwon CW, Le AD (2010) Human gingiva-derived mesenchymal stem cells elicit polarization of m2 macrophages and enhance cutaneous wound healing. Stem Cells 28(10):1856–1868. doi:10.1002/stem.503
77. Su WR, Zhang QZ, Shi SH, Nguyen AL, Le AD (2011) Human gingiva-derived mesenchymal stromal cells attenuate contact hypersensitivity via prostaglandin E2-dependent mechanisms. Stem Cells 29(11):1849–1860. doi:10.1002/stem.738
78. Ren G, Su J, Zhang L, Zhao X, Ling W, L'Huillie A, Zhang J, Lu Y, Roberts AI, Ji W, Zhang H, Rabson AB, Shi Y (2009) Species variation in the mechanisms of mesenchymal stem cell-mediated immunosuppression. Stem Cells 27(8):1954–1962. doi:10.1002/stem.118
79. Sattler C, Steinsdoerfer M, Offers M, Fischer E, Schierl R, Heseler K, Daubener W, Seissler J (2011) Inhibition of T-cell proliferation by murine multipotent mesenchymal stromal cells is mediated by CD39 expression and adenosine generation. Cell Transplant 20(8):1221–1230. doi:10.3727/096368910X546553

80. Tasli PN, Aydin S, Yalvac ME, Sahin F (2014) Bmp 2 and Bmp 7 ınduce odonto- and osteogenesis of human tooth germ stem cells. Appl Biochem Biotechnol 172(6):3016–3025. doi:10.1007/s12010-013-0706-0
81. Kawanabe N, Murata S, Fukushima H, Ishihara Y, Yanagita T, Yanagita E, Ono M, Kurosaka H, Kamioka H, Itoh T (2012) Stage-specific embryonic antigen-4 identifies human dental pulp stem cells. Exp Cell Res 318(5):453–463
82. Dennis JE, Carbillet J-P, Caplan AI, Charbord P (2001) The STRO-1+ marrow cell population is multipotential. Cells Tissues Organs 170(2–3):73–82
83. Laino G, Carinci F, Graziano A, d'Aquino R, Lanza V, De Rosa A, Gombos F, Caruso F, Guida L, Rullo R (2006) In vitro bone production using stem cells derived from human dental pulp. J Craniofac Surg 17(3):511–515
84. Laino G, Graziano A, d'Aquino R, Pirozzi G, Lanza V, Valiante S, De Rosa A, Naro F, Vivarelli E, Papaccio G (2006) An approachable human adult stem cell source for hard-tissue engineering. J Cell Physiol 206(3):693–701
85. Lin L, Rosenberg P (2011) Repair and regeneration in endodontics. Int Endod J 44(10): 889–906
86. Leprince J, Zeitlin B, Tolar M, Peters O (2012) Interactions between immune system and mesenchymal stem cells in dental pulp and periapical tissues. Int Endod J 45(8):689–701
87. Deans RJ, Moseley AB (2000) Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 28(8):875–884
88. Docheva D, Haasters F, Schieker M (2008) Mesenchymal stem cells and their cell surface receptors. Curr Rheumatol Rev 4(3):155–160
89. Huang CH, Tseng WY, Yao CC, Jeng JH, Young TH, Chen YJ (2010) Glucosamine promotes osteogenic differentiation of dental pulp stem cells through modulating the level of the transforming growth factor-β type I receptor. J Cell Physiol 225(1):140–151
90. Huang G-J, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 88(9):792–806
91. Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, Sippel C, Hoffmann K (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 24(2):155–165
92. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT-J (2008) Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 34(2):166–171
93. Suzuki T, Lee C, Chen M, Zhao W, Fu S, Qi J, Chotkowski G, Eisig S, Wong A, Mao J (2011) Induced migration of dental pulp stem cells for in vivo pulp regeneration. J Dent Res 90(8):1013–1018
94. Aggarwal S, Pittenger MF (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105(4):1815–1822
95. Klyushnenkova E, Mosca JD, Zernetkina V, Majumdar MK, Beggs KJ, Simonetti DW, Deans RJ, McIntosh KR (2005) T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance, and suppression. J Biomed Sci 12(1):47–57
96. Tomic S, Djokic J, Vasilijic S, Vucevic D, Todorovic V, Supic G, Colic M (2010) Immunomodulatory properties of mesenchymal stem cells derived from dental pulp and dental follicle are susceptible to activation by toll-like receptor agonists. Stem Cells Dev 20(4):695–708
97. Ding G, Liu Y, An Y, Zhang C, Shi S, Wang W, Wang S (2010) Suppression of T cell proliferation by root apical papilla stem cells in vitro. Cells Tissues Organs 191(5):357
98. Pierdomenico L, Bonsi L, Calvitti M, Rondelli D, Arpinati M, Chirumbolo G, Becchetti E, Marchionni C, Alviano F, Fossati V (2005) Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. Transplantation 80(6): 836–842
99. Wada N, Menicanin D, Shi S, Bartold PM, Gronthos S (2009) Immunomodulatory properties of human periodontal ligament stem cells. J Cell Physiol 219(3):667–676

100. NIH (2014) Prochymal™ adult human mesenchymal stem cells for treatment of moderate-to-severe crohn's disease. https://clinicaltrials.gov/ct2/show/NCT00294112?term=mesenchymal+stem+cells+immunosuppressive&rank=9. Accessed 12 Aug 2015
101. NIH (2014) Treatment of chronic graft-versus-host disease with mesenchymal stromal cells (MSC-cGvHD). https://clinicaltrials.gov/ct2/show/NCT02291770?term=mesenchymal+stem+cells+immunosuppression&rank=7. Accessed 12 Aug 2015
102. NIH (2013) Mesenchymal stem cells after renal or liver transplantation. https://clinicaltrials.gov/ct2/show/NCT01429038?term=mesenchymal+stem+cells+immunosuppression&rank=3. Accessed 12 Aug 2015
103. Yalvac ME, Rizvanov AA, Kilic E, Sahin F, Mukhamedyarov MA, Islamov RR, Palotas A (2009) Potential role of dental stem cells in the cellular therapy of cerebral ischemia. Curr Pharm Des 15(33):3908–3916
104. Bansal R, Jain A (2015) Current overview on dental stem cells applications in regenerative dentistry. J Nat Sci Biol Med 6(1):29
105. Nosrat IV, Smith CA, Mullally P, Olson L, Nosrat CA (2004) Dental pulp cells provide neurotrophic support for dopaminergic neurons and differentiate into neurons in vitro; implications for tissue engineering and repair in the nervous system. Eur J Neurosci 19(9):2388–2398. doi:10.1111/j.0953-816X.2004.03314.x
106. Nosrat IV, Widenfalk J, Olson L, Nosrat CA (2001) Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons in vitro, and rescue motoneurons after spinal cord injury. Dev Biol 238(1):120–132. doi:10.1006/dbio.2001.0400
107. Akira S, Sato S (2003) Toll-like receptors and their signaling mechanisms. Scand J Infect Dis 35(9):555–562
108. Miggin SM, O'Neill LA (2006) New insights into the regulation of TLR signaling. J Leukoc Biol 80(2):220–226. doi:10.1189/jlb.1105672
109. Takeda K, Kaisho T, Akira S (2003) Toll-like receptors. Annu Rev Immunol 21:335–376. doi:10.1146/annurev.immunol.21.120601.141126
110. Waterman RS, Tomchuck SL, Henkle SL, Betancourt AM (2010) A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an ımmunosuppressive MSC2 phenotype. PLoS One 5(4):e10088. doi:10.1371/journal.pone.0010088
111. Liotta F, Angeli R, Cosmi L, Fili L, Manuelli C, Frosali F, Mazzinghi B, Maggi L, Pasini A, Lisi V, Santarlasci V, Consoloni L, Angelotti ML, Romagnani P, Parronchi P, Krampera M, Maggi E, Romagnani S, Annunziato F (2008) Toll-like receptors 3 and 4 are expressed by human bone marrow-derived mesenchymal stem cells and can inhibit their T-cell modulatory activity by impairing Notch signaling. Stem Cells 26(1):279–289. doi:10.1634/stemcells.2007-0454
112. Ding G, Liu Y, An Y, Zhang C, Shi S, Wang W, Wang S (2010) Suppression of T cell proliferation by root apical papilla stem cells in vitro. Cells Tissues Organs 191(5):357–364. doi:10.1159/000276589
113. Shi Y, Hu G, Su J, Li W, Chen Q, Shou P, Xu C, Chen X, Huang Y, Zhu Z, Huang X, Han X, Xie N, Ren G (2010) Mesenchymal stem cells: a new strategy for immunosuppression and tissue repair. Cell Res 20(5):510–518. doi:10.1038/cr.2010.44

Chapter 3
miRNA Regulation in Dental Stem Cells: From Development to Terminal Differentiation

Sukru Gulluoglu, Emre Can Tuysuz, and Omer Faruk Bayrak

3.1 Introduction

Dental tissues are a rich source of mesenchymal stem cells (MSCs), which can be utilized not only for dental regenerative medicine but also other types of stem cell-based therapy applications due to their remarkable ability to differentiate into several cell types such neural progenitors, adipocytes and chondrocytes [1, 2]. Tooth development occurs through epithelial-ectomesenchymal interactions mainly powered by transforming growth factors (TGFs), fibroblast growth factor (FGF), bone morphogenic protein (BMP), Hedgehog, Notch and Wnt pathways. These factors are differentially expressed during the stages of tooth development which are initiation (5 weeks), lamina (6 weeks), placodes (7 weeks), bud (8 weeks), cap (11 weeks), bell (14 weeks) and late bell (18 weeks) stages.

MicroRNAs (miRNAs) are small RNA sequences which are thought to play important roles in regulating gene expression. Since their discovery in 1993, miRNAs have attracted increasing interest by researchers, as microarrays and sequencing techniques are improved. An extensive number of miRNAs have been recognized

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for their roles in embryonic development and adult stem cells (ASCs), orchestrating differentiation and self-renewal by regulating target genes, gene families and pathways. DSCs both during development and after birth until adulthood undergo through dynamic processes of signaling cascades with gene levels fluctuating in defined periods of time, during which miRNAs play their part in these complex series of events.

In this book chapter, the role of numerous miRNAs in DSC differentiation and maintenance from the first stages of development until the adult stage will be investigated in detail.

3.2 Stem Cells and miRNAs

Stem cells science is an important field since regenerative medicine is an emerging area that needs a lot of understanding and different perspectives. Stem cells are undifferentiated cells with a potential to transform into various number of specialized cell types. Embryonic stem cells (ESCs), derived from inner cell mass of blastocysts, are pluripotent stem cells that can differentiate into all types of specialized cells. They can generate three primary germ layers: ectoderm, endoderm and mesoderm. Ectoderm localizes at the outer layer of germ line, which later gives rise to the nervous system, tooth enamel and epidermis, etc. Mesoderm is the middle layer localized between endoderm and ectoderm, which form muscle, bone, circulatory system, notochord and connective tissue. Endoderm gives rise to epithelial lineage cells and organs such as stomach, colon, liver, pancreas and lung.

Induced pluripotent stem (iPS) cells are pluripotent stem cells generated firstly by Takashi & Yamanaka by overexpression of defined transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) from embryonic and adult mouse fibroblast [3]. iPS cells have similar properties and potency as ESCs since they have the ability to be differentiated with the same conventional differentiation agents but not causing the ethical controversy encountered in collection of ESCs. ASCs are another source of stem cells which are easy to obtain and produce, with the advantage of autografting into patients. They are multipotent stem cells found in mature tissues at low ratios and produce limited number of specialized cells. ASCs have the ability of self-renewal, maintaining the population balance of specialized and reserve cells in the tissues that they are found in. Main roles of ASCs are regeneration and maintenance of tissues or organs in which they are located. Differentiation potency of ASCs is less than ESCs. Currently, a lot of progress has been made on the isolation, characterization and manipulation of MSCs, which are in use for research and therapy since their discovery in the 1960s at the bone marrow [4]. Some of the other sources for MSCs includes adipose tissue, umbilical cord blood, dental tissues, synovial fluid and parathyroid gland [5–9].

3.2.1 Regulation of Stemness Properties by miRNAs

miRNAs not only play a crucial role for maintaining the stemness properties in various types of stem cells such as ESCs and MSCs, they also act as regulators of stem cell differentiation both in the embryonic and adult stages, and iPS cell production. As potent regulators of individual genes, gene families and gene networks, miRNAs should always be taken under the scope to better understand the dynamic mechanisms of stem cell biology.

ESCs are pluripotent cells, and this potency is regulated by defined genes such as Oct3/4, Klf4, c-myc, Sox2 and Lin28b. Several studies have suggested that miRNAs have an important role in maintenance of stem cell properties in ESCs [10, 11]. ESCs have a shorter G1 phase than somatic cells, and ESC specific cell cycle miRNAs have important roles in regulating this property [12, 13]. For example, miR-92b is highly expressed in human ESCs (hESCs), and differentiation of hESCs, which resulted in a significant decline in miR-92b level [14]. As miR-92b regulates the p57 gene which inhibits G1 to S phase transition in hESCs [15], it could be suggested that abundant miR-92b expression in hESCs suppresses the p57 protein, providing shorter G1 phase. Furthermore, Kosik et al. compared miRNA level of hESCs and differentiated embryoid bodies, and reported that miR-145 expression diminished in hESCs. Bioinformatical analysis showed that Klf4, Oct4 and Sox2 were candidate targets for miR-145, and ectopic expression of miR-145 reduced gene expression and/or protein levels of these markers. In line with these findings, miR-145 overexpression resulted in differentiation of hESCs, and inhibited self-renewal capacity and colony formation [16]. Another study has shown that miR-290-295 cluster provides stem cell maintenance in ESCs [13]. Briefly, this cluster inhibits Retinoblastoma-Like 2 (Rbl2) protein which is a negative regulator of cell cycle and is a critical factor in maintaining stemness properties of ESCs.

During iPS cell generation, ESC-specific cell cycle (ESCC) family of miRNAs enhances the efficiency of the process [17]. Blelloch et al. showed that ESCC miRNA orthologs miR-302b, and mir-372 promote iPS cell generation from human somatic cells [18]. In another study, inhibition of miR-21 and miR-29, highly expressed miRNAs in mouse embryonic fibroblasts (MEFs), enhanced the efficiency of iPS cell generation from MEFs [19]. Additionally, miR-29 downregulated p53 protein through p85a and CDC42 pathway. miR-21 can activate mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway in cardiac fibroblasts [20], which is particularly significant since inhibition of MAPK/ERK pathway enhances iPS cell generation [21]. Similar to cardiac fibroblasts, miR-21 inhibits MAPK/ERK pathway and enhances iPS cell generation from MEFs.

ASCs are undifferentiated somatic cells found throughout the body, which are responsible for tissue or organ maintenance and regeneration. Unsurprisingly, miRNAs are important regulators of ASCs as in other types of stem cells. miR-29a provides maintenance of hematopoietic stem cell self-renewal through regulating DNA (cytosine-5)-methyltransferase 3a (DNMT3a) expression [22]. Briefly, mice harboring miR-29a/b-1 bicistronic cluster deletion in their genome exhibited

decreased ratio of $Lin^{-}c\text{-}Kit^{+}Sca\text{-}1^{+}$ population in their bone marrow when compared with the wild type group. Ectopic expression of miR-25 in neural stem cells enhances neural stem cell proliferation through regulating insulin growth factor 1 (IGF1) gene [23]. Furthermore, miR-205 is highly expressed in skin stem cells in neonates via regulation the phosphoinositide 3-kinase (PI3K) pathway [24].

3.2.2 miRNAs in MSCs

MSCs are multipotent ASCs that can differentiate into limited number of specialized cells such as bone, adipose and muscle cell. MSCs can be isolated from various cell sources such as bone marrow, cord blood, peripheral blood, adipose tissue and amniotic fluid. MSCs can be used in tissue regeneration approaches and treatments of immune disorders. As a stem cell source for tissue regeneration, MSC differentiation into several cell types has been widely studied; some of those studies have investigated the roles of miRNA in the differentiation process. The miRNA profiles of MSC derived from healthy donors and osteogenically differentiated MSCs have been compared by which 12 miRNAs were discovered to be down-regulated including miR-142-3p, miR-451 and miR-146a, and 17 miRNAs up-regulated including miR-376a, miR-378, miR-193a, miR-100 and miR-31 during differentiation process [25]. In another study, miR-138 inhibited osteoblast differentiation of MSCs derived from bone marrow (BMMSCs). Briefly, pre-mir-138 and antimiR-138 were transfected to MSCs and osteoblast related genes, RUNX2 and OSX2, as well as alkaline phosphatase (ALP) activity were evaluated. Inhibition of miR-138 increased the abundance of osteoblast-related genes and ALP activity, whereas overexpression of miR-138 decreased these parameters [26]. During adipogenic differentiation of MSCs derived from adipose tissue (ADSCs), miR-21 expression was found to have increased. In the same study, the authors report that lentiviral-mediated over-expression of miR-21 enhanced adipogenic differentiation of MSCs. Furthermore, overexpression of miR-21 downregulated TGF beta receptor II at both gene and protein levels [27]. In another study, Wagner et al. showed that adipogenic differentiation of BMMSCs can be regulated by miR-369-5p and miR-371. They concluded that transfection of MSCs with miR-369-5p inhibited adipogenesis, whereas transfection with miR-371 enhanced adipogenesis [28]. Expression of miR-145 decreased gradually during chondrogenic differentiation in murine BMMSCs. In the study, miR-145 was found to regulate the SRY (sex determining region Y)-box 9 (SOX9) gene, an important chondrogenesis mediator [29].

3.2.3 miRNA Regulation of Pathways Active in MSCs

Signaling cascades are well-defined processes during embryonic development and stem cell differentiation. These processes concurrently activate several genes, in which miRNAs can both regulate and be regulated by this activity. Wnt signal

transduction pathway has several functions such as embryonic development, carcinogenesis, heart and neural tissue formation and sex determination. It is also a prominent regulator of stemness and differentiation of MSCs. Wnt pathway mediators, predominantly the WNT2, WNT4, WNT5A, WNT11 and WNT16 genes, are highly active in BMMSCs [30]. WNT3A overexpression in MSCs increased self-renewal capacity and inhibited apoptosis [31, 32]. miR-128 inhibited differentiation of MSCs into neuron-like cells through regulating WNT3A expression [33]. miR-346 expression increases during osteogenic differentiation of BMMSCs through the activation of WNT/β-catenin pathway, leading to enhanced osteogenesis [34]. To sum up, Wnt pathway which can both maintain stem cell phenotype and enhance differentiation of MSCs can be regulated by various miRNAs.

BMPs are growth factors that belong to the TGF-β superfamily. They have vital roles in bone and cartilage formations as well as various biological processes such as maintenance of stem cell properties in ESCs and MSCs. BMP receptor type IB (BMPR1B) signaling induces osteogenic differentiation, whereas BMPR1A inhibits osteogenic differentiation [35, 36]. Kung et al. showed that miR-20a regulates the levels of peroxisome proliferator-activated receptor gamma (PPARG) which is a negative regulator of BMP/RUNX2 signaling. Overexpression of miR-20a enhanced differentiation of human BMMSCs into osteoblast [37]. miRNA profiling of ADSCs exhibited that levels of miR-17-5p and miR-106a reduced during osteogenic differentiation while they enhanced during adipogenesis [38]. It has also been shown that BMP2 is directly regulated by miR-17-5p and miR-106a. Furthermore, another study has suggested that BMP2 can be regulated by miR-149* and miR-654-5p during osteogenic differentiation [39].

TGF-β is a vital cytokine that coordinates differentiation, cell growth, apoptosis and homeostasis. When TGF-β binds to its receptor, an intracellular cascade results in phosphorylation of receptor-regulated Smad2/3 proteins that associate with the common mediator Smad4. In MSCs, TGF-β pathway drives lineage differentiation and determination. For example, MSCs derived from hair follicle could differentiate into smooth muscle cells, and during TGF-β induced differentiation, miR-18b expression decreased. Overexpression of miR-18b caused inhibition of smooth muscle cell differentiation and downregulation of smooth muscle cell associated genes including smooth muscle actin and calponin-1 [40]. van Zoelen et al. showed that TGF-β and BMP induced differentiation of human BMMSCs into chondrogenic lineage causes a significant change in miRNA profile. They found that 485 miRNAs downregulated during differentiation of MSCs into chondrocyte including miR-494, miR-298, miR-500 and miR-524-5p [41]. In another study, miR-21 regulated the TGFBRII gene level during differentiation of MSCs into adipogenic lineages [27].

Notch pathway is highly conserved, and it plays a crucial role in development and homeostasis. The Notch pathway mediates juxtacrine cellular signaling wherein both the signal sending and receiving cells are affected through ligand-receptor crosstalk by which an array of cell fate decisions in neuronal, cardiac, immune, and endocrine development are regulated. Overexpression of miR-126 in mouse BMMSCs increased the expression of the delta like ligand 4 (DLL4), which is a

Notch ligand [42]. miR-1 regulates the transcription factor, hairy and enhancer of split-1 (Hes1) gene which is a downstream target molecule of Notch1, and overexpression of miR-1 in mouse BMMSCs promoted gene expression of cardiac differentiation markers including NK2 homeobox 5 (NKX2-5), GATA binding protein 4 (GATA-4), Cardiac Troponin T (TNNT2), and Connexin43 (CX43). Platelet-derived growth factor (PDGF) takes part in various biological processes such as angiogenesis, cellular proliferation and development [43]. Qu et al. has shown that PDGF inhibits osteogenic differentiation of MSCs. Administration of PDGF to hMSCs caused upregulation of miR-138 [44], indicating that miR-138 can be regulated by PDGF.

3.3 miRNAs in DSCs, Dental Tissues and Their Roles

After development, once the tooth is erupted both in juveniles and adults, the organ possesses diverse types of tissues and structures such as the enamel, dentin, pulp and periodontal ligaments. In infants and adults, dental stem cells (DSCs) can mainly be classified into five main types. The presence of stem cells in dental tissues has first been discovered in the human dental pulp tissue (DPSCs) [45]. More stem cells types have been discovered later in the exfoliated deciduous teeth (SHEDs) [46], periodontal ligament (PDLSCs) [47], the apical papilla (SCAPs)[48], and dental follicle (DFSCs) [49]. Such diversity of stem cell-derived tissues suggests that there are differences in the gene regulation of these stem cell rich areas. Thus, miRNA regulation is also expected to differ.

As potential sources of pluripotent stem cells, DSCs are expected to exhibit similar biological patterns as ESCs or other types of ASCs. A number of miRNAs shows great potential to single handedly control important cascades by targeting one or more key genes. A good example of how a single miRNA may regulate multiple processes in stem cell biology can be seen in the study by Kuboki et al. Regulation of NANOG gene which is responsible for maintenance of stemness properties, differentiation and gene methylation has been observed to be carried out by miRNAs in side populations of DPSCs and PDLSCs [50]. After checking for the potential stem cell markers (NANOG, Oct4 and ABCG2), the side populations of DPSCs and PDLSCs were used to compare miRNA profiles with the main cell population. Among the differentially expressed miRNAs between the two groups, miR-1260b, miR-1280, miR-491-3p, miR-1260a, miR-138-1 and miR-720 down-regulated, and miR-200b, miR-515-5p, miR-1245, miR-3919, miR-182 and miR-607 up-regulated the most in the side populations which are considered to be rich in stem cells. miR-720 which is a regulator of the stem cell marker NANOG has been found to have a binding affinity to its target in DPSCs. DNA methyltransferases (DNMTs) play crucial roles in stem cell differentiation. DNMT3a and DNMT1 have increased upon miR-720 mimic transfection into DPSCs. Since single miRNAs may target members of the same gene family, as in the referred study, they can be considered as molecules that not only target single mRNAs but also gene networks, families

and pathways. Replacement of miR-720 in DPSCs has also decreased cell proliferation and promoted odontogenic differentiation, indicating that miR-720 is a potent regulator controlling the differentiation and proliferation of DPSCs. Another example can be seen in the Notch pathway which is highly active in stem cells. Unsurprisingly, the Notch pathway was found to have elevated activity in SCAPs. miR-34a negatively regulated Notch signaling in SCAPs which resulted in the loss of stemness properties, leading to odontogenic and osteogenic differentiation. Moreover, Notch activation in these cells inhibited differentiation and triggered elevated miR-34a production, suggesting that there is a feedback mechanism between the Notch pathway molecules and miR-34a [51]. In mice, a fine-tuning mechanism to maintain homeostasis in the dental epithelium, which contains epithelial stem cells, has been proposed by Juuri et al. miR-720 has been found to control FGF8, while miR-200b controls SOX2 which are both important genes in stem cell biology.

While similarities in major stem cell pathways are expected between DSCs and other types of stem cells, differences in miRNA patterns is inevitable. BMMSCs have been compared with DPSCs in terms of miRNA profiles, and a total of 48 differentially expressed miRNAs have been discovered between the two groups. Among these miRNAs, miR-516a which is upregulated in DPSCs compared with BMMSCs has been proposed to have an indirect inhibitory effect on the WNT pathway via knock-down of the WNT5A gene. In the same study, miR-7-5p was found to be effective on epidermal growth factor receptor (EGFR) expression [52]. In another study, the miRNA expression profiles of cells derived from deciduous and adult wisdom teeth were reported to be extremely similar. However, they had significant differences with umbilical cord-derived MSCs. Fifteen miRNAs including hsa-miR-196b, hsa-miR-10a, hsa-miR-146a, hsa-miR-335 were downregulated in DSCs, and 26 miRNAs up-regulated including hsa-miR-138, hsa-let7b, hsa-miR-98 and has-miR-199b-5p [53].

3.4 Regulation of Developmental Stages of Tooth by miRNAs

Tooth development mainly occurs in 7 stages: Initiation, Lamina, Placodes, Bud, Cap, Bell, and Late Bell Stages. Tooth develops primarily from the mesoderm layer of embryonic development with the exception of the enamel that is derived from the ectoderm. In this section we will focus on the miRNA regulation during developmental stages of tooth.

3.4.1 Initiation, Lamina and Placode Stages

Initiation stage of tooth starts at the end of the 5th weeks in humans with condensation of jaw epithelium. Condensed dental epithelium interacts with mesenchyme derived from neural crest, and forms dental lamina. Distinction of oral ectoderm

from dental ectoderm is very important during initiation of tooth development. Sharpe et al. showed that WNT7B, expressed in oral ectoderm, repressed Sonic Hedgehog, expressed in the dental ectoderm, and Ptc, expressed in the ectomesenchyme, downregulated [54]. Lamina stage starts at the end of the 6th weeks. BMP is expressed in developing tooth and has a key role in multiple steps of tooth development. Chen et al. showed that the negative regulator of BMP, Noggin, enhanced in dental epithelium and overexpression of Noggin caused arresting at the lamina/early bud stage [55]. Furthermore, CP27 localized in epithelial-mesenchymal interface of dental lamina during the lamina stage. However, during later stages, CP27 localized in stellate reticulum, the oral mucosa mesenchyme, and alveolar bone [56]. Placode is an ectodermal organ consisting of thickened epithelium underlying neural crest derived mesenchyme. It is known that WNT and FGF signals are the activators of placode formation, whereas BMP signals inhibit of placode formation.

3.4.2 Bud Stage

miRNAs have important roles during the bud stage. For example, 37, 124 and 105 miRNAs were differentially expressed in the bud stage compared to the cap, bell and late bell stages, respectively, in miniature pigs. In addition, ssc-miR-125b expression decreased in the bell and late bell stages with respect to the bud stage. Bioinformatic analysis revealed that ssc-125b is a candidate regulator of the TGF-β pathway. Furthermore, ssc-miR-128 diminished in bud stage and bioinformatics analysis revealed that ssc-miR-128 can regulate the WNT pathway [57]. In a study conducted on mice tooth, miR-135 expression level was found to increase in bud stage. miR-135 targets Bmpr-1a and Bmpr-1b receptors [58].

3.4.3 Cap Stage

In the cap stage, the tooth bud forms a cap as a result of the differential proliferation capacity of cells present in the cell population followed by the folding of epithelium [59]. The enamel knot occurs during cap stage where WNT3 expression is observed specifically in the enamel [60]. Thirty-seven, 4 and 16 miRNAs were differentially expressed in the cap stage in comparison with the bud, bell and late bell stages, respectively, in miniature pigs. ssc-miR-199a-3p down-regulated in the cap stage, and ssc-miR-199a-3p has been proposed to regulate the WNT pathway. Furthermore, TGF-β pathway regulator, let-7f, downregulated when compared with the bell stage [57].

3.4.4 Bell Stage

Bell stage, also known as histodifferentiation of morphodifferentiation, is the stage in which the dental organ is bell-like, and cells in the enamel organ differentiate into outer enamel epithelial covering the enamel organ and inner enamel epithelial cells which differentiate into ameloblast cells. Outer enamel epithelial cells provide nutrition of ameloblast cells.

As mentioned above, ssc-miR-125b regulating the TGF-β pathway downregulated in the early bell stage when compared with the bud stage. Reduction in ssc-miR-125b level in the early bell stage provides activation of the pathway which might imply that TGF-β pathway is an important regulator of this differentiation stage [57]. In a comparison of miRNA levels between the early bell stage and the late bell stage in humans, 29 miRNAs were found to be differentially expressed. hsa-miR-34a reduced in the early bell stage, and BMP2, BMP7, Notch1 and Notch7 were found to be putative targets of hsa-miR-34a. Moreover, hsa-miR-224 increased in the early bell stage and amelogenin, X isoform (AMELX), Hes5, ameloblastin (AMBN) and BMP3 were found to be the putative targets for this miRNA. The study revealed that downregulation of has-miR-34a in the early bell stage provided activation of Notch and TGF-β pathway, with the activation or suppression of key genes responsible for tooth development. Furthermore, hsa-miR-34a was found to regulate Notch1 and BMP7 in dental papilla cells [61].

3.4.5 Late Bell Stage

Dentin formation and mineralization starts at the late bell stage. Inner dental epithelium and odondoblast outlines the future dentino-enamel junctions. Dental organ, which is responsible for providing nutrition for the residing cells, consists of outer dental epithelium, stellate reticulum, stratum intermedium and inner dental epithelium.

He et al. showed that in miniature pigs, 105, 16 and 40 miRNAs were dysregulated in the late bell stage when compared with the bud, cup and early bell stages, respectively. ssc-miR-99b and ssc-miR-206 enhanced in late bell stage when compared to the bud stage. Furthermore, ssc-miR-214, which has been associated with the WNT pathway, downregulated in late bell stage when compared with bud stage [57]. hsa-miR-34a also upregulated in late bell stage with respect to the early bell stage, and it was shown that hsa-miR-34a regulated Notch and TGF-β pathways [61]. As a result, it can be concluded that during the late bell stage has-miR-34a expression can act as a switch to downregulate Notch and TGF-β pathways to facilitate the transition from the early to late bell stage.

3.4.6 Tooth Eruption

Tooth eruption is the final process by which the tooth becomes visible and functional for the organism. In newborn humans, tooth eruption starts 6 months to 2 years after birth. Periodontal ligament and dental follicle are the main tissues that take part in the process. Several studies showed that EGF, TGF-β, interleukin-l (IL-1), colony-stimulating factor-l (CSF-l) have crucial roles during tooth eruption [62]. Khan et al. showed that decreasing the abundance of miR-214 by mandibular injection of antimiR in mice prevented tooth eruption. Additionally, after the injection of antimiR-214, TGFβ1 and clusterin expressions decreased [63]. It is evidenced that salivary miRNAs function as critical players in tooth development and eruption in rodents [64, 65]. miRNA analysis of saliva from different dentition stage of children including edentulous, deciduous and permanent teeth showed that 47 miRNAs dysregulated between three groups. Briefly, expression of 19 miRNAs increased and expression of 27 miRNAs decreased gradually during transition from edentulous stage to permanent stage [66].

It has been observed that the RUNX2 molecule takes plays an important role in tooth eruption since a mutated form of RUNX2 gene caused a delay in tooth eruption. miRNA profile of mutated and wild-type hDFSCs revealed that 123 miRNAs were differentially expressed (69 upregulated, 54 downregulated) in mutated RUNX2-hDFSCs. miR-146a can be highlighted in this study since it can target the RUNX2 gene [67].

3.5 Role of miRNAs in Differentiation of DSCs

Having multipotent characteristics, DSCs display the potential to be used in tissue engineering and stem cell therapy. As the knowledge about the molecular pathways behind DSC differentiation grows, the role of miRNAs in these gene networks also begins to be unveiled. Acquisition of such knowledge will not only help us to understand the biology of this process, but also will help to optimize the in vitro differentiation techniques into necessary lineages for therapy purposes. Table 3.1 presents selected miRNAs that play various roles in DSC differentiation and have promising validated and predicted targets.

3.5.1 Odontogenic Differentiation

Odontoblast cells are differentiated mesenchymal cells responsible in tooth development and regeneration, primarily by producing dentin. They are located near the dental pulp and rich in collagen type 1 (COL1). Several studies suggested that isolated DSCs can differentiate into odontoblast cells in vitro and in vivo [78–81]. In the study conducted by Ling et al., miRNAs have been shown to affect the

Table 3.1 Role of miRNAs in dental stem cell (DSC) differentiation and their relevant predicted or validated targets

miRNA	Differentiation inhibition	Predicted gene targets	Validated gene targets	Source of dental stem cells	Reference
miR-135	Odontogenesis	APC, MEF2C and COL5A1		DPSCs	[68]
miR-145	Odontogenesis		KLF, OSX	DPSCs	[69]
miR-720	Odontogenesis		NANOG	DPSCs	[50]
miR-338-3p	Odontogenesis		RUNX2	mDPC6T Cell Line	[70]
mir32, mir885-5p and mir586	Odontogenesis		DSPP	DPSCs	[71]
Let-7	Odontogenesis		DMP1	DPSCs	[72]
miR-27	Odontogenesis		APC	MDPC-23 Cell Line	[73]
miR-17	Osteogenesis		SMURF1	PDLSCs	[74]
miR-218	Osteogenesis		RUNX2	PDLSCs, DPSCs, gingiva-derived MSCs	[75]
miR-26a	Osteogenesis	SMAD1		PDLSCs	[76]
miR-18a	Osteogenesis	CCN2		PDLSCs	[76]
miR-141	Osteogenesis	DLX5		PDLSCs	[76]
miR-200b	Osteogenesis	SMAD5		PDLSCs	[76]
miR-183	Insulin-producing cell differentiation		FOXO1	DPSCs	[77]

DPSCs Dental pulp stem cells, *PDLSCs* Periodontal ligament stem cells

differentiation of DPSCs into odontoblast cells. DPSCs isolated from pre-molar and impacted third molar teeth from health donors were differentiated into odontoblast cells, and the miRNA expression profiles of undifferentiated DPSCs and differentiated DPSCs were compared. According to the results, 22 miRNAs were dysregulated between two groups. hsa-miR-135b expression can be singled out as a reduced miRNA during osteogenic differentiation [68]. Adenomatous polyposis coli (APC), myocyte enhancer factor 2C (MEF2C) and COL5A1 are reported to be candidate targets for hsa-miR-135b. APC can inhibit WNT pathway which provides maintenance of DPSC properties, while MEF2C is responsible in terminal differentiation of odontoblast [82, 83]. In a study on mice, miR-145 and miR-143 were found to regulate odontoblast cell differentiation. DPSCs derived from mouse were differentiated into odontoblast-like cells in vitro, which resulted in differential expressions of 27 miRNAs. They also found that mmu-miR-145 and mmu-miR-143 expressions reduced during odontoblast differentiation which was in parallel with the increasing levels of KLF4 and osterix (OSX) genes whose expression is known to increase

during odontoblast cell differentiation [84–86]. Following a microarray study, it was discovered that miR-338-3p could induce odontoblast differentiation through regulating expression of RUNX2, and further functional tests revealed that this miRNA targets RUNX2 in both gene and protein levels. Therefore, targeting of KLF4 by miR-338-3p may be a crucial step towards odontogenic differentiation [70]. In another study, miR-720 expression was found to decrease side population of DPSCs when compared with main population. Overexpression of miR-720 increased differentiation of hDPSCs into odontogenic lineage through regulating the expression of NANOG which is responsible for stemness maintenance [50]. Besides, 10 different predicted miRNAs determined using 4 different online target prediction databases have been shown to regulate expression of dentin sialophosphoprotein (DSPP), an important marker to identify odontogenic differentiation. Among the 10 miRNAs, miR-32, miR-885-5p and miR-586 regulated DSPP expression in 293-T cells, suggesting that such an interaction can be possible in differentiation of DPSCs as well [71]. Dentin matrix protein 1 which is essential in odontogenesis can be regulated by let-7 [72]. During the differentiation of odontoblastic cells, miR-27 was up-regulated. Briefly, transfection of MDPC-23 cell line with miR-27 mimic increased the odontogenic differentiation. Furthermore, miR-27 was found to inhibit APC, a negative regulator of WNT/β-catenin pathway, and WNT/β-catenin pathway induced differentiation of odontoblastic cell line MDPC-23 [73].

3.5.2 Osteogenic Differentiation

Several studies explored that DSCs have the ability for osteogenic differentiation. For example, MacDougall et al. showed that PDLSCs can differentiate into osteogenic lineage [87]. miRNA regulation of DSCs during osteogenic differentiation has been studied by several groups. miR-17 level decreased during osteogenic differentiation of PDLSCs of healthy donors [74]. miR-17 targets the SMAD specific E3 ubiquitin protein ligase 1 (Smurf1) gene, a negative regulator of osteogenic differentiation as presented in other studies [88–90]. On the other hand, when miR-17 is overexpressed, expression of osteogenic differentiation related gene RUNX2 and functional osteogenic markers increase. Another study showed that miR-218 level decreased during osteogenic differentiation of PDLSCs, DPSCs and gingiva-derived MSCs. After DSCs were differentiated into osteogenic lineage, an increase in the expression of RUNX2 were observed, confirmed by qPCR. They also compared miRNA profile of both undifferentiated and differentiated DSCs and reported that expression levels of 8 miRNAs decreased during osteogenic differentiation. Among the differentially expressed miRNAs, the most downregulated miRNA, miR-218, targeted RUNX2 expression [75]. In another study, effects of the osteoporosis drug, ibandronate, on cell proliferation and miRNA profile of PDLSCs have been investigated. Ibandronate treatment resulted in differentiation of PDLSCs into osteogenic lineage [76]. Eighteen miRNAs (2 upregulated, 16 downregulated) were

dysregulated during the process. Expressions of miR-26a, miR-18a, miR-141 and miR-200b decreased during ibandronate-induced osteogenic differentiation of DSCs, and SMAD1, connective tissue growth factor (CCN2), DLX5 and SMAD5 were the candidate targets for these miRNAs.

3.5.3 Other Lineages

Between the miRNA profile of undifferentiated DSCs and differentiated insulin-producing cells, 7 miRNAs including mir101a, miR-101b, miR-181c, miR-29a, miR-29b, miR-29c and miR-30e upregulated, and miR-183 downregulated during differentiation [77]. After differentiation of DPSCs into insulin-producing cells, it was found that forkhead box O1 (FOXO1), a key gene in pancreatic differentiation, upregulated in differentiated insulin-producing cells, indicating that miR-183 could regulate FOXO1 expression. Taken together, we can interpret that downregulation of miR-183 during pancreatic cell differentiation provides more abundant of FOXO1 which can facilitate the differentiation.

There is lack of miRNA research for many lineages that DSCs can differentiate into. Further studies should strictly be conducted to elucidate the roles of miRNAs in DSC differentiation into other lineages such as chondrocytes, neurons and adipocytes.

3.6 Expression of miRNA in Dental Tissues and Stem Cell Niche

Although stem cells have self-sufficiency to a certain extent in terms self-renewal, preservation of stemness, migration and differentiation, the tissue environment in which stem cells are positioned is very important for the stem cells to function properly. Stem cell niches are local tissue environments that have the duties of maintenance and regulation of the stem around them. Stem cell dormancy and activation upon injury are also regulated by the niche.

Understanding the stem cell niche around DSCs will not only help us to better understand the biological mechanisms behind DSCs but also help us to acquire potentially more potent stem cells from various tooth tissues. Additionally, explanation of how the niche acts to help stem cells self-renew may lead to grow these cells more efficiently in vitro. In the dental pulp, multiple stem cell niches have been identified in the perivascular area by determining the microenvironments that produce nestin, vimentin, Oct3 and Oct4 [91]. Another widely studied stem cell niche is the cervical loop of rodent incisors, in which epithelial stem cells are maintained and regulated by mainly FGF signaling through the Notch pathway [92].

miRNAs produced in dental tissues and DSC microenvironment can directly affect target mRNAs intracellularly or can function extracellularly, affecting neighboring cells. As the importance of stem cell niches has been realized, specific

miRNA markers and their potential roles have become an emerging field of study. For instance, in the testis, there is a huge potential of miRNAs regulating the surrounding somatic cells, and the spermotogonial cells with the possibility of intercellular regulation [93]. Although MSCs that have been isolated from bone marrow, adipose tissue and cord blood had similar miRNA profiles, each tissue type had specific miRNA levels that may be connected to their surrounding tissue [94].

Studies on the miRNA profile of dental tissues, their comparison and potential effects on DSCs have been widely carried out in several researches. In one study, fibroblasts from dental pulp, gingiva and periodontal ligament have been shown to display different miRNA profiles. miR-146a and miR-155 have been singled out to show this tissue dependent expressions. A number of miRNAs that have been detected have known functions in extracellular matrix turnover and inflammation. Stimulation by lipopolysaccharides derived from *Escherichia coli* resulted in the increase of miR-146a and decrease of miR-155 solely in the gingival fibroblasts [95].

The adult mouse incisor tooth is a good model organ not only for DSC studies but also for other ASCs. Unlike any human teeth, mouse incisors grow continuously during adulthood. Labial and lingual cervical loops are two epithelial stem cell niches that give rise to this continuously growing tooth by presenting progeny that migrate out of the niche and move towards the tip of the tooth, and eventually differentiate into enamel-producing ameloblasts. miRNAs have potentially play an important role in maintaining the balance of self-renewal and differentiation in the stem cell niche of these cells. Labial cervical loop forming ameloblast stem cells, lingual cervical loop containing stem cells that do not give rise to ameloblasts, and ameloblasts were used in a miRNA profile comparison study. Twenty-six miRNAs were differentially expressed between labial and lingual cervical loops which would highlight the importance of miRNAs in ameloblast stem cell renewal. Thirty-five miRNAs were differentially expressed between labial cervical loop and ameloblast cells which would highlight the significance of miRNAs in enamel differentiation. The labial cervical loop samples had increased miR-31, miR-96, miR-182, miR-200c and miR-429, and decreased miR-21 levels compared to the lingual cervical loop samples. miR-138, miR-141, miR-200c and miR-429 were highly expressed in ameloblasts, whereas miR-143 and miR-145 levels decreased, when compared to labial cervical loop samples. miR-31 localized as expected in the labial cervical loop region especially in the T-A cell region [96]. Similarly, comparison of miRNA profiles of mouse incisors with molars proved that continuously developing incisors have a distinct miRNA profile than the non-developing molars, suggesting an extensive role of miRNAs in the process. In the same study, miR-200c has been identified as a regulator of Noggin, suggesting that this miRNA is essential for normal tooth development, and dental epithelial cell differentiation [97].

3.7 Future Directions

Although ESCs are considered as the most functional cells in stem cell technology, ethical debate and difficulties in cell collection and long term storage still persist. This undesirable situation forces researchers to improve the knowledge on ASCs.

Moreover, the emergence of iPSCs technology may help scientists to increase the potency of ASCs. As dental tissues emerge as reliable, sustainable and non-invasive sources for ASCs, understanding the molecular mechanisms of DSC stemness and differentiation is crucial in order to optimize the manipulation of these cells in vitro and in vivo to be used in stem cells therapies and tissue engineering approaches. miRNAs are known to be important mediators of stem cell biology in any stage of life ranging from the embryonic stage into adult development. miRNAs are natural RNA interfering molecules that have been extensively studied in areas ranging from cancer to stem cell biology. Undoubtedly, to fully understand the mechanisms behind DSC biology, the miRNA profiles of each DSC source under every differentiation process must be monitored. Such studies could help to connect the missing links in canonical pathways and gene networks that transcriptomics or proteomics cannot unveil. However, it is hard to miss the fact that miRNA studies have been neglected in the DSC field. It is hard to find satisfactory studies of miRNA profiles after DSC differentiation into particular lineages such as the neurons. Furthermore, present studies do not provide solid data due to lack of repetition and sample number. The need for further miRNA profiling studies also results from the increasing number of newly identified miRNAs. Roughly, the number of miRNAs presented in the miRBase doubles in every 18 months thanks to high-throughput RNA sequencing technologies. As the database is updated, new versions of miRNA microarray ChIPs are presented; thus, past studies using older chip versions remain inadequate.

miRNAs have the potential to be utilized as molecular transfecting agents as well. Fully understanding the specific miRNAs for DSC differentiation would make it possible to use miRNAs to direct differentiation toward the desired path. Since differentiation is a dynamic process in which genes are turned on and off periodically and at well-defined times, stable transfections would not be very helpful. Besides, a stably transfected cell would contain extra pieces of DNA, which is not desired for cells that will be transferred to humans. Moreover, the usage of viruses for stable transfection is risky. miRNAs are excellent molecules for transient transfection, easier than other types of therapeutic nucleic acids. There is no need for vectors, and lipofection-mediated transfection is a well-defined method for miRNA transfection with high yield and minimum toxicity. Since the stability of mature miRNAs is very low, it would be very easy to control the timing of target gene downregulation. All these factors make miRNAs ideal for controlling gene regulation is DSC differentiation.

References

1. Yan X, Qin H, Qu C, Tuan RS, Shi S, Huang GT (2010) IPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. Stem Cells Dev 19(4):469–480. doi:10.1089/scd.2009.0314
2. Stevens A, Zuliani T, Olejnik C, LeRoy H, Obriot H, Kerr-Conte J, Formstecher P, Bailliez Y, Polakowska RR (2008) Human dental pulp stem cells differentiate into neural crest-derived

melanocytes and have label-retaining and sphere-forming abilities. Stem Cells Dev 17(6):1175–1184. doi:10.1089/scd.2008.0012
3. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676. doi:10.1016/j.cell.2006.07.024
4. Friedenstein AJ, Piatetzky S II, Petrakova KV (1966) Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morphol 16(3):381–390
5. Lee OK, Kuo TK, Chen WM, Lee KD, Hsieh SL, Chen TH (2004) Isolation of multipotent mesenchymal stem cells from umbilical cord blood. Blood 103(5):1669–1675. doi:10.1182/blood-2003-05-1670
6. Huang GT, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 88(9):792–806. doi:10.1177/0022034509340867
7. Gimble JM, Katz AJ, Bunnell BA (2007) Adipose-derived stem cells for regenerative medicine. Circ Res 100(9):1249–1260. doi:10.1161/01.RES.0000265074.83288.09
8. Jones EA, English A, Henshaw K, Kinsey SE, Markham AF, Emery P, McGonagle D (2004) Enumeration and phenotypic characterization of synovial fluid multipotential mesenchymal progenitor cells in inflammatory and degenerative arthritis. Arthritis Rheum 50(3):817–827. doi:10.1002/art.20203
9. Shih YR, Kuo TK, Yang AH, Lee OK, Lee CH (2009) Isolation and characterization of stem cells from the human parathyroid gland. Cell Prolif 42(4):461–470. doi:10.1111/j.1365-2184.2009.00614.x
10. Stadler B, Ivanovska I, Mehta K, Song S, Nelson A, Tan Y, Mathieu J, Darby C, Blau CA, Ware C, Peters G, Miller DG, Shen L, Cleary MA, Ruohola-Baker H (2010) Characterization of microRNAs involved in embryonic stem cell states. Stem Cells Dev 19(7):935–950. doi:10.1089/scd.2009.0426
11. Qi J, Yu JY, Shcherbata HR, Mathieu J, Wang AJ, Seal S, Zhou W, Stadler BM, Bourgin D, Wang L, Nelson A, Ware C, Raymond C, Lim LP, Magnus J, Ivanovska I, Diaz R, Ball A, Cleary MA, Ruohola-Baker H (2009) microRNAs regulate human embryonic stem cell division. Cell Cycle 8(22):3729–3741
12. Becker KA, Ghule PN, Therrien JA, Lian JB, Stein JL, van Wijnen AJ, Stein GS (2006) Self-renewal of human embryonic stem cells is supported by a shortened G1 cell cycle phase. J Cell Physiol 209(3):883–893. doi:10.1002/jcp.20776
13. Wang Y, Baskerville S, Shenoy A, Babiarz JE, Baehner L, Blelloch R (2008) Embryonic stem cell-specific microRNAs regulate the G1-S transition and promote rapid proliferation. Nat Genet 40(12):1478–1483. doi:10.1038/ng.250
14. Bar M, Wyman SK, Fritz BR, Qi J, Garg KS, Parkin RK, Kroh EM, Bendoraite A, Mitchell PS, Nelson AM, Ruzzo WL, Ware C, Radich JP, Gentleman R, Ruohola-Baker H, Tewari M (2008) MicroRNA discovery and profiling in human embryonic stem cells by deep sequencing of small RNA libraries. Stem Cells 26(10):2496–2505. doi:10.1634/stemcells.2008-0356
15. Sengupta S, Nie J, Wagner RJ, Yang C, Stewart R, Thomson JA (2009) MicroRNA 92b controls the G1/S checkpoint gene p57 in human embryonic stem cells. Stem Cells 27(7):1524–1528. doi:10.1002/stem.84
16. Xu N, Papagiannakopoulos T, Pan G, Thomson JA, Kosik KS (2009) MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. Cell 137(4):647–658. doi:10.1016/j.cell.2009.02.038
17. Judson RL, Babiarz JE, Venere M, Blelloch R (2009) Embryonic stem cell-specific microRNAs promote induced pluripotency. Nat Biotechnol 27(5):459–461. doi:10.1038/nbt.1535
18. Subramanyam D, Lamouille S, Judson RL, Liu JY, Bucay N, Derynck R, Blelloch R (2011) Multiple targets of miR-302 and miR-372 promote reprogramming of human fibroblasts to induced pluripotent stem cells. Nat Biotechnol 29(5):443–448. doi:10.1038/nbt.1862
19. Yang CS, Li Z, Rana TM (2011) microRNAs modulate IPS cell generation. RNA 17(8):1451–1460. doi:10.1261/rna.2664111

20. Thum T, Gross C, Fiedler J, Fischer T, Kissler S, Bussen M, Galuppo P, Just S, Rottbauer W, Frantz S, Castoldi M, Soutschek J, Koteliansky V, Rosenwald A, Basson MA, Licht JD, Pena JT, Rouhanifard SH, Muckenthaler MU, Tuschl T, Martin GR, Bauersachs J, Engelhardt S (2008) MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature 456(7224):980–984. doi:10.1038/nature07511
21. Silva J, Barrandon O, Nichols J, Kawaguchi J, Theunissen TW, Smith A (2008) Promotion of reprogramming to ground state pluripotency by signal inhibition. PLoS Biol 6(10), e253. doi:10.1371/journal.pbio.0060253
22. Hu W, Dooley J, Chung SS, Chandramohan D, Cimmino L, Mukherjee S, Mason CE, de Strooper B, Liston A, Park CY (2015) miR-29a maintains mouse hematopoietic stem cell self-renewal by regulating Dnmt3a. Blood 125(14):2206–2216. doi:10.1182/blood-2014-06-585273
23. Brett JO, Renault VM, Rafalski VA, Webb AE, Brunet A (2011) The microRNA cluster miR-106b~25 regulates adult neural stem/progenitor cell proliferation and neuronal differentiation. Aging (Albany NY) 3(2):108–124
24. Wang D, Zhang Z, O'Loughlin E, Wang L, Fan X, Lai EC, Yi R (2013) MicroRNA-205 controls neonatal expansion of skin stem cells by modulating the PI(3)K pathway. Nat Cell Biol 15(10):1153–1163. doi:10.1038/ncb2827
25. Baglio SR, Devescovi V, Granchi D, Baldini N (2013) MicroRNA expression profiling of human bone marrow mesenchymal stem cells during osteogenic differentiation reveals Osterix regulation by miR-31. Gene 527(1):321–331. doi:10.1016/j.gene.2013.06.021
26. Eskildsen T, Taipaleenmaki H, Stenvang J, Abdallah BM, Ditzel N, Nossent AY, Bak M, Kauppinen S, Kassem M (2011) MicroRNA-138 regulates osteogenic differentiation of human stromal (mesenchymal) stem cells in vivo. Proc Natl Acad Sci U S A 108(15):6139–6144. doi:10.1073/pnas.1016758108
27. Kim YJ, Hwang SJ, Bae YC, Jung JS (2009) MiR-21 regulates adipogenic differentiation through the modulation of TGF-beta signaling in mesenchymal stem cells derived from human adipose tissue. Stem Cells 27(12):3093–3102. doi:10.1002/stem.235
28. Bork S, Horn P, Castoldi M, Hellwig I, Ho AD, Wagner W (2011) Adipogenic differentiation of human mesenchymal stromal cells is down-regulated by microRNA-369-5p and up-regulated by microRNA-371. J Cell Physiol 226(9):2226–2234. doi:10.1002/jcp.22557
29. Yang B, Guo H, Zhang Y, Chen L, Ying D, Dong S (2011) MicroRNA-145 regulates chondrogenic differentiation of mesenchymal stem cells by targeting Sox9. PLoS One 6(7), e21679. doi:10.1371/journal.pone.0021679
30. Etheridge SL, Spencer GJ, Heath DJ, Genever PG (2004) Expression profiling and functional analysis of wnt signaling mechanisms in mesenchymal stem cells. Stem Cells 22(5):849–860. doi:10.1634/stemcells.22-5-849
31. Boland GM, Perkins G, Hall DJ, Tuan RS (2004) Wnt 3a promotes proliferation and suppresses osteogenic differentiation of adult human mesenchymal stem cells. J Cell Biochem 93(6):1210–1230. doi:10.1002/jcb.20284
32. Cho HH, Kim YJ, Kim SJ, Kim JH, Bae YC, Ba B, Jung JS (2006) Endogenous Wnt signaling promotes proliferation and suppresses osteogenic differentiation in human adipose derived stromal cells. Tissue Eng 12(1):111–121. doi:10.1089/ten.2006.12.111
33. Wu R, Tang Y, Zang W, Wang Y, Li M, Du Y, Zhao G, Xu Y (2014) MicroRNA-128 regulates the differentiation of rat bone mesenchymal stem cells into neuron-like cells by Wnt signaling. Mol Cell Biochem 387(1–2):151–158. doi:10.1007/s11010-013-1880-7
34. Wang Q, Cai J, Cai XH, Chen L (2013) miR-346 regulates osteogenic differentiation of human bone marrow-derived mesenchymal stem cells by targeting the Wnt/beta-catenin pathway. PLoS One 8(9):e72266. doi:10.1371/journal.pone.0072266
35. Ying QL, Nichols J, Chambers I, Smith A (2003) BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell 115(3):281–292
36. Chen D, Ji X, Harris MA, Feng JQ, Karsenty G, Celeste AJ, Rosen V, Mundy GR, Harris SE (1998) Differential roles for bone morphogenetic protein (BMP) receptor type IB and IA in

differentiation and specification of mesenchymal precursor cells to osteoblast and adipocyte lineages. J Cell Biol 142(1):295–305
37. Zhang JF, Fu WM, He ML, Xie WD, Lv Q, Wan G, Li G, Wang H, Lu G, Hu X, Jiang S, Li JN, Lin MC, Zhang YO, Kung HF (2011) MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. RNA Biol 8(5):829–838. doi:10.4161/rna.8.5.16043
38. Li H, Li T, Wang S, Wei J, Fan J, Li J, Han Q, Liao L, Shao C, Zhao RC (2013) miR-17-5p and miR-106a are involved in the balance between osteogenic and adipogenic differentiation of adipose-derived mesenchymal stem cells. Stem Cell Res 10(3):313–324. doi:10.1016/j.scr.2012.11.007
39. Wei J, Zhang B, Zheng X, Chen H, Tian X, Tang P, Song Q, Li T (2012) Changes of microRNA and target gene expression levels in osteogenic differentiation of human bone marrow mesenchymal stem cells. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 26(4):483–488
40. Liu X, Song L, Liu J, Wang S, Tan X, Bai X, Bai T, Wang Y, Li M, Song Y, Li Y (2013) miR-18b inhibits TGF-beta1-induced differentiation of hair follicle stem cells into smooth muscle cells by targeting SMAD2. Biochem Biophys Res Commun 438(3):551–556. doi:10.1016/j.bbrc.2013.07.090
41. Weber M, Sotoca AM, Kupfer P, Guthke R, van Zoelen EJ (2013) Dynamic modelling of microRNA regulation during mesenchymal stem cell differentiation. BMC Syst Biol 7:124. doi:10.1186/1752-0509-7-124
42. Huang F, Zhu X, Hu XQ, Fang ZF, Tang L, Lu XL, Zhou SH (2013) Mesenchymal stem cells modified with miR-126 release angiogenic factors and activate Notch ligand Delta-like-4, enhancing ischemic angiogenesis and cell survival. Int J Mol Med 31(2):484–492. doi:10.3892/ijmm.2012.1200
43. Hoch RV, Soriano P (2003) Roles of PDGF in animal development. Development 130(20):4769–4784. doi:10.1242/dev.00721
44. Qu B, Xia X, Wu HH, Tu CQ, Pan XM (2014) PDGF-regulated miRNA-138 inhibits the osteogenic differentiation of mesenchymal stem cells. Biochem Biophys Res Commun 448(3):241–247. doi:10.1016/j.bbrc.2014.04.091
45. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97(25):13625–13630. doi:10.1073/pnas.240309797
46. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100(10):5807–5812. doi:10.1073/pnas.0937635100
47. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364(9429):149–155. doi:10.1016/S0140-6736(04)16627-0
48. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Wang S, Shi S (2006) Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One 1, e79. doi:10.1371/journal.pone.0000079
49. Morsczeck C, Gotz W, Schierholz J, Zeilhofer F, Kuhn U, Mohl C, Sippel C, Hoffmann KH (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 24(2):155–165. doi:10.1016/j.matbio.2004.12.004
50. Hara ES, Ono M, Eguchi T, Kubota S, Pham HT, Sonoyama W, Tajima S, Takigawa M, Calderwood SK, Kuboki T (2013) miRNA-720 controls stem cell phenotype, proliferation and differentiation of human dental pulp cells. PLoS One 8(12):e83545. doi:10.1371/journal.pone.0083545
51. Sun F, Wan M, Xu X, Gao B, Zhou Y, Sun J, Cheng L, Klein OD, Zhou X, Zheng L (2014) Crosstalk between miR-34a and notch signaling promotes differentiation in apical papilla stem cells (SCAPs). J Dent Res 93(6):589–595. doi:10.1177/0022034514531146
52. Vasanthan P, Govindasamy V, Gnanasegaran N, Kunasekaran W, Musa S, Abu Kasim NH (2015) Differential expression of basal microRNAs' patterns in human dental pulp stem cells. J Cell Mol Med 19(3):566–580. doi:10.1111/jcmm.12381

53. Chen HC, Lee YS, Sieber M, Lu HT, Wei PC, Wang CN, Peng HH, Chao AS, Cheng PJ, Chang SD, Chen SJ, Wang TH (2012) MicroRNA and messenger RNA analyses of mesenchymal stem cells derived from teeth and the Wharton jelly of umbilical cord. Stem Cells Dev 21(6):911–922. doi:10.1089/scd.2011.0186
54. Sarkar L, Cobourne M, Naylor S, Smalley M, Dale T, Sharpe PT (2000) Wnt/Shh interactions regulate ectodermal boundary formation during mammalian tooth development. Proc Natl Acad Sci U S A 97(9):4520–4524
55. Wang Y, Li L, Zheng Y, Yuan G, Yang G, He F, Chen Y (2012) BMP activity is required for tooth development from the lamina to bud stage. J Dent Res 91(7):690–695. doi:10.1177/0022034512448660
56. Diekwisch TG, Luan X, McIntosh JE (2002) CP27 localization in the dental lamina basement membrane and in the stellate reticulum of developing teeth. J Histochem Cytochem 50(4):583–586
57. Li A, Song T, Wang F, Liu D, Fan Z, Zhang C, He J, Wang S (2012) MicroRNAome and expression profile of developing tooth germ in miniature pigs. PLoS One 7(12), e52256. doi:10.1371/journal.pone.0052256
58. Kim EJ, Lee MJ, Li L, Yoon KS, Kim KS, Jung HS (2014) Failure of Tooth Formation Mediated by miR-135a Overexpression via BMP Signaling. J Dent Res 93(6):571–575. doi:10.1177/0022034514529303
59. Koussoulakou DS, Margaritis LH, Koussoulakos SL (2009) A curriculum vitae of teeth: evolution, generation, regeneration. Int J Biol Sci 5(3):226–243
60. Sarkar L, Sharpe PT (1999) Expression of Wnt signalling pathway genes during tooth development. Mech Dev 85(1–2):197–200
61. Wan M, Gao B, Sun F, Tang Y, Ye L, Fan Y, Klein OD, Zhou X, Zheng L (2012) microRNA miR-34a regulates cytodifferentiation and targets multi-signaling pathways in human dental papilla cells. PLoS One 7(11):e50090. doi:10.1371/journal.pone.0050090
62. Marks SC Jr, Gorski JP, Wise GE (1995) The mechanisms and mediators of tooth eruption--models for developmental biologists. Int J Dev Biol 39(1):223–230
63. Khan QE, Sehic A, Khuu C, Risnes S, Osmundsen H (2013) Expression of Clu and Tgfb1 during murine tooth development: effects of in-vivo transfection with anti-miR-214. Eur J Oral Sci 121(4):303–312. doi:10.1111/eos.12056
64. Cao H, Wang J, Li X, Florez S, Huang Z, Venugopalan SR, Elangovan S, Skobe Z, Margolis HC, Martin JF, Amendt BA (2010) MicroRNAs play a critical role in tooth development. J Dent Res 89(8):779–784. doi:10.1177/0022034510369304
65. Jevnaker AM, Osmundsen H (2008) MicroRNA expression profiling of the developing murine molar tooth germ and the developing murine submandibular salivary gland. Arch Oral Biol 53(7):629–645. doi:10.1016/j.archoralbio.2008.01.014
66. Formosa A, Piro MC, Docimo R, Maturo P, Sollecito DR, Kalimutho M, Sancesario G, Barlattani A, Melino G, Candi E, Bernardini S (2011) Salivary miRNAome profiling uncovers epithelial and proliferative miRNAs with differential expression across dentition stages. Cell Cycle 10(19):3359–3368. doi:10.4161/cc.10.19.17647
67. Chen P, Wei D, Xie B, Ni J, Xuan D, Zhang J (2014) Effect and possible mechanism of network between microRNAs and RUNX2 gene on human dental follicle cells. J Cell Biochem 115(2):340–348. doi:10.1002/jcb.24668
68. Gong Q, Wang R, Jiang H, Lin Z, Ling J (2012) Alteration of microRNA expression of human dental pulp cells during odontogenic differentiation. J Endod 38(10):1348–1354. doi:10.1016/j.joen.2012.06.016
69. Liu H, Lin H, Zhang L, Sun Q, Yuan G, Zhang L, Chen S, Chen Z (2013) miR-145 and miR-143 regulate odontoblast differentiation through targeting Klf4 and Osx genes in a feedback loop. J Biol Chem 288(13):9261–9271. doi:10.1074/jbc.M112.433730
70. Sun Q, Liu H, Lin H, Yuan G, Zhang L, Chen Z (2013) MicroRNA-338-3p promotes differentiation of mDPC6T into odontoblast-like cells by targeting Runx2. Mol Cell Biochem 377(1–2):143–149. doi:10.1007/s11010-013-1580-3

71. Huang X, Xu S, Gao J, Liu F, Yue J, Chen T, Wu B (2011) miRNA expression profiling identifies DSPP regulators in cultured dental pulp cells. Int J Mol Med 28(4):659–667. doi:10.3892/ijmm.2011.721
72. Yue J, Wu B, Gao J, Huang X, Li C, Ma D, Fang F (2012) DMP1 is a target of let-7 in dental pulp cells. Int J Mol Med 30(2):295–301. doi:10.3892/ijmm.2012.982
73. Park MG, Kim JS, Park SY, Lee SA, Kim HJ, Kim CS, Kim JS, Chun HS, Park JC, Kim do K (2014) MicroRNA-27 promotes the differentiation of odontoblastic cell by targeting APC and activating Wnt/beta-catenin signaling. Gene 538(2):266–272. doi:10.1016/j.gene.2014.01.045
74. Liu Y, Liu W, Hu C, Xue Z, Wang G, Ding B, Luo H, Tang L, Kong X, Chen X, Liu N, Ding Y, Jin Y (2011) MiR-17 modulates osteogenic differentiation through a coherent feed-forward loop in mesenchymal stem cells isolated from periodontal ligaments of patients with periodontitis. Stem Cells 29(11):1804–1816. doi:10.1002/stem.728
75. Gay I, Cavender A, Peto D, Sun Z, Speer A, Cao H, Amendt BA (2014) Differentiation of human dental stem cells reveals a role for microRNA-218. J Periodontal Res 49(1):110–120. doi:10.1111/jre.12086
76. Zhou Q, Zhao ZN, Cheng JT, Zhang B, Xu J, Huang F, Zhao RN, Chen YJ (2011) Ibandronate promotes osteogenic differentiation of periodontal ligament stem cells by regulating the expression of microRNAs. Biochem Biophys Res Commun 404(1):127–132. doi:10.1016/j.bbrc.2010.11.079
77. Nozaki T, Ohura K (2014) Regulation of miRNA during direct reprogramming of dental pulp cells to insulin-producing cells. Biochem Biophys Res Commun 444(2):195–198. doi:10.1016/j.bbrc.2014.01.030
78. Wang J, Liu X, Jin X, Ma H, Hu J, Ni L, Ma PX (2010) The odontogenic differentiation of human dental pulp stem cells on nanofibrous poly(L-lactic acid) scaffolds in vitro and in vivo. Acta Biomater 6(10):3856–3863. doi:10.1016/j.actbio.2010.04.009
79. Ravindran S, Zhang Y, Huang CC, George A (2014) Odontogenic induction of dental stem cells by extracellular matrix-inspired three-dimensional scaffold. Tissue Eng Part A 20(1–2):92–102. doi:10.1089/ten.TEA.2013.0192
80. Nam S, Won JE, Kim CH, Kim HW (2011) Odontogenic differentiation of human dental pulp stem cells stimulated by the calcium phosphate porous granules. J Tissue Eng 2011:812547. doi:10.4061/2011/812547
81. Bakopoulou A, Leyhausen G, Volk J, Tsiftsoglou A, Garefis P, Koidis P, Geurtsen W (2011) Comparative analysis of in vitro osteo/odontogenic differentiation potential of human dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP). Arch Oral Biol 56(7):709–721. doi:10.1016/j.archoralbio.2010.12.008
82. Scheller EL, Chang J, Wang CY (2008) Wnt/beta-catenin inhibits dental pulp stem cell differentiation. J Dent Res 87(2):126–130
83. Buchaille R, Couble ML, Magloire H, Bleicher F (2000) A substractive PCR-based cDNA library from human odontoblast cells: identification of novel genes expressed in tooth forming cells. Matrix Biol 19(5):421–430
84. Lin H, Xu L, Liu H, Sun Q, Chen Z, Yuan G, Chen Z (2011) KLF4 promotes the odontoblastic differentiation of human dental pulp cells. J Endod 37(7):948–954. doi:10.1016/j.joen.2011.03.030
85. Chen Z, Couble ML, Mouterfi N, Magloire H, Chen Z, Bleicher F (2009) Spatial and temporal expression of KLF4 and KLF5 during murine tooth development. Arch Oral Biol 54(5):403–411. doi:10.1016/j.archoralbio.2009.02.003
86. Chen S, Gluhak-Heinrich J, Wang YH, Wu YM, Chuang HH, Chen L, Yuan GH, Dong J, Gay I, MacDougall M (2009) Runx2, osx, and dspp in tooth development. J Dent Res 88(10):904–909. doi:10.1177/0022034509342873
87. Gay IC, Chen S, MacDougall M (2007) Isolation and characterization of multipotent human periodontal ligament stem cells. Orthod Craniofac Res 10(3):149–160. doi:10.1111/j.1601-6343.2007.00399.x

88. Guo R, Yamashita M, Zhang Q, Zhou Q, Chen D, Reynolds DG, Awad HA, Yanoso L, Zhao L, Schwarz EM, Zhang YE, Boyce BF, Xing L (2008) Ubiquitin ligase Smurf1 mediates tumor necrosis factor-induced systemic bone loss by promoting proteasomal degradation of bone morphogenetic signaling proteins. J Biol Chem 283(34):23084–23092. doi:10.1074/jbc.M709848200
89. Zhao L, Huang J, Guo R, Wang Y, Chen D, Xing L (2010) Smurf1 inhibits mesenchymal stem cell proliferation and differentiation into osteoblasts through JunB degradation. J Bone Miner Res 25(6):1246–1256. doi:10.1002/jbmr.28
90. Zhao M, Qiao M, Harris SE, Oyajobi BO, Mundy GR, Chen D (2004) Smurf1 inhibits osteoblast differentiation and bone formation in vitro and in vivo. J Biol Chem 279(13):12854–12859. doi:10.1074/jbc.M313294200
91. Lizier NF, Kerkis A, Gomes CM, Hebling J, Oliveira CF, Caplan AI, Kerkis I (2012) Scaling-up of dental pulp stem cells isolated from multiple niches. PLoS One 7(6), e39885. doi:10.1371/journal.pone.0039885
92. Thesleff I, Wang XP, Suomalainen M (2007) Regulation of epithelial stem cells in tooth regeneration. C R Biol 330(6–7):561–564. doi:10.1016/j.crvi.2007.03.005
93. Yang QE, Racicot KE, Kaucher AV, Oatley MJ, Oatley JM (2013) MicroRNAs 221 and 222 regulate the undifferentiated state in mammalian male germ cells. Development 140(2):280–290. doi:10.1242/dev.087403
94. Ragni E, Montemurro T, Montelatici E, Lavazza C, Vigano M, Rebulla P, Giordano R, Lazzari L (2013) Differential microRNA signature of human mesenchymal stem cells from different sources reveals an "environmental-niche memory" for bone marrow stem cells. Exp Cell Res 319(10):1562–1574. doi:10.1016/j.yexcr.2013.04.002
95. Sipert CR, Morandini AC, Dionisio TJ, Trachtenberg AJ, Kuo WP, Santos CF (2014) MicroRNA-146a and microRNA-155 show tissue-dependent expression in dental pulp, gingival and periodontal ligament fibroblasts in vitro. J Oral Sci 56(2):157–164
96. Jheon AH, Li CY, Wen T, Michon F, Klein OD (2011) Expression of microRNAs in the stem cell niche of the adult mouse incisor. PLoS One 6(9), e24536. doi:10.1371/journal.pone.0024536
97. Cao H, Jheon A, Li X, Sun Z, Wang J, Florez S, Zhang Z, McManus MT, Klein OD, Amendt BA (2013) The Pitx2:miR-200c/141:noggin pathway regulates Bmp signaling and ameloblast differentiation. Development 140(16):3348–3359. doi:10.1242/dev.089193

Chapter 4
Signaling Pathways in Dental Stem Cells During Their Maintenance and Differentiation

Genxia Liu, Shu Ma, Yixiang Zhou, Yadie Lu, Lin Jin, Zilu Wang, and Jinhua Yu

4.1 Introduction

Dental stem cells (DSCs) residing in dental tissues possess the self-renewal and multipotential differentiation ability, and are essential in the process of tooth homeostasis, repair and regeneration. The maintenance, proliferation and differentiation of DSCs are directly or indirectly regulated by a variety of factors, such as microenvironment, growth factors and donor ages. The complex network of signaling pathways, including fibroblast growth factor (FGF), bone morphogenetic protein (BMP), Notch, nuclear transcription factor kappa-B (NF-κB), mitogen-activated protein kinases (MAPKs), transforming growth factor-β (TGF-β), mammalian target of rapamycin (mTOR), phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B (PI3K/AKT) and sonic hedgehog (SHH) signaling pathways, participate in regulating the formation, homeostasis, and differentiation of DSCs in the developing tooth and throughout the adulthood. Researches over the past years have given rise to the meaningful progress on the understanding of the signaling network.

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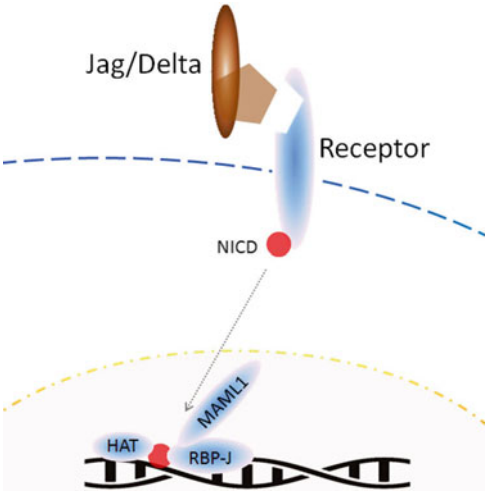

Fig. 4.1 Notch signaling pathway. Notch ligands (Jagged1, Jagged2, Delta1, Delta2 and Delta3) interact with Notch receptors and then initiate the Notch pathway. The activation of Notch subsequently gives rise to a release of Notch intracellular domain (NICD) into the cytoplasm where it translocates to the nucleus. In the nucleus, NICD binds to RBP-J and MAML1, recruits the transcriptional co-activators and leads to the transcription of target genes

4.2 Signaling Pathways in DSC Maintenance/Homeostasis

The signaling pathway is a series of cellular proteins that transfer a biological signal from a receptor on the cell membrane to the DNA in the cell nucleus. The pathway begins with a signaling molecule binding to the membrane receptor and ends when the nuclear DNA generates respective proteins and brings about some cellular changes (*e.g.,* cell differentiation).

During tooth development, DSCs can maintain the stable state, referred to as homeostasis, and many signaling pathways (*e.g.*, Notch, BMP-SHH, MAPK and Eph/Ephrin signaling pathways) control the maintenance of stem cells in tooth.

4.2.1 Notch Signaling Pathway

The Notch signaling pathway is a highly conserved signaling cascade and plays a key role in the stem cell maintenance and fate determination. There are usually four kinds of Notch receptors, i.e., Notch1, Notch2, Notch3 and Notch4. These receptors are single-pass transmembrane receptors. Notch ligands (Jagged1, Jagged2, Delta1, Delta2 and Delta3) interact with these membrane-bound Notch receptors and directly initiate Notch signaling pathway and downstream molecules to mediate the expression level of target genes (Fig. 4.1).

Previous studies have shown that Notch receptors are absent in the adult rat pulp tissues but the expression level will be reactivated during the repair of tooth injury [1]. Notch signaling is also essential for the development of dental epithelium and enamel organ [2]. Notch receptors as well as Notch ligands are expressed in both dental epithelial and mesenchymal cells during the odontogenesis, and initiate the stage of epithelial-mesenchymal interactions for tooth morphogenesis [3, 4]. Notch and FGF signaling pathways are associated with dental epithelial stem cells in regulating their fate and FGF10 maintains the stem cell population during the development of mouse incisors [5, 6].

4.2.2 SHH Signaling Pathway

SHH signaling pathway is a chain of proteins that transfer the information to cells for proper embryonic development. In addition, it is highly active in cell proliferation and differentiation of both epithelial and mesenchymal stem cells (MSCs).

In mice, dental epithelial stem cells residing in the cervical loop at the proximal end of the labial side of incisors are maintained along with the MSCs, and they allow the incisors to grow continuously throughout life [7]. Researchers have focused on the molecular mechanisms of this phenomenon, and find that SHH signaling pathway is related to the stem cell homeostasis [8]. Moreover, BMP-Smad4-SHH signaling can regulate the epithelial stem cell maintenance in tooth development. $Sox2^{+}$ epithelial stem cells exist transiently during the molar development, and sonic hedgehog-glioma-associated oncogene 1 (Shh-Gli1) activity provides a niche for maintenance of these stem cells. However, loss of Smad4 results in ectopic SHH-Gli1 signaling and maintenance of $Sox2^{+}$ cells [9]. This study has proved the importance of crosstalk between BMP and SHH signaling pathways in the regulation of epithelial stem cell fate during odontogenesis. Moreover, SHH pathway can inhibit the osteo/dentinogenic differentiation of stem cells from apical papilla [10].

4.2.3 MAPK Signaling Pathway

MAPK signaling pathway (also known as the Ras-Raf-MEK-ERK pathway) contains several proteins, including MAPK (mitogen-activated protein kinases), that communicate by driving the phosphate groups into a neighboring protein (work as an "on" or "off" switch manner). This pathway is involved in cell apoptosis, survival, migration, proliferation, differentiation as well as other cellular processes. Three main MAPK family members (extracellular signal-regulated kinase (ERK), c-Jun-N-terminal kinase (JNK) and p38) are distinctly referred to these processes [11].

Recent literatures have provided convincing evidences that MAPK signaling pathway plays a critical role in the maintenance, migration, proliferation and differentiation of DSCs. Two-hydroxyethyl methacrylate (HEMA), a kind of resin-based dental materials, can inhibit the cell migration of dental pulp stem cells (DPSCs) by phosphorylation of p38 but not ERK, or JNK MAPK pathways [12]. p38 MAPK and insulin-like growth factor 1 receptor (IGF-1R) are responsible for the mitotic quiescence of DPSCs. The inhibitors of IGF-1R can improve the sphere-forming capacity of DPSCs and decrease the colony-forming capacity without causing cell death, in contrast to the p38 inhibitors. IGF-1R and p38 MAPK signaling pathways are interrelated at the molecular levels in DPSCs. Signals from these pathways converge as signal transducers and activators of transcription 3 (STAT3), and oppositely modulate its activity to maintain the quiescence or enhance the self-renewal and differentiation of cells [13].

Previous studies have proposed that interleukin 8 (IL-8) might be involved in regulating the immune response of DPSCs and promoting the recruitment process of neighboring DPSCs to the site of injury [14]. Lipopolysaccharide (LPS), which mediates IL-8 expression in DPSCs, is associated with toll-like receptor 4 (TLR4), myeloid differentiation marker 88 (MyD88), MAPK and NF-κB signaling pathways. Overall results of the study indicate that NF-κB and MAPK signaling pathways are closely involved in dental pulp inflammation and maintaining of the homeostasis of DPSCs niche. Another study reveals that DPSCs may play important roles in the immune responses during the pulp infection via activating NF-κB signaling pathway [15].

4.2.4 Eph-Ephrin Signaling Pathway

Eph-Ephrin signaling pathway includes Ephs and their corresponding ephrin ligands (ephrins), which are both membrane-bound proteins. Thus, the activation of Eph-Ephrin intracellular pathways can only happen through the direct cell-cell interactions. Eph-Ephrin signaling regulates diverse biological processes during the embryonic development (*e.g.*, formation of tissue boundaries, cell migration, angiogenesis, and stem cell differentiation).

Tooth development occurs through interactions between cranial neural crest-derived mesenchymal and epithelial cells [16], while DPSCs reside mainly within the perivascular niche of dental pulp tissue. The Eph family of receptor tyrosine kinases and their ligands, ephrin molecules, are reported to play an imperative role in the migration of neural crest cells throughout the development and maintenance of stem cell niche (Fig. 4.2) [17].

DPSCs exposed to EphB2-Fc and EphB1-Fc can exhibit a significantly rounder and smaller morphology than hDPSCs treated with human IgG-Fc controls. EphB2-Fc treated DPSCs present the same migration speed as human IgG-Fc treated DPSCs while the migration ability of EphB1-Fc treated DPSCs decreases significantly. The ERK inhibitor U0126 can partially reverse the reduction of migration speed of EphB1-Fc treated DPSCs [18]. These data suggest that EphB-EphrinB

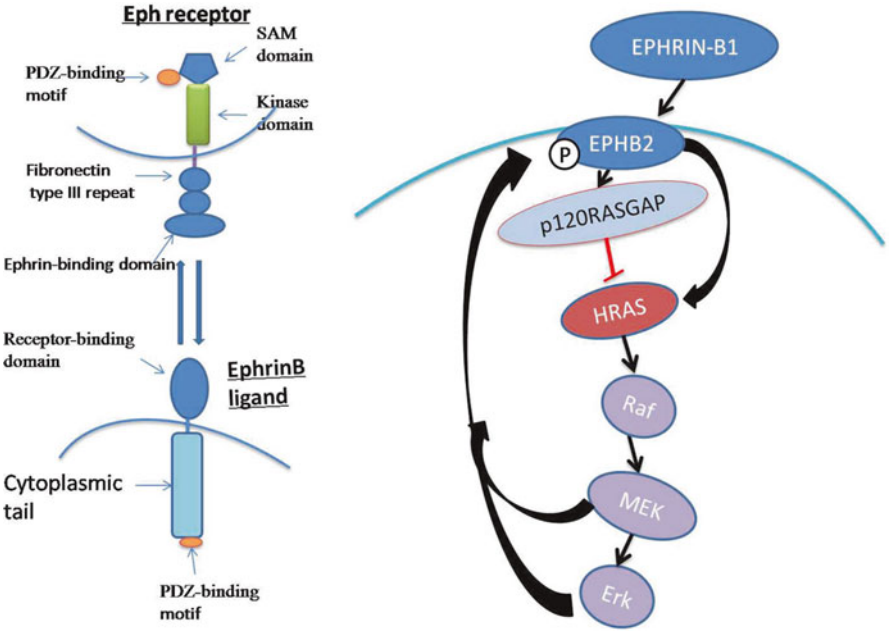

Fig. 4.2 Eph-Ephrin signaling pathway. Eph family is composed of receptor tyrosine kinases and their ligands. The activation of EphB2 bound by EphrinB1 stimulates the HRAS-Erk signaling pathway, and the increase in MEK and/or Erk activity, reversely enables the enhanced expression of EPHB2 under the stimulation of EphrinB1. The phosphorylation of EphB2 can also activate the expression of p120RASGAP, leading to the inhibition of HRAS

pathway also mediates human DPSCs attachment, spreading and migration in DPSC niche, in which ERK-MAPK signaling are involved in the regulation of these processes.

4.3 Signaling Pathways in DSC Migration

Stem cells can adhere, grow and migrate to the damaged areas during inflammatory response or wound healing. There are some critical signaling pathways that have great impacts on the migration of DSCs.

4.3.1 MAPK Signaling Pathway

ERK, JNK and p38 MAPKs can be activated by a variety of environmental factors. Activated ERK, JNK and p38 can translocate to the nucleus where they phosphorylate the transcription factors (c-Jun, c-Fos, Elk-1 and Sp1), and then regulate the downstream gene expression (Fig. 4.3).

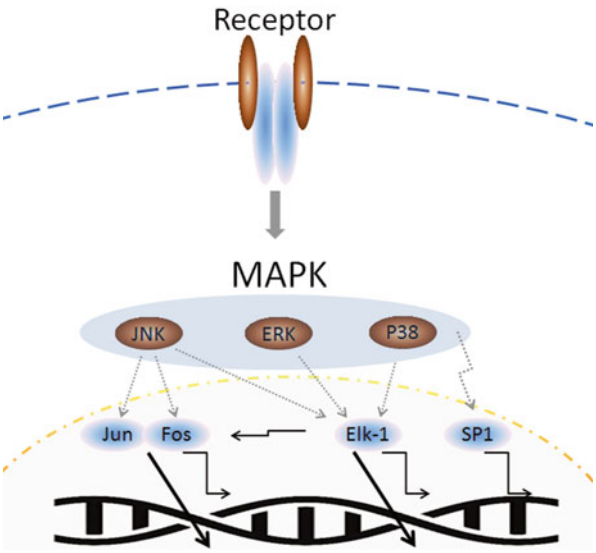

Fig. 4.3 MAPK signaling pathway. ERK, JNK and p38 MAPKs are members of MAPK family which can be activated by a variety of environmental factors. Activated ERK, JNK and p38 can translocate to the nucleus where they phosphorylate transcription factors (c-Jun, c-Fos, Elk-1 and Sp1) and then regulate the downstream gene expression

Previous studies have proved that MAPKs, including JNK, p38 and ERK are involved in the cell migration process [19]. In particular, JNK regulates cell migration by phosphorylating paxillin, doublecortin X-linked (DCX), Jun and microtubule-associated proteins. The antimicrobial peptide LL37 promotes the migration of DPSCs via activating the epidermal growth factor receptor (EGFR)-JNK signaling pathway, which may lead to the increased regeneration of pulp-dentin complexes [20]. MAPK regulates the directional migration of cells via the phosphorylation of MAPK-activated protein kinase 2/3 (MAPKAP 2/3). Some studies have demonstrated that HEMA inhibits the migration of DPSCs at non-toxic doses, and such inhibition is associated with the p38 signaling pathway [12]. Moreover, LPS can promote the adhesion and migration of DPSCs by upregulating the expression of adhesion molecules and chemotactic factors, while inhibition of MAPK and NF-κB significantly antagonizes LPS-induced adhesion and migration [21].

The inhibition of JNK or p38 pathways in DPSCs significantly decreases cell proliferation, alkaline phosphatase (ALP) activity, and mineralization ability stimulated by hepatocyte growth factor (HGF). JNK and p38 inhibitors can affect F-actin remodeling induced by HGF and thus, contribute to HGF-induced migration [22]. The activation of fibroblast growth factor receptor (FGFR), ERK, JNK, and AKT can modulate the upregulation of focal adhesion molecules, stress fiber assembly, and enhance cell migration induced by iRoot BP Plus [23]. ERK determines cell movement by the phosphorylation of myosin light chain kinase (MLCK), calpain or focal adhesion kinase (FAK). Overall, the different kinds of kinases in MAPK family all appear to be capable of regulating cell migration via particular mechanisms.

4.3.2 PI3K/AKT Signaling Pathway

The PI3K/AKT pathway mainly contains the phosphatidylinositol 3-kinase (PI3K) and AKT. The pathway begins with an activation of a membrane receptor and phosphorylation of PI3K. Then, PI3K phosphorylates the lipids and generates the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP3) which subsequently activates the AKT. Activated AKT mediates the downstream responses by phosphorylating a series of intracellular proteins.

PI3K/AKT signaling pathway is critical in cell growth and migration. Firstly identified in osteoblast-like cell line MC3T3-E1, periostin is a kind of matrix-cellular protein expressed in multiple tissues like bone, periodontal ligament, skin and various cancers [24, 25]. Periostin interacts with integrin molecule on the cell surface, mediating cell adhesion and migration of various kinds of cells. In periodontal tissues, periostin is localized between the cytoplasmic processes of cementoblasts/periodontal fibroblasts and the adjacent collagen fibers [26]. Periostin can induce cell proliferation and cell migration of periodontal ligament (PDL) cells by activating the PI3K/AKT signaling pathway (higher phosphorylation of AKT and the ribosomal protein S6) [27].

Cartilage oligomeric matrix protein (COMP) is another kind of matrix-cellular protein that is firstly detected in cartilage tissues [28]. Recent researches have revealed that COMP is essential in different diseases such as bone tissue disorders and atherosclerosis [29, 30]. Combination of recombinant angiopoietin 1 (Ang1), an important factor for endothelial survival and proliferation [31], COMP (COMP-Ang1) can promote the migration of periodontal ligament stem cells (PDLSCs) through the activation of PI3K/AKT signaling pathway [32]. Moreover, fibroblast growth factor-2 can stimulate the directed migration of PDLSCs via PI3K/AKT pathway [33].

4.3.3 Eph-Ephrin Signaling Pathway

The EphB-EphrinB family consists of contact-dependent molecules that mediate various inhibitory or repulsive cellular responses depending on the model of signaling. The EphB-EphrinB family has shown to be expressed in tooth development and plays critical roles in dental cell migration and tooth repair. EphrinB1 expression is downregulated in the dental pulp tissue of injured tooth, and it can inhibit the migration of DPSCs in vitro [18, 34]. EphB-EphrinB molecules are paramount for the perivascular DPSCs migration toward the dentin surfaces and differentiation into functional odontoblasts after the injury of dentin matrix [34]. The interaction between EphB and its corresponding ephrin ligand (EphrinB) is required for the attachment, spreading and migration of human DPSCs in its niche. However, the major role of EphB-EphrinB pathway in these processes is the induction of inhibitory responses [18].

Other signaling pathway may also interact with the Eph signaling pathway. EphrinB1-induced DPSCs migration inhibition can be partially reversed by the suppression of MAPK signaling pathway [18]. The actions of PI3K signaling pathway on endothelial cell migration and proliferation can be mediated by EphB receptors [35].

4.4 Signaling Pathways in DSC Proliferation

DSCs have a long-term proliferation capacity and generate many identical copies of themselves, which are regulated by several related signaling pathways.

4.4.1 MAPK Signaling Pathway

MAPK pathway consists of many signaling molecules that can be activated by diverse extracellular stimuli. Activation of MAPK pathway can give rise to a variety of physiological effects, including cell apoptosis and proliferation. Many studies have revealed that chemical and mechanical stress can affect the proliferation of DSCs via activation of MAPK signaling pathway. For instance, cisplatin, a commonly used chemotherapeutic agent, can induce a greater genotoxic stress response in DPSCs in comparison to human dermal fibroblasts (HDFs). Cisplatin in higher concentrations can initiate the activation of all three main MAPK families (*e.g.*, ERK, JNK and p38) and cell apoptosis in DPSCs [36]. Dental tissues are subjected to various kinds of mechanical stress such as compression fluid-sheer stress and uniaxial vertical and horizontal stretch during jaw movement and occlusal forces. Mechanical stress can activate several intracellular signals such as MAPK through mechanoreceptors [37, 38]. Mechanical stretch can enhance the proliferation while suppressing the osteogenic differentiation of DPSCs. The stretch significantly enhances the phosphorylation of AKT, ERK1/2, and p38 MAPK as well as upregulating the proliferation of DPSCs [39].

Epiregulin (EREG), a member of epidermal growth factor family, can enhance the proliferation ability of stem cells from apical papilla (SCAPs) by activating JNK MAPK pathway [40]. In addition, mechanical stress stimuli can augment the proliferation of SCAPs by activating ERK 1/2 and JNK pathway [41]. Some researchers have established PDL tissue model under compression, and found that the prolonged compression can inhibit the cell proliferation by the activation of MAPK pathway [42].

4.4.2 PI3K/AKT Signaling Pathway

PI3K pathway is one of the key pathways in the regulation of crucial cellular processes such as cell survival, growth, migration, apoptosis, transcription and translation. Stem cell factor (SCF), one of the prominent homing factors, can bind to c-Kit receptor (CD117) and recruit stem cells toward homing sites [43]. Both SCF and c-Kit are highly expressed in differentiation of DPSCs. SCF treatment in dental pulp progenitors may enhance the phosphorylation of ERK and/or AKT, and stimulate the cyclin D3 and CDK4 (cell cycle proteins) expression in DPSCs [44]. In addition, the increasing fluid shear stress (FSS) and periostin may regulate the proliferation of human PDLSCs via the PI3K/AKT/mTOR signaling axis [27, 45].

4.4.3 NF-κB Signaling Pathway

NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) is a protein complex that modifies the transcription of DNA in almost all animal cell types. Canonical NF-κB pathway is regulated by the inhibition of IκB kinase complex (IKK-a, IKK-b and IKK-c). The IKK complex phosphorylates/degrades the IκB, and releases NF-κB subunits, mainly p65 and p50. These phosphorylated subunits enter the cell nucleus and bind to DNA, which subsequently bring about a variety of biological processes including cell proliferation, cell apoptosis, and cell differentiation. Moreover, NF-κB signaling pathway is greatly involved in the process of DSC proliferation. DPSCs derived from injured pulps present a lower proliferative capacity than normal DPSCs, and this process is proposed to be related with NF-κB signaling pathway [46]. Moreover, donor sodium nitroprusside (SNP) can induce nitric oxide (NO) production, and downregulate the proliferation of hPDLSCs. Blockade of NF-κB signaling suppresses the SNP-induced growth inhibition, showing that the influence of NO on the proliferation of hPDLSCs is conducted by NF-κB signaling pathway [47].

4.4.4 Notch Signaling Pathway

Notch signaling governs the cell fate determination of adult and embryonic tissues. The Notch ligand, Delta1, is known to affect the proliferation and differentiation of various tissue specific stem cells. Studies have revealed that Notch receptors and Delta1 ligand are identified and expressed in DPSCs. The proliferation index (PI) and colonies of dental pulp cells are significantly upregulated in Delta1 transduced DPSCs than the control groups (wt- and vector transduced DPSCs). Therefore, it can be proposed that Notch-Delta1 signaling is essentially associated with the proliferation of DSCs [48].

4.4.5 Wnt/β-Catenin Signaling Pathway

The canonical Wnt pathway is a key component in the induction of epithelial-mesenchymal interactions, and actively participates in tooth morphogenesis and development. WNT10A, a member of Wnt family, can promote the proliferation ability and negatively regulate the odontoblastic differentiation of DPSCs [49]. Moreover, the canonical Wnt/β-catenin pathway can facilitate the proliferation of SCAPs [50]. In addition, bioactive scaffolds containing lithium ions can enhance the proliferation of PDLSCs via the activation of Wnt/β-catenin pathway [51]. Recent studies have revealed that stress-associated periodontal disturbance may be due to GC-induced changes in PDLSCs. Dexamethasone treatment can induce the expression of several genes including dickkopf-1 (DKK-1) in PDLSCs, and then inhibit Wnt-mediated activation of β-catenin signaling as well as their growth rate [52].

4.4.6 Other Signaling Pathways

TGF-β2 may influence the growth and differentiation of DPSCs through an autocrine way via the activation of ALK/Smad2/3-signal transduction pathways [53]. Small molecules (Pluripotin (SC1), 6-bromoindirubin-3-oxime and rapamycin) can decrease the DPSC proliferation, which may be mediated by mTOR signaling pathway [54]. ITGA5 down-regulation inhibits the proliferative capacity of hDPSCs, and promotes their odontogenic differentiation, suggesting that ITGA5 signaling pathway can negatively affect the odontogenic differentiation of hDPSCs and may help hDPSCs to remain in a proliferative and undifferentiated state [55].

4.5 Signaling Pathways in DSC Differentiation

DSCs are undifferentiated cells that have a special capacity to differentiate into specialized cell types. More and more studies have found that many kinds of signaling pathways are involved in the multiple differentiation abilities of DSCs.

4.5.1 TGF-β Signaling Pathway

TGF-β1 is a multifunctional cytokine and intimately involved in the metabolism of several tissues, including dental pulps. TGF-β signaling pathway is crucial for epithelial-mesenchymal interactions, especially in those vital interactions during tooth morphogenesis. Interaction of TGF-β with the membrane TGF-β receptor I

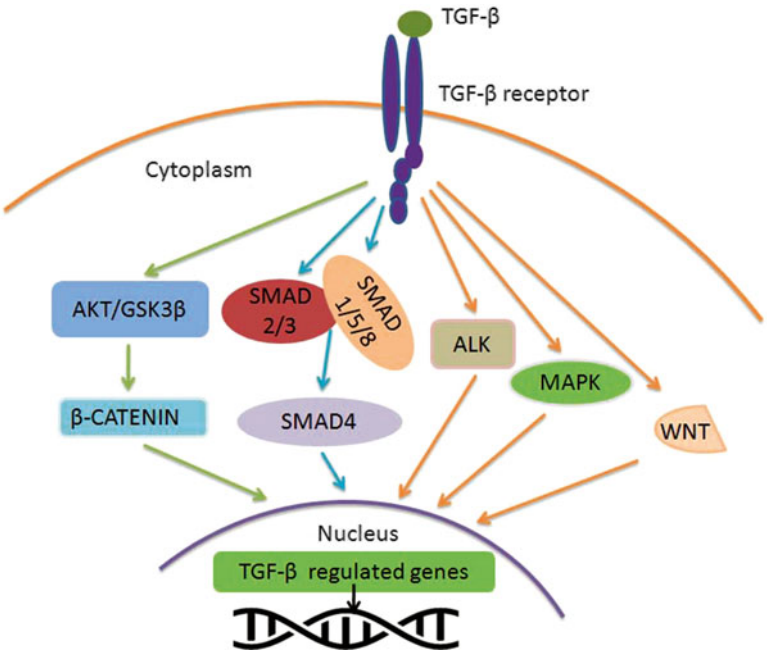

Fig. 4.4 TGF-β signaling pathway. TGF-β signaling pathway is crucial for the tooth morphogenesis and repair. Interaction of TGF-β with the membrane TGF-β receptor I and II mediates the activities of multiple kinds of signaling pathways (*e.g.*, MAPK, Wnt, Smad and PI3K/AKT pathways), and then regulates the expression levels of TGF-β related genes via the cascade interactions among these pathways

and II mediates the activities of multiple kinds of signaling pathways, and then regulates the expression levels of TGF-β related genes via the cascade interactions among these pathways (Fig. 4.4). TGF-β1, TGF-β2, and a small quantity of TGF-β3 mRNAs are expressed in DPSCs [53]. TGF-β receptors I/II are both expressed in odontoblasts and pulp cells, and they response to subtle variations in expression levels and participate in the tissues' response to injury [56].

Exogenous TGF-β2 can upregulate the expression levels of nestin and dentin sialophosphoprotein (DSPP) in DPSCs, indicating that TGF-β signaling controls the odontoblast differentiation and dentin formation ability during tooth morphogenesis [57]. TGF-β2 possibly mediates the differentiation of DPSCs at specific stages, which cooperates with other factors through multiple signaling pathways, especially with the ALK/Smad2/3-signal transduction pathways [53].

TGF-β signaling also participates in nerve growth factor (NGF) regulation during pulp tissue repair. TGF-β can up-regulate NGF in hDPSCs via p38 and JNK MAPK pathways [58]. Some studies suggest that TGF-β1 can inhibit the proliferation of SCAPs and their mineralization by decreasing the osteogenic/dentinogenic gene expressions [59]. In detail, TGF-β1 promotes the cell growth, collagen content

and ALP activity at lower concentrations (0.1–1 ng/mL) but down-regulates the activity at higher concentrations (>5 ng/mL) by regulating ERK1/2 and Smad2 signaling pathways [60].

4.5.2 BMPs Signaling Pathway

BMP2 and BMP4 genes are proved to be expressed and play essential roles during embryonic tooth development. The BMP2 gene is also expressed in post-natal odontoblasts and ameloblasts during tooth differentiation period from birth to approximately 3 weeks after birth. Dentin-derived BMP2 possesses the ability to drive the differentiation of DSCs from exfoliated deciduous teeth (SHEDs) into mature dentin-forming odontoblasts [61]. BMP2 transcripts are restricted in dental papillae, and remarkably upregulated during odontoblastic differentiation [62].

Both SHEDs and adult DPSCs express BMP receptors, including BMPR-IA, BMPR-IB and BMPR-II. The blockade of BMP2 signaling inhibits the expression of odontoblastic differentiation markers in SHEDs. Similarly, BMP2 drives the differentiation of SHEDs into odontoblasts [63]. Some studies suggest that lentiviral-mediated BMP2 gene transfection can accelerate the odontogenic differentiation capability of human SCAPs in vitro [64]. Meanwhile, hPDLSCs/rAd-BMP2 effectively promote the osteogenesis both in vitro and in vivo. Thus, hPDLSCs/rAd-BMP2 can be applied in a novel therapeutic approach for the regeneration of deteriorated bony defects [65].

BMP7 can induce the gene expression of several markers of cementoblasts and cementocytes, such as protein tyrosine phosphatase-like member/cementum attachment protein (PTPLA/CAP) and cementum protein 1 (CEMP1) [66]. BMP7 treatment upregulates the transcription of Sp7/Osterix and PTPLA/CAP by binding to specific short motifs termed as GC-rich Smad-binding elements (GC-SBEs) located in the human PTPLA/CAP and CEMP1 promoter. The gene expression levels of RUNX2 and ALP are increased afterward while the expression of odontogenic markers such as DSPP, bone sialoprotein (BSP) and dentin matrix acidic phosphoprotein 1 (DMP1) are not affected [67].

4.5.3 NF-κB Signaling Pathway

NF-κB signaling not only participates in regulating immune responses and inflammation, but also plays critical roles in differentiation of MSCs including DSCs (Fig. 4.5). NF-κB signaling pathway is activated in case of estrogen deficiency and subsequently decreases the osteo/odontogenic differentiation of DPSCs. Inhibitors of the NF-κB effectively rescues the down-regulated differentiation potential of DPSCs [68]. DPSCs derived from the injured pulps exhibit the robust osteogenic potential and weak odontogenic capacity as compared with healthy DPSCs. The

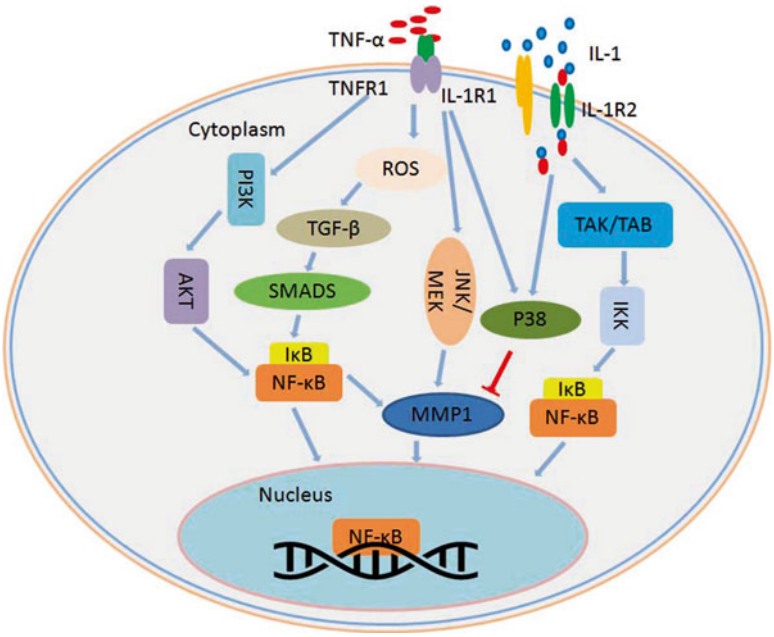

Fig. 4.5 NF-κB signaling pathway. Small molecules like TNF-α and IL-1 can induce PI3K/AKT, TAK/TAB/IKK and JNK/MEK/MMP1 signaling pathways in the cytoplasm. All these signalings will converge to the IκB/NF-κB at the cytoplasmic level and then translocate to the nucleus to regulate the expression of NF-κB target genes

inhibitors of NF-κB pathway can reverse the process that the osteogenic potential of DPSCs is significantly reduced while the odontogenic differentiation is enhanced. Therefore, the NF-κB signaling pathway can be proposed to be associated with the osteo/odentogenic differentiation of DPSCs [46].

LPS can activate TLR4, and regulate NF-κB pathway of human PDLSCs, leading to decrease in osteogenic potential. Thus, blockage of TLR4 or NF-κB pathway might provide a new approach for periodontitis treatment [69]. NF-κB pathway-activated SCAPs present higher proliferation/migration capacity and increased odonto/osteogenic ability than control cells. Likewise, NF-κB pathway-suppressed SCAPs inversely display lower proliferation/migration ability as well as decreased odonto/osteogenic ability than control group [70].

4.5.4 MAPK Signaling Pathway

p38-MAPK is involved in the inflammatory response of PDLSCs during the chronic periodontitis in which p38 is strongly induced in PDLSCs derived from the infected periodontal tissues. The p38 inhibition markedly suppresses the osteogenic differentiation of PDLSCs in a chronic inflammatory microenvironment [71].

Natural mineralized scaffolds (*e.g.*, demineralized dentin matrix-DDM, ceramic bovine bone-CBB) can induce DPSCs to exhibit higher levels of ALP activity and mRNA expression of osteo/odontogenetic markers than other scaffolds via the activation of MAPK signaling pathway. However, the inhibitors of ERK1/2 and p38 can down-regulate the odontogenic differentiation ability of DPSCs cultured on DDM and CBB [72]. BMP9 can promote the bone formation of PDLSCs. p38 and ERK1/2 MAPKs are involved in BMP9-induced osteogenic differentiation of PDLSCs. The inhibitors of ERK1/2 and p38 increase BMP9-induced osteogenic differentiation of PDLSCs [73]. Moreover, IGF-1can induce the phosphorylation of ERK and JNK in PDLSCs, and promote the osteogenic differentiation of PDLSCs, suggesting the involvement of MAPK signaling pathway in the IGF-1-based differentiation of PDLSCs [74]. Stretch can increase the proliferation rate of DPSCs via the activation of ERK pathway, and inhibit the osteogenic differentiation in which PI3K/AKT and ERK pathways are partly involved [39]. Mechanical stress can enhance the odonto/osteogenic differentiation of SCAPs via the activation of ERK 1/2 and JNK MAPK signaling pathways [75]. In addition, hypoxia can affect the osteogenic potential, mineralization and paracrine release of therapeutic factors from PDLSCs, and the process is closely related to ERK and p38 MAPK signaling pathways [76].

MAPK signaling pathway also plays an important role in the revascularization of dental-pulp complex. LPS stimulates the expression level of vascular endothelial growth factor (VEGF) in DPSCs and human dental pulp fibroblasts via ERK1/2 MAPK signaling pathway [77].

4.5.5 mTOR Signaling Pathway

mTOR kinase is the catalytic subunit of at least two distinct signaling complexes: target of rapamycin complex 1 and 2 (TORC 1 and 2) [78]. TORC 1 is a popular regulator of protein translation [79], and is essential for cell growth, cell proliferation, and cell cycle. On the other hand, TORC 2 is involved in the cytoskeleton reorganization and cell survival [78]. In the concept of DSC differentiation, the mTOR signaling pathway is activated in the process of osteogenic differentiation of hDPSCs [80].

Both TORC1 and TORC2 play critical roles in the modulation of DPSCs while TORC1 is essential in SHEDs differentiation. Inhibition of the TORC1 complex proteins (mTOR or raptor) can effectively decrease the mineralized matrix deposition of SHEDs. Conversely, when the TORC2 complex proteins are downregulated, both mineralization and differentiation markers are increased in SHEDs. Furthermore, the increased mineralization of SHEDs is dependent on functioning TORC1 complex [81].

Pluripotin can affect the maintenance of hDPSCs properties, decreasing cell proliferation, increasing the expression of STRO-1, NANOG, OCT4, and SOX2, and diminishing cell differentiation through various signaling pathways including mTOR-signaling pathway [54].

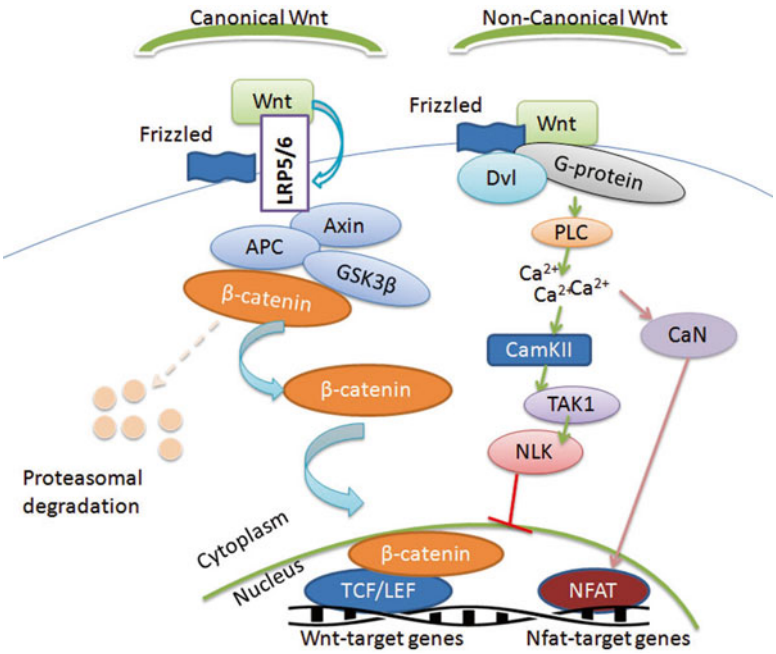

Fig. 4.6 Wnt/β-catenin signaling pathway. Wnt signaling pathway is divided into the canonical Wnt signaling and non-canonical Wnt signaling. The former plays a crucial role in tooth development. Wnt protein binds its receptor Frizzled and co-receptor LRP5/6, and stimulates the LRP5/6 phosphorylation. Phosphorylated LRP5/6 recruits Axin to the membrane and disrupts the Axin complex that containing APC and GSK3β. GSK3β phosphorylates β-catenin, subsequently, the phosphorylated β-catenin enters the nucleus, where it binds TCF/LEF and co-activators, and activates the downstream gene expression

4.5.6 Wnt/β-Catenin Signaling Pathway

Nineteen Wnt family proteins are divided into two main categories, canonical and non-canonical wnt signaling pathways, based on their role in cytosolic β-catenin stabilization [82]. Canonical Wnt signaling transduces their signals via regulation of β-catenin levels and is thought to be of much importance in the tooth development and self-renewal of stem cells (Fig. 4.6).

After transduction with canonical Wnt-1 by retrovirus-mediated infection, matrix-cellular protein osteopontin and type I collagen are upregulated while ALP activity and the mineralization of DPSCs are inhibited. Over-expression of β-catenin can effectively inhibit the differentiation and mineralization of DPSCs, indicating that DPSC differentiation is downregulated via the activation of Wnt/β-catenin signaling pathway [83]. Wnt3A effectively induces ALP activity in immortalized SCAPs (iSCAPs), and BMP9 also induces the expression of osteocalcin and osteopontin as well as matrix mineralization of iSCAPs. Moreover, BMP9 and Wnt3A

can act synergistically, and their ability to induce the osteo/odontogenic differentiation will be diminished by knockdown of β-catenin [84].

Zinc-bioglass (ZnBG) incorporated within calcium phosphate cements (CPC) can activate the odontogenic differentiation and promote the angiogenesis of DPSCs in vitro. ZnBG upregulates the integrins and their downstream signaling pathways including canonical and non-canonical Wnt signaling pathways [85].

After osteogenic genes in PDLSCs are increased by down-regulating anti-differentiation noncoding RNA (ANCR), the osteogenic differentiation of PDLSCs is improved. When the canonical WNT signaling pathway is suppressed, the osteogenic differentiation of PDLSC/ANCR-RNAi cells is inhibited too, indicating that Wnt/β-catenin signaling pathway may play a crucial role in the ANCR-mediated osteogenic differentiation of PDLSCs [86]. Nicotine and TNF-α can induce the osteogenic differentiation deficiency of PDLSCs by activating WNT signaling [87, 88], and down-regulation of β-catenin level can activate the non-canonical Wnt/Ca^{2+} pathway, leading to the promotion of osteogenic differentiation in PDLSCs [89]. The β-catenin also plays an important role in the osteo/odontogenic differentiation of SCAPs. Silencing of β-catenin in SCAPs can reduce BMP9/WNT3A-induced expression of osteocalcin/osteopontin and matrix mineralization in vitro and ectopic bone formation in vivo [90].

4.5.7 Other Signaling Pathways

Shh signaling pathway is related to cell differentiation and osteogenesis which is negatively modulated by BMP signaling. It can repress the osteo/dentinogenic differentiation of SCAPs [91]. Moreover, Notch signaling also participates in the odontoblastic differentiation of DSCs [2], which permits DPSCs differentiating into odontoblast-like cells in the appropriate inductive conditions. Notch signaling pathway is also important in maintaining the correct balance between proliferation and differentiation of DPSCs. Activation of Notch signaling by Delta1 ligand can enhance the proliferation and odontogenic ability of DPSCs due to the increasing of the proliferation index (PI), DSPP protein expression level and calcified nodules number in Delta1-DPSCs [48]. However, another study reports that the activation of Notch signaling by either Jagged1 or N1ICD can depress the differentiation of DPSCs into odontoblasts without interrupting cell proliferation [92]. In addition, Notch signaling pathway modulates the osteogenic differentiation of dental follicle stem cells (DFSCs) [93]. Therefore, we can conclude that distinct Notch ligand may induce different effects of Notch signaling on the differentiation of DSCs. The mechanism of these distinct effects remains puzzled and needs more explorations.

Trichostatin A (TSA) is an efficient histone deacetylase (HDAC) inhibitor with a wide spectrum of epigenetic activities known to mediate many kinds of cellular behaviors, including MSC differentiation. It can significantly upregulate the expression levels of phospho-Smad2/3, Smad4, and nuclear factor I-C, while specific

inhibitor of Smad3 suppresses TSA-based differentiation of hDPSCs, suggesting that Smad signaling pathway is also involved in the differentiation of DPSCs [94].

Basic FGF has been found to increase the neurosphere size and upregulate the expression of neurogenic markers of DPSCs. Inhibition of FGFR or Phospholipase Cγ (PLCγ) signaling can abolish the basic FGF-mediated neuronal differentiation of DPSCs [95].

4.6 Signaling Pathway Networks

Crosstalk between cellular processes and molecular signaling pathways is frequent in any biological system. Signaling pathways can affect each other synergistically in maintaining cell survival, apoptosis, proliferation, differentiation as well as other cellular processes of DSCs.

Some similar stem cell–related genes can be detected in DPSCs and PDLSCs during their odontogenic/osteogenic differentiation. The genes exhibit considerable overlap with minor difference between DPSCs and PDLSCs. Numerous regulatory genes in odonto/osteogenic differentiation interact or crosstalk through Notch, Wnt, TGF-β/BMP, and cadherin signaling pathways [96]. Extracellular phosphate (Pi) can regulate the BMP2 expression level by cAMP/protein kinase A and ERK1/2 MAPK signaling pathways in human DPSCs [97]. TGF-β1 can downregulate the differentiation ability of human DPSCs through ALK5/Smad2/3 signaling pathways [98]. Furthermore, p38 MAPK pathway is involved in regulating ALP activity of hDPSCs and may interact with Smad pathway [99]. As the main element of many pulp capping materials, calcium ions can upregulate the odontoblastic differentiation and mineralization of DPSCs. Calcium ions activate the BMP2-mediated Smad1/5/8 and ERK1/2 pathways to control the odontoblastic differentiation of DPSCs in which Smad1/5/8 and ERK1/2 signaling converge at Runx2 in DPSCs [100].

5' adenosine monophosphate-activated protein kinase (AMPK), AKT and mTOR signaling pathways act synergistically in the differentiation process of human DPSCs. AMPK, the upstream mechanism of AKT and mTOR signaling pathways, can regulate the osteogenic differentiation of human DPSCs via both early mTOR suppression-modulated autophagy and late activation of AKT/mTOR signaling axis. AKT inhibition restrains mTOR activation without influencing AMPK phosphorylation [101]. PIN1, a peptidyl-prolyl cis/trans isomerase, acts as an important modulator of odontogenic and adipogenic differentiation of hDPSCs. BMP, Wnt/β--catenin, MAPK and NF-κB pathway are involved in PIN1-mediated differentiation of hDPSCs [102]. Moreover, WNT5α mRNA and protein expressions rapidly increased in response to LPS treatment in a time- and dose-dependent manner. LPS-induced WNT5α expression is mediated through the TLR4/MyD88/PI3K/AKT signaling pathways, which subsequently activate NF-κB signaling pathway in hDPSCs [103].

4.7 Conclusions and Prospects

Overall data has shown that the maintenance, proliferation, migration, and differentiation of DSCs are regulated by a variety of signaling pathways. Although larger amount of recent studies have led to rapid expansion of knowledge of signaling molecular mechanisms in stem cell biology, this field is still full of confusions and challenges. The complex signaling networks participating in the homeostasis, migration, proliferation and differentiation of DSCs are still in its infancy. DSCs are thought to be an appropriate and sufficient candidate for tooth regeneration. However, their clinical applications remain much immature and difficult. Therefore, more laboratorial and clinical researches need to be conducted to explore the further pathway mechanisms, which are important to clarify the signaling-related behaviors of dental stem cells. Moreover, the upstream and downstream transcription factors as well as their detailed functions in these signaling pathways should be extensively investigated, so that we can easily and efficiently smooth the potential difficulties in stem cell-based tooth regeneration.

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References

1. Lovschall H, Mitsiadis TA, Poulsen K, Jensen KH, Kjeldsen AL (2007) Coexpression of Notch3 and Rgs5 in the pericyte-vascular smooth muscle cell axis in response to pulp injury. Int J Dev Biol 51(8):715–721. doi:10.1387/ijdb.072393hl
2. Cai X, Gong P, Huang Y, Lin Y (2011) Notch signalling pathway in tooth development and adult dental cells. Cell Prolif 44(6):495–507. doi:10.1111/j.1365-2184.2011.00780.x
3. Mitsiadis TA, Lardelli M, Lendahl U, Thesleff I (1995) Expression of Notch 1, 2 and 3 is regulated by epithelial-mesenchymal interactions and retinoic acid in the developing mouse tooth and associated with determination of ameloblast cell fate. J Cell Biol 130(2):407–418
4. Mitsiadis TA, Henrique D, Thesleff I, Lendahl U (1997) Mouse Serrate-1 (Jagged-1): expression in the developing tooth is regulated by epithelial-mesenchymal interactions and fibroblast growth factor-4. Development 124(8):1473–1483
5. Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I (1999) Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. J Cell Biol 147(1):105–120
6. Harada H, Toyono T, Toyoshima K, Yamasaki M, Itoh N, Kato S, Sekine K, Ohuchi H (2002) FGF10 maintains stem cell compartment in developing mouse incisors. Development 129(6):1533–1541
7. Biehs B, Hu JK, Strauli NB, Sangiorgi E, Jung H, Heber RP, Ho S, Goodwin AF, Dasen JS, Capecchi MR, Klein OD (2013) BMI1 represses Ink4a/Arf and Hox genes to regulate stem cells in the rodent incisor. Nat Cell Biol 15(7):846–852. doi:10.1038/ncb2766
8. Zhao H, Feng J, Seidel K, Shi S, Klein O, Sharpe P, Chai Y (2014) Secretion of shh by a neurovascular bundle niche supports mesenchymal stem cell homeostasis in the adult mouse incisor. Cell Stem Cell 14(2):160–173. doi:10.1016/j.stem.2013.12.013

9. Zhang J, Huang FF, Wu DS, Li WJ, Zhan HE, Peng MY, Fang P, Cao PF, Zhang MM, Zeng H, Chen FP (2015) SUMOylation of insulin-like growth factor 1 receptor, promotes proliferation in acute myeloid leukemia. Cancer Lett 357(1):297–306. doi:10.1016/j.canlet.2014.11.052
10. Jiang Q, Du J, Yin X, Shan Z, Ma Y, Ma P, Du J, Fan Z (2013) Shh signaling, negatively regulated by BMP signaling, inhibits the osteo/dentinogenic differentiation potentials of mesenchymal stem cells from apical papilla. Mol Cell Biochem 383(1–2):85–93
11. Johnson GL, Lapadat R (2002) Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. Science 298(5600):1911–1912. doi:10.1126/science.1072682
12. Williams DW, Wu H, Oh JE, Fakhar C, Kang MK, Shin KH, Park NH, Kim RH (2013) 2-Hydroxyethyl methacrylate inhibits migration of dental pulp stem cells. J Endod 39(9):1156–1160. doi:10.1016/j.joen.2013.06.004
13. Vandomme J, Touil Y, Ostyn P, Olejnik C, Flamenco P, El Machhour R, Segard P, Masselot B, Bailliez Y, Formstecher P, Polakowska R (2014) Insulin-like growth factor 1 receptor and p38 mitogen-activated protein kinase signals inversely regulate signal transducer and activator of transcription 3 activity to control human dental pulp stem cell quiescence, propagation, and differentiation. Stem Cells Dev 23(8):839–851. doi:10.1089/scd.2013.0400
14. Kim SM, Kim DS, Jeong CH, Kim DH, Kim JH, Jeon HB, Kwon SJ, Jeun SS, Yang YS, Oh W, Chang JW (2011) CXC chemokine receptor 1 enhances the ability of human umbilical cord blood-derived mesenchymal stem cells to migrate toward gliomas. Biochem Biophys Res Commun 407(4):741–746. doi:10.1016/j.bbrc.2011.03.093
15. Chang J, Zhang C, Tani-Ishii N, Shi S, Wang CY (2005) NF-kappaB activation in human dental pulp stem cells by TNF and LPS. J Dent Res 84(11):994–998
16. Chai Y, Jiang X, Ito Y, Bringas P Jr, Han J, Rowitch DH, Soriano P, McMahon AP, Sucov HM (2000) Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development 127(8):1671–1679
17. Smith A, Robinson V, Patel K, Wilkinson DG (1997) The EphA4 and EphB1 receptor tyrosine kinases and ephrin-B2 ligand regulate targeted migration of branchial neural crest cells. Curr Biol 7(8):561–570
18. Stokowski A, Shi S, Sun T, Bartold PM, Koblar SA, Gronthos S (2007) EphB/ephrin-B interaction mediates adult stem cell attachment, spreading, and migration: implications for dental tissue repair. Stem Cells 25(1):156–164. doi:10.1634/stemcells.2006-0373
19. Huang C, Jacobson K, Schaller MD (2004) MAP kinases and cell migration. J Cell Sci 117(Pt 20):4619–4628. doi:10.1242/jcs.01481
20. Kajiya M, Shiba H, Komatsuzawa H, Ouhara K, Fujita T, Takeda K, Uchida Y, Mizuno N, Kawaguchi H, Kurihara H (2010) The antimicrobial peptide LL37 induces the migration of human pulp cells: a possible adjunct for regenerative endodontics. J Endod 36(6):1009–1013. doi:10.1016/j.joen.2010.02.028
21. Li D, Fu L, Zhang Y, Yu Q, Ma F, Wang Z, Luo Z, Zhou Z, Cooper PR, He W (2014) The effects of LPS on adhesion and migration of human dental pulp stem cells in vitro. J Dent 42(10):1327–1334. doi:10.1016/j.jdent.2014.07.007
22. Su Y, Xie W, Wang C, Peng L, Zhou X, Ye L (2012) JNK/P38 mitogen-activated protein kinase used for hepatocyte growth factor-induced proliferation, differentiation, and migration in human dental papilla cells. J Endod 38(9):1207–1213. doi:10.1016/j.joen.2012.06.011
23. Zhang J, Zhu LX, Cheng X, Lin Y, Yan P, Peng B (2015) Promotion of dental pulp cell migration and pulp repair by a bioceramic putty involving FGFR-mediated signaling pathways. J Dent Res. doi:10.1177/0022034515572020
24. Takeshita S, Kikuno R, Tezuka K, Amann E (1993) Osteoblast-specific factor 2: cloning of a putative bone adhesion protein with homology with the insect protein fasciclin I. Biochem J 294(Pt 1):271–278
25. Zhou HM, Wang J, Elliott C, Wen W, Hamilton DW, Conway SJ (2010) Spatiotemporal expression of periostin during skin development and incisional wound healing: lessons for

human fibrotic scar formation. J Cell Commun Signal 4(2):99–107. doi:10.1007/s12079-010-0090-2
26. Polizzotti BD, Arab S, Kuhn B (2012) Intrapericardial delivery of gelfoam enables the targeted delivery of Periostin peptide after myocardial infarction by inducing fibrin clot formation. PloS one 7(5), e36788. doi:10.1371/journal.pone.0036788
27. Padial-Molina M, Volk SL, Rios HF (2014) Periostin increases migration and proliferation of human periodontal ligament fibroblasts challenged by tumor necrosis factor -alpha and Porphyromonas gingivalis lipopolysaccharides. J Periodontal Res 49(3):405–414. doi:10.1111/jre.12120
28. Hedbom E, Antonsson P, Hjerpe A, Aeschlimann D, Paulsson M, Rosa-Pimentel E, Sommarin Y, Wendel M, Oldberg A, Heinegard D (1992) Cartilage matrix proteins. An acidic oligomeric protein (COMP) detected only in cartilage. J Biol Chem 267(9):6132–6136
29. Wang L, Zheng J, Du Y, Huang Y, Li J, Liu B, Liu CJ, Zhu Y, Gao Y, Xu Q, Kong W, Wang X (2010) Cartilage oligomeric matrix protein maintains the contractile phenotype of vascular smooth muscle cells by interacting with alpha(7)beta(1) integrin. Circ Res 106(3):514–525. doi:10.1161/CIRCRESAHA.109.202762
30. Das BR, Roy A, Khan FR (2015) Cartilage oligomeric matrix protein in monitoring and prognostication of osteoarthritis and its utility in drug development. Perspect Clin Res 6(1):4–9. doi:10.4103/2229-3485.148792
31. Abdel-Malak NA, Srikant CB, Kristof AS, Magder SA, Di Battista JA, Hussain SN (2008) Angiopoietin-1 promotes endothelial cell proliferation and migration through AP-1-dependent autocrine production of interleukin-8. Blood 111(8):4145–4154. doi:10.1182/blood-2007-08-110338
32. Kook SH, Lim SS, Cho ES, Lee YH, Han SK, Lee KY, Kwon J, Hwang JW, Bae CH, Seo YK, Lee JC (2014) COMP-angiopoietin 1 increases proliferation, differentiation, and migration of stem-like cells through Tie-2-mediated activation of p38 MAPK and PI3K/Akt signal transduction pathways. Biochem Biophys Res Commun 455(3–4):371–377. doi:10.1016/j.bbrc.2014.11.025; S0006-291X(14)02035-X [pii]
33. Shimabukuro Y, Terashima H, Takedachi M, Maeda K, Nakamura T, Sawada K, Kobashi M, Awata T, Oohara H, Kawahara T, Iwayama T, Hashikawa T, Yanagita M, Yamada S, Murakami S (2011) Fibroblast growth factor-2 stimulates directed migration of periodontal ligament cells via PI3K/AKT signaling and CD44/hyaluronan interaction. J Cell Physiol 226(3):809–821. doi:10.1002/jcp.22406
34. Arthur A, Koblar S, Shi S, Gronthos S (2009) Eph/ephrinB mediate dental pulp stem cell mobilization and function. J Dent Res 88(9):829–834. doi:10.1177/0022034509342363
35. Steinle JJ, Meininger CJ, Chowdhury U, Wu G, Granger HJ (2003) Role of ephrin B2 in human retinal endothelial cell proliferation and migration. Cell Signal 15(11):1011–1017
36. Seifrtova M, Havelek R, Cmielova J, Jiroutova A, Soukup T, Bruckova L, Mokry J, English D, Rezacova M (2012) The response of human ectomesenchymal dental pulp stem cells to cisplatin treatment. Int Endod J 45(5):401–412. doi:10.1111/j.1365-2591.2011.01990.x
37. Cook B, Hardy RW, McConnaughey WB, Zuker CS (2008) Preserving cell shape under environmental stress. Nature 452(7185):361–364. doi:10.1038/nature06603
38. Kanzaki M, Nagasawa M, Kojima I, Sato C, Naruse K, Sokabe M, Iida H (1999) Molecular identification of a eukaryotic, stretch-activated nonselective cation channel. Science 285(5429):882–886
39. Hata M, Naruse K, Ozawa S, Kobayashi Y, Nakamura N, Kojima N, Omi M, Katanosaka Y, Nishikawa T, Tanaka Y, Matsubara T (2013) Mechanical stretch increases the proliferation while inhibiting the osteogenic differentiation in dental pulp stem cells. Tissue Eng Part A 19(5–6):625–633. doi:10.1089/ten.tea.2012.0099
40. Cao Y, Xia DS, Qi SR, Du J, Ma P, Wang SL, Fan ZP (2013) Epiregulin can promote proliferation of stem cells from the dental apical papilla via MEK/Erk and JNK signalling pathways. Cell Prolif 46(4):447–456. doi:10.1111/cpr.12039

41. Mu C, Lv T, Wang Z, Ma S, Ma J, Liu J, Yu J, Mu J (2014) Mechanical stress stimulates the osteo/odontoblastic differentiation of human stem cells from apical papilla via erk 1/2 and JNK MAPK pathways. Biomed Res Int 2014:494378. doi:10.1155/2014/494378
42. Li Y, Li M, Tan L, Huang S, Zhao L, Tang T, Liu J, Zhao Z (2013) Analysis of time-course gene expression profiles of a periodontal ligament tissue model under compression. Arch Oral Biol 58(5):511–522. doi:10.1016/j.archoralbio.2012.10.006
43. Yu XY, Geng YJ, Li XH, Lin QX, Shan ZX, Lin SG, Song YH, Li Y (2009) The effects of mesenchymal stem cells on c-kit up-regulation and cell-cycle re-entry of neonatal cardiomyocytes are mediated by activation of insulin-like growth factor 1 receptor. Mol Cell Biochem 332(1–2):25–32. doi:10.1007/s11010-009-0170-x
44. Pan S, Dangaria S, Gopinathan G, Yan X, Lu X, Kolokythas A, Niu Y, Luan X (2013) SCF promotes dental pulp progenitor migration, neovascularization, and collagen remodeling - potential applications as a homing factor in dental pulp regeneration. Stem Cell Rev 9(5):655–667. doi:10.1007/s12015-013-9442-7
45. Qi L, Zhang Y (2014) The microRNA 132 regulates fluid shear stress-induced differentiation in periodontal ligament cells through mTOR signaling pathway. Cell Physiol Biochem 33(2):433–445. doi:10.1159/000358624
46. Wang Y, Yan M, Wang Z, Wu J, Zheng Y, Yu J (2013) Dental pulp stem cells from traumatically exposed pulps exhibited an enhanced osteogenic potential and weakened odontogenic capacity. Arch Oral Biol 58(11):1709–1717. doi:10.1016/j.archoralbio.2013.09.001
47. Lee SK, Choi HI, Yang YS, Jeong GS, Hwang JH, Lee SI, Kang KH, Cho JH, Chae JM, Kim YC, Kim EC (2009) Nitric oxide modulates osteoblastic differentiation with heme oxygenase-1 via the mitogen activated protein kinase and nuclear factor-kappaB pathways in human periodontal ligament cells. Biol Pharm Bull 32(8):1328–1334
48. He F, Yang Z, Tan Y, Yu N, Wang X, Yao N, Zhao J (2009) Effects of Notch ligand Delta1 on the proliferation and differentiation of human dental pulp stem cells in vitro. Arch Oral Biol 54(3):216–222. doi:10.1016/j.archoralbio.2008.10.003
49. Zhang Z, Guo Q, Tian H, Lv P, Zhou C, Gao X (2014) Effects of WNT10A on proliferation and differentiation of human dental pulp cells. J Endod 40(10):1593–1599. doi:10.1016/j.joen.2014.07.009
50. Wang J, Liu B, Gu S, Liang J (2012) Effects of Wnt/beta-catenin signalling on proliferation and differentiation of apical papilla stem cells. Cell Prolif 45(2):121–131. doi:10.1111/j.1365-2184.2012.00806.x
51. Han P, Wu C, Chang J, Xiao Y (2012) The cementogenic differentiation of periodontal ligament cells via the activation of Wnt/beta-catenin signalling pathway by Li+ ions released from bioactive scaffolds. Biomaterials 33(27):6370–6379. doi:10.1016/j.biomaterials.2012.05.061
52. Choi SS, Park EK, Kwack MH, Sung YK (2015) Effects of dexamethasone, a synthetic glucocorticoid, on human periodontal ligament stem cells. Naunyn Schmiedebergs Arch Pharmacol. doi:10.1007/s00210-015-1151-0
53. Tai TF, Chan CP, Lin CC, Chen LI, Jeng JH, Chang MC (2008) Transforming growth factor beta2 regulates growth and differentiation of pulp cells via ALK5/Smad2/3. J Endod 34(4):427–432. doi:10.1016/j.joen.2008.02.007
54. Al-Habib M, Yu Z, Huang GT (2013) Small molecules affect human dental pulp stem cell properties via multiple signaling pathways. Stem Cells Dev 22(17):2402–2413. doi:10.1089/scd.2012.0426
55. Cui L, Xu S, Ma D, Gao J, Liu Y, Yue J, Wu B (2014) The role of integrin-alpha5 in the proliferation and odontogenic differentiation of human dental pulp stem cells. J Endod 40(2):235–240. doi:10.1016/j.joen.2013.08.011
56. Sloan AJ, Couble ML, Bleicher F, Magloire H, Smith AJ, Farges JC (2001) Expression of TGF-beta receptors I and II in the human dental pulp by in situ hybridization. Adv Dent Res 15:63–67

57. Oka S, Oka K, Xu X, Sasaki T, Bringas P Jr, Chai Y (2007) Cell autonomous requirement for TGF-beta signaling during odontoblast differentiation and dentin matrix formation. Mech Dev 124(6):409–415. doi:10.1016/j.mod.2007.02.003
58. Yongchaitrakul T, Pavasant P (2007) Transforming growth factor-beta1 up-regulates the expression of nerve growth factor through mitogen-activated protein kinase signaling pathways in dental pulp cells. Eur J Oral Sci 115(1):57–63. doi:10.1111/j.1600-0722.2007.00420.x
59. He W, Zhang J, Niu Z, Yu Q, Wang Z, Zhang R, Su L, Fu L, Smith AJ, Cooper PR (2014) Regulatory Interplay between NFIC and TGF-beta 1 in Apical Papilla-derived Stem Cells. J Dent Res 93(5):496–501. doi:10.1177/0022034514525200
60. Chang HH, Chang MC, Wu IH, Huang GF, Huang WL, Wang YL, Lee SY, Yeh CY, Guo MK, Chan CP, Hsien HC, Jeng JH (2015) Role of ALK5/Smad2/3 and MEK1/ERK signaling in transforming growth factor beta 1-modulated growth, collagen turnover, and differentiation of stem cells from apical papilla of human tooth. J Endod 41(8):1272–1280. doi:10.1016/j.joen.2015.03.022
61. Vainio S, Karavanova I, Jowett A, Thesleff I (1993) Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development. Cell 75(1):45–58
62. Lyons KM, Pelton RW, Hogan BL (1990) Organogenesis and pattern formation in the mouse: RNA distribution patterns suggest a role for bone morphogenetic protein-2A (BMP-2A). Development 109(4):833–844
63. Casagrande L, Demarco FF, Zhang Z, Araujo FB, Shi S, Nor JE (2010) Dentin-derived BMP-2 and odontoblast differentiation. J Dent Res 89(6):603–608. doi:10.1177/0022034510364487
64. Zhang W, Zhang X, Ling J, Liu W, Zhang X, Ma J, Zheng J (2014) Proliferation and odontogenic differentiation of BMP2 genetransfected stem cells from human tooth apical papilla: an in vitro study. Int J Mol Med 34(4):1004–1012. doi:10.3892/ijmm.2014.1862
65. Jung IH, Lee SH, Jun CM, Oh N, Yun JH (2014) Characterization of the enhanced bone regenerative capacity of human periodontal ligament stem cells engineered to express the gene encoding bone morphogenetic protein 2. Tissue Eng Part A 20(15–16):2189–2199. doi:10.1089/ten.TEA.2013.0648
66. Torii D, Konishi K, Watanabe N, Goto S, Tsutsui T (2015) Cementogenic potential of multipotential mesenchymal stem cells purified from the human periodontal ligament. Odontology 103(1):27–35. doi:10.1007/s10266-013-0145-y
67. Torii D, Tsutsui TW, Watanabe N, Konishi K (2014) Bone morphogenetic protein 7 induces cementogenic differentiation of human periodontal ligament-derived mesenchymal stem cells. Odontology. doi:10.1007/s10266-014-0182-1
68. Wang Y, Yan M, Yu Y, Wu J, Yu J, Fan Z (2013) Estrogen deficiency inhibits the odonto/osteogenic differentiation of dental pulp stem cells via activation of the NF-kappaB pathway. Cell Tissue Res 352(3):551–559. doi:10.1007/s00441-013-1604-z
69. Li CH, Li B, Dong ZW, Gao L, He XN, Liao L, Hu CH, Wang QT, Jin Y (2014) Lipopolysaccharide differentially affects the osteogenic differentiation of periodontal ligament stem cells and bone marrow mesenchymal stem cells through Toll-like receptor 4 mediated nuclear factor kappa B pathway. Stem Cell Res Ther 5. doi:Artn 67; Doi10.1186/Scrt456
70. Li J, Yan M, Wang Z, Jing S, Li Y, Liu G, Yu J, Fan Z (2014) Effects of canonical NF-kappaB signaling pathway on the proliferation and odonto/osteogenic differentiation of human stem cells from apical papilla. Biomed Res Int 2014:319651. doi:10.1155/2014/319651
71. Nie J, Zhang B, Gu B, Liu N (2015) Effects of p38 mitogen-activated protein kinase on osteogenic differentiation of human periodontal ligament stem cells in inflammatory microenvironment. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 37(1):1–7
72. Zhang H, Liu S, Zhou Y, Tan J, Che H, Ning F, Zhang X, Xun W, Huo N, Tang L, Deng Z, Jin Y (2012) Natural mineralized scaffolds promote the dentinogenic potential of dental pulp stem cells via the mitogen-activated protein kinase signaling pathway. Tissue Eng Part A 18(7–8):677–691. doi:10.1089/ten.TEA.2011.0269

73. Ye G, Li C, Xiang X, Chen C, Zhang R, Yang X, Yu X, Wang J, Wang L, Shi Q, Weng Y (2014) Bone morphogenetic protein-9 induces PDLSCs osteogenic differentiation through the ERK and p38 signal pathways. Int J Med Sci 11(10):1065–1072
74. Yu Y, Mu J, Fan Z, Lei G, Yan M, Wang S, Tang C, Wang Z, Yu J, Zhang G (2012) Insulin-like growth factor 1 enhances the proliferation and osteogenic differentiation of human periodontal ligament stem cells via ERK and JNK MAPK pathways. Histochem Cell Biol 137(4):513–525. doi:10.1007/s00418-011-0908-x
75. Mu C, Lv T, Wang Z, Ma S, Ma J, Liu J, Yu J, Mu J (2014) Mechanical stress stimulates the osteo/odontoblastic differentiation of human stem cells from apical papilla via erk 1/2 and JNK MAPK pathways. Biomed Res Int 2014:494378
76. Wu Y, Yang Y, Yang P, Gu Y, Zhao Z, Tan L, Zhao L, Tang T, Li Y (2013) The osteogenic differentiation of PDLSCs is mediated through MEK/ERK and p38 MAPK signalling under hypoxia. Arch Oral Biol 58(10):1357–1368
77. Botero TM, Son JS, Vodopyanov D, Hasegawa M, Shelburne CE, Nor JE (2010) MAPK signaling is required for LPs-induced VEGF in pulp stem cells. J Dent Res 89(3):264–269. doi:10.1177/0022034509357556
78. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM (2005) Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science 307(5712):1098–1101. doi:10.1126/science.1106148
79. Foster KG, Fingar DC (2010) Mammalian target of rapamycin (mTOR): conducting the cellular signaling symphony. J Biol Chem 285(19):14071–14077. doi:10.1074/jbc.R109.094003
80. Feng X, Huang D, Lu X, Feng G, Xing J, Lu J, Xu K, Xia W, Meng Y, Tao T, Li L, Gu Z (2014) Insulin-like growth factor 1 can promote proliferation and osteogenic differentiation of human dental pulp stem cells via mTOR pathway. Dev Growth Differ 56(9):615–624. doi:10.1111/dgd.12179
81. Kim JK, Baker J, Nor JE, Hill EE (2011) mTor plays an important role in odontoblast differentiation. J Endod 37(8):1081–1085. doi:10.1016/j.joen.2011.03.034
82. He B, Barg RN, You L, Xu Z, Reguart N, Mikami I, Batra S, Rosell R, Jablons DM (2005) Wnt signaling in stem cells and non-small-cell lung cancer. Clin Lung Cancer 7(1):54–60. doi:10.3816/CLC.2005.n.022
83. Scheller EL, Chang J, Wang CY (2008) Wnt/beta-catenin inhibits dental pulp stem cell differentiation. J Dent Res 87(2):126–130
84. Zhang H, Wang J, Deng F, Huang E, Yan Z, Wang Z, Deng Y, Zhang Q, Zhang Z, Ye J, Qiao M, Li R, Wang J, Wei Q, Zhou G, Luu HH, Haydon RC, He TC, Deng F (2015) Canonical Wnt signaling acts synergistically on BMP9-induced osteo/odontoblastic differentiation of stem cells of dental apical papilla (SCAPs). Biomaterials 39:145–154. doi:10.1016/j.biomaterials.2014.11.007
85. Zhang J, Park YD, Bae WJ, El-Fiqi A, Shin SH, Lee EJ, Kim HW, Kim EC (2014) Effects of bioactive cements incorporating zinc-bioglass nanoparticles on odontogenic and angiogenic potential of human dental pulp cells. J Biomater Appl. doi:10.1177/0885328214550896
86. Jia Q, Jiang W, Ni L (2015) Down-regulated non-coding RNA (lncRNA-ANCR) promotes osteogenic differentiation of periodontal ligament stem cells. Arch Oral Biol 60(2):234–241
87. Zhou Z, Li B, Dong Z, Liu F, Zhang Y, Yu Y, Shang F, Wu L, Wang X, Jin Y (2013) Nicotine deteriorates the osteogenic differentiation of periodontal ligament stem cells through alpha7 nicotinic acetylcholine receptor regulating Wnt pathway. PLoS One 8(12), e83102
88. Liu W, Konermann A, Guo T, Jager A, Zhang L, Jin Y (2014) Canonical Wnt signaling differently modulates osteogenic differentiation of mesenchymal stem cells derived from bone marrow and from periodontal ligament under inflammatory conditions. Biochim Biophys Acta 1840(3):1125–1134
89. Liu N, Shi S, Deng M, Tang L, Zhang G, Liu N, Ding B, Liu W, Liu Y, Shi H, Liu L, Jin Y (2011) High levels of beta-catenin signaling reduce osteogenic differentiation of stem cells in inflammatory microenvironments through inhibition of the noncanonical Wnt pathway. J Bone Miner Res 26(9):2082–2095

90. Zhang H, Wang J, Deng F, Huang E, Yan Z, Wang Z, Deng Y, Zhang Q, Zhang Z, Ye J, Qiao M, Li R, Wang J, Wei Q, Zhou G, Luu HH, Haydon RC, He T-C, Deng F (2015) Canonical Wnt signaling acts synergistically on BMP9-induced osteo/odontoblastic differentiation of stem cells of dental apical papilla (SCAPs). Biomaterials 39:145–154
91. Jiang Q, Du J, Yin X, Shan Z, Ma Y, Ma P, Fan Z (2013) Shh signaling, negatively regulated by BMP signaling, inhibits the osteo/dentinogenic differentiation potentials of mesenchymal stem cells from apical papilla. Mol Cell Biochem 383(1–2):85–93. doi:10.1007/s11010-013-1757-9
92. Zhang C, Chang J, Sonoyama W, Shi S, Wang CY (2008) Inhibition of human dental pulp stem cell differentiation by Notch signaling. J Dent Res 87(3):250–255. doi:87/3/250 [pii]
93. Viale-Bouroncle S, Gosau M, Morsczeck C (2014) NOTCH1 signaling regulates the BMP2/DLX-3 directed osteogenic differentiation of dental follicle cells. Biochem Biophys Res Commun 443(2):500–504. doi:10.1016/j.bbrc.2013.11.120
94. Jin H, Park JY, Choi H, Choung PH (2013) HDAC inhibitor trichostatin A promotes proliferation and odontoblast differentiation of human dental pulp stem cells. Tissue Eng Part A 19(5–6):613–624. doi:10.1089/ten.TEA.2012.0163
95. Osathanon T, Nowwarote N, Pavasant P (2011) Basic fibroblast growth factor inhibits mineralization but induces neuronal differentiation by human dental pulp stem cells through a FGFR and PLCgamma signaling pathway. J Cell Biochem 112(7):1807–1816. doi:10.1002/jcb.23097
96. Liu L, Ling J, Wei X, Wu L, Xiao Y (2009) Stem cell regulatory gene expression in human adult dental pulp and periodontal ligament cells undergoing odontogenic/osteogenic differentiation. J Endod 35(10):1368–1376. doi:10.1016/j.joen.2009.07.005
97. Tada H, Nemoto E, Foster BL, Somerman MJ, Shimauchi H (2011) Phosphate increases bone morphogenetic protein-2 expression through cAMP-dependent protein kinase and ERK1/2 pathways in human dental pulp cells. Bone 48(6):1409–1416. doi:10.1016/j.bone.2011.03.675
98. Lin PS, Chang MC, Chan CP, Lee SY, Lee JJ, Tsai YL, Tseng HC, Tai TF, Lin HJ, Jeng JH (2011) Transforming growth factor beta1 down-regulates Runx-2 and alkaline phosphatase activity of human dental pulp cells via ALK5/Smad2/3 signaling. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 111(3):394–400. doi:10.1016/j.tripleo.2010.09.079
99. Wang FM, Hu T, Zhou X (2006) p38 mitogen-activated protein kinase and alkaline phosphatase in human dental pulp cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 102(1):114–118. doi:10.1016/j.tripleo.2005.08.007
100. Li S, Hu J, Zhang G, Qi W, Zhang P, Li P, Zeng Y, Zhao W, Tan Y (2015) Extracellular Ca2+ promotes odontoblastic differentiation of dental pulp stem cells via BMP2-mediated Smad1/5/8 and Erk1/2 pathways. J Cell Physiol 230(9):2164–2173. doi:10.1002/jcp.24945
101. Pantovic A, Krstic A, Janjetovic K, Kocic J, Harhaji-Trajkovic L, Bugarski D, Trajkovic V (2013) Coordinated time-dependent modulation of AMPK/Akt/mTOR signaling and autophagy controls osteogenic differentiation of human mesenchymal stem cells. Bone 52(1):524–531. doi:10.1016/j.bone.2012.10.024
102. Lee YM, Shin SY, Jue SS, Kwon IK, Cho EH, Cho ES, Park SH, Kim EC (2014) The role of PIN1 on odontogenic and adipogenic differentiation in human dental pulp stem cells. Stem Cells Dev 23(6):618–630. doi:10.1089/scd.2013.0339
103. He WX, Wang ZH, Zhou ZY, Zhang YQ, Zhu QL, Wei KW, Lin Y, Cooper PR, Smith AJ, Yu Q (2014) Lipopolysaccharide enhances Wnt5a expression through toll-like receptor 4, myeloid differentiating factor 88, phosphatidylinositol 3-OH kinase/AKT and nuclear factor kappa B pathways in human dental pulp stem cells. J Endod 40(1):69–75

Chapter 5
Genetically Engineered Dental Stem Cells for Regenerative Medicine

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5.1 Introduction

Mesenchymal stem cells (MSCs) of adult organism have multilineage differentiation potential, secrete trophic growth factors that influence the microenvironment, and promote angiogenesis and tissue regeneration, as well as reduce inflammation. MSCs naturally express cell surface markers (Cluster of Differentiation - CD markers) of CD44, CD73, CD90, CD105, but not markers of hematopoietic stem cell CD45, CD34 and CD14. Of particular interest are immunosuppressive properties of MSCs, moreover, they usually do not form teratomas [1]. All these properties make MSCs promising materials to be used in regenerative medicine and tissue engineering. MSCs can be isolated from various sources such as bone marrow, adipose tissue, cord blood, liver, lung, dental tissues etc.

Actively explored MSCs from dental and related tissues are dental pulp stem cells (DPSCs), dental follicle precursor cells (DFSCs), periodontal ligament stem cells (PDLSCs), stem cells from human exfoliated deciduous teeth (SHEDs), stem cells from the apical papilla (SCAPs) and human tooth germ stem cells (hTGSCs) [2, 3].

Stem cells derived from human dental pulp [4] and postnatal dental follicle of wisdom tooth [5] have properties of MSCs, in which they retain the capacity of self-renewal and in vitro differentiation toward different cell types, including osteoblasts, cementoblasts, chondroblasts, adipocytes, muscle and nerve cells [6, 7]. In addition, DPSCs secrete neurotrophic factors (Nerve growth factor - NGF, Brain-derived neurotrophic factor - BDNF, Glial cell line-derived neurotrophic factor - GDNF) and promote the survival of sensory neurons in vitro and motor neurons in vivo [8].

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Therapeutic application of MSCs can be enhanced by genetic modification using various approaches of delivering recombinant genetic material. Introduction of recombinant genes into the target cell allows controlling of proliferation, migration, differentiation, cell-cell and cell-matrix interaction, secretion of soluble signaling molecules and apoptosis.

This chapter will describe current knowledge and recent developments in genetically engineered dental stem cells (DSCs) and their application in regenerative medicine.

5.2 Genetic Modification of MSCs and Their Biomedical Applications

One of the limitations of MSC application in regenerative medicine is the low survival of transplanted cells. Genetic modification is a key tool for improving the therapeutic potential and viability of MSCs. Overexpression of various factors increases viability of MSCs under hypoxic conditions, protects from apoptosis, increases the proliferation, migration and differentiation capacity, and improves metabolic characteristics and angiogenic properties of the modified cells. Secreted products (proteins, factors) by modified MSCs may have paracrine and endocrine actions, which help to exert the therapeutic effect.

To increase survival of MSCs in vivo, a variety of pro-angiogenic and anti-apoptotic genes (*AKT1*, *HO-1*, *BCL2* etc.) are used for genetic modification of these cells [1]. For example, it has been shown that autologous transplantation of MSCs overexpressing AKT1 improves cardiac function in pigs with myocardial infarction [9]. It has also been found that overexpression of chemokine (C-X-C motif) receptor 4 (CXCR4) or C-C chemokine receptor type-1 (CCR-1) stimulates migration of MSCs [10, 11]. Currently, research is focused on the use of MSCs for bone regeneration and stimulation of vascularization. For genetic modification of the cells, scientists use genes encoding bone morphogenetic proteins (BMPs), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1) and transforming growth factor-β (TGF-β) [12]. Kumar et al. studied the effect of MSCs transduced with recombinant adenovirus encoding BMP2 and VEGF on the process of bone formation in a mouse model of segmental bone defect. The research has found that modified MSCs have greater therapeutic effect, stimulating the bone formation and vascularization process [13].

Modern methods of cell and molecular biology allow manipulation of the genotype of stem cells and engineer their functional properties. Therefore, the development of effective gene delivery systems is one of the major challenges of regenerative medicine. Gene delivery technology made significant progress in recent years. However, there are multiple problems associated with low efficiency, high cost and complexity of vector delivery systems. Issues include toxicity, immunogenicity, carcinogenicity of the vectors and transient transgene expression [14]. Gene delivery systems can be divided into two groups: viral and non-viral.

5.2.1 Viral Nucleic Acid Transfer Techniques

The life cycle of a virus involves several stages. Infection begins with recognition of target cell by the virus, the viral surface proteins recognize specific receptors on the cell plasma membrane resulting in the attachmenent of the virus to cell surface. The viral particle or the genetic material of the virus penetrates the cell where replication (viral gene expression, replication of viral genome and synthesis of viral proteins) and formation of new virions occurs. Virions are released from cells by lysis or budding, and begin a new process of infection of neighboring cells or circulate in the bloodstream until they meet receptive target cell [15].

In the context of viral delivery of nucleic acids, the viral transduction can be defined as non-replicative or unproductive infection delivering heterologous (*i.e.* not viral) genetic material into the target cell. To achieve this, the viral genome has to be subjected to major modifications in order to eliminate the genes required for replication and pathogenicity. Thus, the virus becomes a simple carrier of genetic information [16].

Viral systems are based on the use of recombinant viruses (retroviruses, lentiviruses, adenoviruses, adeno-associated viruses - AAV, etc.), obtained through genetic engineering and optimized for recombinant gene transfer. The main advantages of viral systems include high efficiency of genetic modification and long-term transgene expression. However, clinical application of viral vectors are often limited by their immunogenicity and oncogenicity [16].

Due to the low pathogenicity (the ability to infect non-dividing and dividing cell) and broad tropism (high-level expression of the viral proteins during viral replication and transport of viral genome into the nucleus), adenoviruses are considered potential candidates for regenerative medicine. However, high immunogenicity of adenovirus proteins and antiviral cellular immune responses reduce the duration of transgene expression and thus limit the use of adenoviral vectors in clinics [17]. To solve this problem, adenovirus vectors are made from rare serotypes that cause mild infections. Assembling of replication-defective vectors and viruses that lack genes required for productive replication of viral particles occurs in special packaging cell lines [18].

Low immunogenicity, site-specific integration, as well as the ability to infect a broad range of dividing and non-dividing cells in vitro and in vivo make AAV a promising vector system for gene delivery. To replicate, they need a helper virus co-infection: adenovirus or herpes simplex virus type 1 (HSV1). High infectivity and the ability to transduce both dividing and non-dividing cells make herpesvirus vectors good candidates for gene transfer. Vectors based on HSV1 have high infectivity in nerve cells due to natural tropism of the virus; in sensory nerve endings, it takes latent state. However, the drawbacks of a HSV1-based vector system are represented by immunogenicity of viral proteins and short duration of transgene expression [19]. Another problem is the presence of latently infected cells that can be transduced with HSV1-based vectors, which may lead to the recombination between wild type and vector genomes [20].

Genetic modification via retroviral vectors leads to integration into the host genome of the cells that can further lead to insertional mutagenesis and activation of oncogenes [21]. Vectors based on retroviruses provide long-term transgene expression for more than 6 months. A limitation of using retroviral vectors is their inability to infect non-dividing cells [22].

The most widely used retroviral vectors are lentiviruses, a subgroup of retroviruses that can efficiently deliver a transgene into non-dividing cells, ensuring long-term expression. In particular, lentiviral vectors derived from the human immunodeficiency virus (HIV) are able to target different cell types known to be difficult to transduce (neurons, hematopoietic progenitor cells, lymphoid cells, macrophages, etc.) [23].

Currently, the research is underway to develop pseudotype retroviral vectors in which the viral envelope glycoproteins are replaced with glycoproteins of the other viruses such as vesicular stomatitis virus (VSV) and rabies virus strain pasteur vaccins/PV. Pseudotyping of recombinant retroviruses allows transduction of a wide range of primary and immortalized human and other mammalian cell lines [24]. Besides, another widely used vector is baculovirus, which has low toxicity and high efficiency in transgenic gene transfer. Another advantage of this type of vectors is that they do not get integrated into target cell genome [25]. For example, Lu et al. have used baculovirus encoding TGF-β3 to modify MSCs from adipose tissue (ADSCs) to demonstrate that these cells increase chondrogenesis and stimulate cartilage formation in vitro [26].

5.2.2 Non-Viral Methods for Nucleic Acids Transfer

Development of effective non-viral nucleic acid delivery approaches is complicated by various intra- and extra-cellular obstacles. After entering cell cytoplasm the DNA, particularly in uncomplexed form, undergoes degradation by various nucleases. The most difficult stage is penetration of DNA through the cell membrane, since both DNA and the cell surface have negative charges. However, the problem can be alleviated by physical and chemical methods that enable delivery of DNA into cells [27].

Physical methods, such as the gene gun, electroporation and sonoporation can create microfractures in the cell membrane allowing DNA penetration. Electroporation reversibly increases cell membrane permeability by means of high voltage impulses passing through the lipid bilayer and forming pores suitable for penetration of different macromolecules [28]. Yalvac and colleagues performed electroporation of human DFSCs and demonstrated higher percentage of transfected cells compared with that of chemical methods of transfection [29]. Non-viral transfection methods of DFSCs are crucial for developing gene and cell therapy technology since DFSCs could serve as a convenient alternative source of MSCs [30]. Nakashima et al. optimized conditions for DFSC electroporation and found that the transfer efficiency of therapeutic genes is best achieved in conditions using three rectangular pulses at a frequency of 1 Hz, pulse length of 999 microseconds and strength of the electric field of 1.05 kV/cm [31].

Sonoporation is a nucleic acid transfer method using ultrasonic waves that increase permeability of the cell membrane. It is worth noting the difference between sonoporation and electroporation; during electroporation DNA moves in the electric field, whereas during sonoporation DNA penetrates into the cells by means of passive diffusion [32].

In recent years, chemical transfection reagents have actively been used for the delivery of nucleic acids due to multiple advantages including safety, low toxicity, ability to carry large size genes and ease of preparation. Cationic lipids and polymers are promising chemical vectors used to form complexes with negatively charged DNA through electrostatic interactions. Formation of complexes can promote absorption by the cell, improve intracellular delivery of genes and protect the DNA from nuclease-driven degradation [27]. The advantages of using the peptides and proteins for gene delivery include simplicity of production and use of recombinant proteins, high purity and homogeneity, targeted transport of polyplexes (DNA/protein complexes) to certain cell types through specific ligand-receptor interactions, and the absence of restrictions on the size and type of the nucleic acid delivered to the cells [33].

Cationic polymers include natural polymers (*i.e.* chitosan), dendrimers (*i.e.* polyamidoamine), polypeptides (*i.e.* poly-L-lysine), polyarginine, polyornithine, histones [34], protamine, polyethylene imine, and others [33]. Gheisari et al. optimized transfection conditions of MSCs derived from rat bone marrow using different cationic polymers with cytotoxicity lower than 20 % [35].

Transfection efficiency of cationic lipids (liposomes) is defined by their structure. In general, cationic lipids include several components: hydrophilic head and hydrophobic domain that are connected via linker [36]. The properties of the linker affect the biodegradability and toxicity of the cationic lipids, as well as formation of lipoplexes and their transfection efficiency [37]. Functionality of lipoplexes is determined by a large number of factors, with the most important one being the charge ratio between cationic lipid and DNA (must be greater than 1) [38]. It is believed that lipoplexes penetrate cells by endocytosis. After internalization (the capture) by the cell, lipoplexes should be released from the endosome into the cytoplasm to avoid degradation. One of the main disadvantages of cationic lipids is their inability to deliver the DNA directly into the cell nucleus. In addition, the large size of lipoplexes prevents them from passing through the nuclear pore. According to the current literature, after transfection using cationic lipids (liposomes) MSCs retain the ability to proliferate and differentiate without the loss of transgene expression [39].

5.3 Genetic Modification for Cell Immortalization

One of the limitations of using adult stem cells in regenerative medicine and tissue engineering is their aging, a phenomenon that reduces the proliferative activity and biological properties of the cells. The main reason for reduced number of cell divisions and aging is the shortening of telomeres that stretches the DNA at the ends of

chromosomes, therefore protecting them from degradation. Sustaining the telomere length depends on the expression of telomerase reverse transcriptase (TERT) gene, encoding the catalytic subunit of telomerase protein complex responsible for addition of telomeric DNA during cell division. The activity of p21, p53, Retinoblastoma-associated protein (RB) and other proteins is also affected during the aging process [40].

The biological role of p53 protein is to ensure genome stability and genetic uniformity of the cells in the whole organism. p53 protein triggers the transcription of a group of genes and is activated in response to accumulation of DNA damage. The activation of p53 leads to the arrest of cell cycle and DNA replication; it also initiates apoptosis under strong stress conditions. p53 activity also depends on the presence of p16 protein regulated by RB protein [41].

To prevent cell aging and enhance proliferation activity of cell cultures, genetic transformation can be performed in a process known as immortalization. There are several methods for immortalization of mammalian cells in culture conditions. Most common methods are based on recombinant lenti-, retro- or adeno-viruses expressing Epstein-Barr virus (EBV), *e6/7* genes of human papilloma virus 16 (HPV-16), SV40 large T antigen, hTERT, short interfering RNA (siRNA) specific to mRNA of *p53* and *RB* genes, as well as mutant forms of ras and myc proteins [42]. The easiest and the most reliable way to induce the immortalization process is to use large T antigen of the SV40 virus. Recent studies have shown that SV40 large T antigen can increase telomerase activity [43]. The *hTERT* gene is also actively used for stem cell immortalization, leading to prolonged cell proliferation without disrupting unique properties. It has been shown that hTERT-induced immortalization of hTGSCs does not lead to chromosomal aberrations or damage to the DNA as in the cells immortalized with SV40 large T antigen, and does not affect the process of cell differentiation in vitro. Cytogenetic study of 20 hTGSC-hTERT cell lines displayed trisomy of chromosome 7 in only one cell line, while the rest of the immortalized cell lines demonstrated normal 46XY karyotype. On the other hand, all investigated hTGSC-SV40 cell lines showed abnormal chromosomal characteristics, namely the association of telomeres, deletions and duplications. The authors have shown that after hTERT immortalization hTGSCs retained their immunophenotypic characteristics and the ability to differentiate as standard MSCs. Moreover, following immortalization, a significant increase was observed in expression level of pluripotent transcription factors (Oct4 and Sox2), in contrast to reduced expression of factor Klf4 that plays a role in chromosome remodeling [44]. The conditioned medium of hTGSC-hTERT cells also demonstrated neuroprotective properties against SH-SY5Y human neuroblastoma cells by reducing oxidative stress-induced neurotoxicity or the effect of cytostatic anticancer drug doxorubicin [44]. It has been shown that ectopic expression of telomerase in hDPSCs prevents aging by downregulation of *p16* and *p53* gene activity, thereby maintaining proliferative ability of the cells and promoting expression of pluripotency transcription factors [45].

Wang and coworkers immortalized mouse SCAPs by using retroviral system expressing SV40 large T antigen flanked by Cre/LoxP sites. Immortalized SCAPs

(iSCAPs) were found to express MSC markers. Further experiments showed that the genetic modification (transduction) of iSCAPs by recombinant adenovirus expressing BMP9 initiated the process of cell differentiation toward osteo-, chondro- and adipo-genic directions in vitro and in vivo [46]. Yokoi et al. immortalized mouse DFSCs using mutant human papillomavirus (HPV)-16 virus *e6* gene lacking PDZ-binding domain motive. mDFSCs expressing mutant *e6* gene had prolonged proliferative activity (150 population doublings, PD) in contrast to that of native cells (10 PD). Immortalized mDFSCs cells expressed genes, important for defining tendon/ligament phenotype, including Scleraxis (Scx), growth and differentiation factor 5 (GDF5), EphA4, Six-1, periostin and collagen type I. In order to analyse the differentiation potential of immortalized mDFSCs they were transplanted into severe combined immunodeficiency mice. Four weeks after transplantation immortalized mDFSCs were capable of generating periodontal-like tissue expressing periostin, Scx, collagen type 12, collagen type I, most probably due to the presence of subpopulation of periodontal progenitor cells [47]. These results indicate that immortalized mDFSCs can act as periodontal progenitor cells and can be used to study the formation of periodontal tissue and also for development of novel regenerative therapies.

5.4 Genetic Modification for Modulation of Cell Phenotype and Osteo- and Odontogenic Differentiation

Currently, there are a large number of experimental studies showing the possibility of using osteogenic potential of MSCs for bone tissue regeneration [3]. BMPs are involved in the initiation and maintenance of odonto- and osteo-genesis. For example, BMP2 and BMP7 are multifunctional cytokines that belong to the TGF-β superfamily. These glycoproteins act as disulfide-linked homo- or hetero-dimers, and are potent regulators of the formation and regeneration of bone and cartilage tissue, they also promote cell proliferation during embryonic development and adult bone homeostasis [48]. Tasli et al. studied the effect of BMP2 and BMP7 on the induction of osteogenic and odontogenic cell differentiation of hTGSCs [49]. Authors carried out genetic modification of hTGSCs via electroporation using genetic constructs encoding cDNAs for BMP2 or BMP7 genes. They studied the mRNA levels of DSPP (Dentin sialophosphoprotein), OCN (Osteocalcin) and COL1A (Collagen, type I, alpha) genes, early markers of osteogenic and odontogenic differentiation. The study shows that overexpression of BMP2 and BMP7 in hTGSCs leads to an increase in alkaline phosphatase (ALP) activity and enhanced mRNA expression of DSPP, OCN and COL1A genes compared to native cells, therefore indicating induction (stimulation) of osteo- and odontogenic differentiation. Interesting to note that rise in ectopic expression of one of the studied BMP proteins increases endogenous expression of the other, even though BMP2 and BMP7 are independently involved in the induction of bone formation and odontogenesis [49]. Yang et al. transfected

DPSCs with BMP2 gene and showed that BMP2 overexpression significantly increased the expression level of genes involved in osteogenic and odontogenic differentiation, such as ALP, OCN, COL1A, BSP (Bone sialoprotein), DSPP and DMP1 (dentin matrix protein 1) [50–52]. For BMP2 gene delivery the authors used both viral [50, 51] and non-viral [52] approaches. Experiments in vitro demonstrated that transfected DPSCs differentiated into odontoblasts-like cells even when cultured in a non-osteogenic medium [51]. The study also showed that ectopic expression of transfected BMP2 gene in mice-implanted DPSCs resulted in formation of mineralized tissue [50].

Zhang et al. investigated the effects of lentivirus-mediated *BMP2* gene delivery on odontogenic differentiation of human SCAPs in vitro [53]. Modification of SCAPs with *BMP2* gene stimulated cell differentiation toward odontogenic lineage by upregulation of ALP, OCN, DSPP and DMP1 genes. Therefore, the BMP2 gene transfection of dental cells can be an effective strategy for developing new methods to be used in tissue engineering and regenerative medicine approaches [53]. Other promising inducers of osteogenesis and odontogenesis are BMP7 and Growth/differentiation factor 11 (GDF11), also known as the BMP11. To study the effect of BMP7 on the differentiation processes, Yang et al. cultured DPSCs on collagen-chitosan scaffolds (matrixes) impregnated with plasmid DNA encoding BMP7 cDNA. In vitro and in vivo investigations have shown that cells cultured using plasmid DNA-impregnated scaffolds were successfully transfected and expressed BMP7. In addition, modified cells displayed higher proliferation rates and odontogenic differentiation potential with respect to the cells cultured on scaffolds without plasmid DNA [54]. One of the potential clinical applications of matrixes implanted with genetically modified cells includes regeneration of dentin-pulp complex through a dental pulp capping procedure.

Nakashima and co-workers optimized sonoporation to deliver plasmid DNA encoding GDF11 cDNA into dog DPSCs in vivo. Modification of DPSCs with GDF11 led to induction of dentin sialoprotein expression, a marker of odontoblasts differentiation, and formation of large amounts of reparative dentine in the pulp isolated from a dog tooth [55]. The authors obtained similar results by modifying mice DPSCs in vitro and in vivo with GDF11 by electroporation [56]. These results demonstrate the feasibility of using BMPs in gene-cell applications for endodontic treatment of teeth. In addition, authors have developed a three-dimensional canine pulp cell culture modified with GDF11. Based on in vivo experiments, it was demonstrated that autologous transplantation of GDF11-transfected cell mass on the surface of the amputated pulp stimulated reparative dentin formation [31].

Transcription factor Runx2 (involved in BMP- and TGF-β1-signaling) plays an important role in skeletal development and differentiation of osteoblasts. Runx2 expression was detected in DFSCs during development of periodontal tissues. Structural and functional analysis shows the presence of five amino acid (aa) motif, VWRPY, at the C-terminus of Runx2 that is responsible for the suppression of transcriptional activation by Runx2 [57, 58]. Pan and colleagues have shown that high levels of Runx2 increased expression of osteoblast-/cementoblast-related genes and

enhanced osteogenic differentiation of DFSCs in vitro. In addition, authors investigated the transcriptional activity of mutant forms of Runx2 with deleted VWRPY motif and showed that it leads to higher expression levels of OPN, ColI and CP23 in DFSCs compared to the full length Runx2 [57].

Expression level of the transcription factor DLX3 (distal-less homeobox 3) has been shown to increase during osteogenic differentiation of DFSCs in vitro [59]. Viale-Bouroncle et al. studied regulation of DLX3 expression in DFSCs and found it to be crucial for viability and proliferative capacity of the cells; DLX3 gene knockdown resulted in an increase of apoptotic cells in culture. Using microarray technology, the authors showed that overexpression of DLX3 in DFSCs leads to upregulation of 73 genes (*e.g.*, IL8, CXCL10, CXCL11, MMP1, BMP2, NR4A2, HES1 and ATF3) and downregulation of 55 genes (*e.g.*, COL3A1, ELN, OMD and PLXNC1). DLX3 regulates osteogenic differentiation and mineralization of DFSCs through BMP2-dependent pathway and feedback control [60].

Other studies have demonstrated that during osteogenic differentiation of SCAPs, expression of EGR1 (early growth response gene 1) is induced. Overexpression of EGR1 results in upregulation of DLX3 and BMP2, whereas knockdown of EGR1 leads to downregulation of DLX3, BMP2 and ALP. Similarly, overexpression of EGR1 in SCAPs contributes to the process of mineralization after osteogenic differentiation [61].

It is known that the function of Runx2 in osteoblast differentiation is regulated by protein Twist1. A shift in equilibrium between transcription factors Runx2 and Twist1 occurs in the beginning of osteoblasts differentiation, allowing expression of genes involved in the process of mineralization. Li et al. show that lentivirus-mediated overexpression of Twist1 in DPSCs increases the expression levels of OCN, DMP1, OPN and DSPP genes that are characteristic of terminally differentiated odontoblasts and markers of late mineralization [62].

The canonical Wnt signaling pathway plays a critical role in the development of teeth and self-renewal of stem cells through β-catenin regulation. Scheller et al. studied the regulation of odontoblast-like differentiation by modulating canonical Wnt signaling in DPSCs and found that overexpression of β-catenin led to inhibition of differentiation and mineralization of DPSCs. DPSCs stably transduced by canonical Wnt-1 and β-catenin demonstrated increased expression of OPN and Col1, and decreased activity of ALP and mineralization. These results suggest that canonical Wnt signaling negatively regulates the odontoblast-like differentiation of DPSCs [63].

5.5 Genetic Modification for Enhanced Angiogenesis

Angiogenesis, the formation of new capillaries from pre-existing blood vessels, is a key process in tissue engineering, wound healing, and reproduction. The angiogenesis process is comprised of the following steps: degradation of the basal membrane and extracellular matrix, proliferation and migration of endothelial cells, their

interaction with pericytes, and tube formation and maturation into functional blood vessels [64]. Pericytes, specialized connective tissue cells, are part of the small blood vessel walls where they perform key functions in the maintenance of blood vessels and angiogenesis [65]. Pericytes dysfunction leads to pathological angiogenesis, which is found in a number of diseases such as diabetic microangiopathy and tissue fibrosis [66].

Molecular and cellular events that occur in the process of angiogenesis are regulated by numerous stimulatory and inhibitory signals, including growth factors and their receptors, enzymes, matrix metalloproteinases, cytokines, endogenous angiogenesis inhibitors, transcription factors, adhesion molecules and extracellular matrix components [64]. The MSC transplantation may stimulate angiogenesis in two ways: either through a paracrine effect (stimulation of the formation of blood vessels from host tissue by secreting angiogenic factors) or by differentiation into endothelial and pericyte-like cells actively involved in neovascularization [67].

hDPSCs secrete a variety of angiogenic factors such as VEGF, platelet derived growth factor (PDGF) and basic fibroblast growth factor (bFGF or FGF2), and can stimulate the formation of capillary-like structures by human umbilical vein endothelial cells (HUVECs) in vitro [68]. As hDPSCs were administered intramyocardially in rat models of myocardial infarction, improvement in cardiac function, reduction of infarct size, and improved neovascularization were noted [69]. In addition, Janebodin et al. showed that DPSCs derived from mouse pulp formed capillary-like structures in vitro as they were co-cultured with mouse endothelial cells and HUVEC cells [70]. To determine the molecular mechanism of DPSCs angiogenic activity authors used an exogenous inhibitor of angiogenesis sFlt (Soluble Fms-like Tyrosine Kinase) that regulates the VEGF function by competitively binding to VEGF-A [71]. The findings showed that sFlt inhibited signaling of VEGF-A through receptor VEGFR2 by blocking the binding of VEGF to the receptor.

Human VEGF is one of the most promising growth factors for clinical use due to its neuroprotective, neurotrophic and pro-angiogenic properties [64, 72, 73]. Besides its strong pro-angiogenic properties, VEGF supports neuronal survival, protects the nervous tissue from oxygen starvation during hypoxia, stimulates neuroblast proliferation and axonal growth, and also supports the survival of neurons in vitro and in vivo [74]. Different cell types, including stem cells, have the ability to secrete high level of endogenous VEGF that may have an advantage as cellular material to develop methods of treatment of neurodegenerative and ischemic diseases in humans. In this context, studies have found that hTGSCs possess high endogenous VEGF gene expression and actively secrete VEGF protein into the culture medium. VEGF concentration in the culture medium reached 367 pg/ml, 2247 pg/ml and 2675 pg/ml after 24 hours, 3 and 5 days of incubation periods, respectively [75]. Modification of hTGSCs with plasmid pBud-VEGF-FGF2 using Turbofect transfection reagent resulted in increased expression of VEGF in culture medium (2442 pg/ml) 24 hours after transfection (unpublished data).

5.6 Conclusion

MSCs, due to their multilineage differentiation capacity, are being actively investigated as promising cell material for tissue regeneration therapies. MSCs isolated from different tissues of the tooth can be used as alternatives to bone marrow- and adipose tissue-derived MSCs. In vivo studies have shown that MSCs from tooth tissues have high regenerative potential and can be used as treatment options for various diseases such as pulpitis, periapical, coronary artery and neurodegenerative diseases. To increase the therapeutic potential of MSCs, scientists actively use different methods of genetic engineering. The most effective ones are the viral delivery methods of recombinant genetic material, however their clinical use is significantly limited by potential immunogenicity and oncogenicity of viral. Currently, studies are underway to develop effective and safe delivery systems for transgene transfer using chemical methods, such as cationic lipids and polymers. Judging from the experimental data described in this chapter, it can be concluded that genetic modification can significantly increase the therapeutic potential of dental MSCs that can serve as basis for the development of new methods of gene-cell therapy for regenerative medicine.

Another important limitation of clinical use of MSCs is their heterogeneous cells population meaning the lack of standardized and certified methods of their selection from a variety of tissues. There is a growing interest in the directed genetic modification of MSCs for use in treatments of various human diseases. In the future, researchers will face the challenge of developing validated methods of isolation and modification of MSCs, overcoming limitations of cell survivability after transplantation and minimizing the adverse effects on the human body.

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References

1. Park JS, Suryaprakash S, Lao YH, Leong KW (2015) Engineering mesenchymal stem cells for regenerative medicine and drug delivery. Methods. doi:10.1016/j.ymeth.2015.03.002
2. Huang GT, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 88(9):792–806. doi:10.1177/0022034509340867
3. Kim JY, Kim MR, Kim SJ (2013) Modulation of osteoblastic/odontoblastic differentiation of adult mesenchymal stem cells through gene introduction: a brief review. J Korean Assoc Oral Maxillofac Surg 39(2):55–62. doi:10.5125/jkaoms.2013.39.2.55
4. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97(25):13625–13630

5. Morsczeck C, Gotz W, Schierholz J, Zeilhofer F, Kuhn U, Mohl C, Sippel C, Hoffmann KH (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 24(2):155–165
6. Kerkis I, Ambrosio CE, Kerkis A, Martins DS, Zucconi E, Fonseca SA, Cabral RM, Maranduba CM, Gaiad TP, Morini AC, Vieira NM, Brolio MP, Sant'Anna OA, Miglino MA, Zatz M (2008) Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic? J Transl Med 6:35. doi:10.1186/1479-5876-6-35
7. Yao S, Pan F, Prpic V, Wise GE (2008) Differentiation of stem cells in the dental follicle. J Dent Res 87(8):767–771
8. Nosrat IV, Widenfalk J, Olson L, Nosrat CA (2001) Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons in vitro, and rescue motoneurons after spinal cord injury. Dev Biol 238(1):120–132
9. Yu YS, Shen ZY, Ye WX, Huang HY, Hua F, Chen YH, Chen K, Lao WJ, Tao L (2010) AKT-modified autologous intracoronary mesenchymal stem cells prevent remodeling and repair in swine infarcted myocardium. Chin Med J (Engl) 123(13):1702–1708
10. Cheng Z, Ou L, Zhou X, Li F, Jia X, Zhang Y, Liu X, Li Y, Ward CA, Melo LG, Kong D (2008) Targeted migration of mesenchymal stem cells modified with CXCR4 gene to infarcted myocardium improves cardiac performance. Mol Ther 16(3):571–579. doi:10.1038/sj.mt.6300374
11. Huang J, Zhang Z, Guo J, Ni A, Deb A, Zhang L, Mirotsou M, Pratt RE, Dzau VJ (2010) Genetic modification of mesenchymal stem cells overexpressing CCR1 increases cell viability, migration, engraftment, and capillary density in the injured myocardium. Circ Res 106(11):1753–1762. doi:10.1161/CIRCRESAHA.109.196030
12. Lu CH, Chang YH, Lin SY, Li KC, Hu YC (2013) Recent progresses in gene delivery-based bone tissue engineering. Biotechnol Adv 31(8):1695–1706. doi:10.1016/j.biotechadv.2013.08.015
13. Kumar S, Wan C, Ramaswamy G, Clemens TL, Ponnazhagan S (2010) Mesenchymal stem cells expressing osteogenic and angiogenic factors synergistically enhance bone formation in a mouse model of segmental bone defect. Mol Ther 18(5):1026–1034. doi:10.1038/mt.2009.315
14. Santos JL, Pandita D, Rodrigues J, Pego AP, Granja PL, Tomas H (2011) Non-viral gene delivery to mesenchymal stem cells: methods, strategies and application in bone tissue engineering and regeneration. Curr Gene Ther 11(1):46–57
15. Vannucci L, Lai M, Chiuppesi F, Ceccherini-Nelli L, Pistello M (2013) Viral vectors: a look back and ahead on gene transfer technology. New Microbiol 36(1):1–22
16. Ibraheem D, Elaissari A, Fessi H (2014) Gene therapy and DNA delivery systems. Int J Pharm 459(1–2):70–83. doi:10.1016/j.ijpharm.2013.11.041
17. Schagen FH, Ossevoort M, Toes RE, Hoeben RC (2004) Immune responses against adenoviral vectors and their transgene products: a review of strategies for evasion. Crit Rev Oncol Hematol 50(1):51–70
18. Campos SK, Barry MA (2007) Current advances and future challenges in Adenoviral vector biology and targeting. Curr Gene Ther 7(3):189–204
19. Latchman DS (2005) Herpes simplex virus-based vectors for the treatment of cancer and neurodegenerative disease. Curr Opin Mol Ther 7(5):415–418
20. de Silva S, Bowers WJ (2009) Herpes virus amplicon vectors. Viruses 1(3):594–629. doi:10.3390/v1030594
21. Wu X, Burgess SM (2004) Integration target site selection for retroviruses and transposable elements. Cell Mol Life Sci 61(19–20):2588–2596
22. Nanou A, Azzouz M (2009) Gene therapy for neurodegenerative diseases based on lentiviral vectors. Prog Brain Res 175:187–200. doi:10.1016/S0079-6123(09)17513-1
23. Dropulic B (2011) Lentiviral vectors: their molecular design, safety, and use in laboratory and preclinical research. Hum Gene Ther 22(6):649–657. doi:10.1089/hum.2011.058
24. Albertini AA, Baquero E, Ferlin A, Gaudin Y (2012) Molecular and cellular aspects of rhabdovirus entry. Viruses 4(1):117–139. doi:10.3390/v4010117

25. Hitchman RB, Possee RD, Crombie AT, Chambers A, Ho K, Siaterli E, Lissina O, Sternard H, Novy R, Loomis K, Bird LE, Owens RJ, King LA (2010) Genetic modification of a baculovirus vector for increased expression in insect cells. Cell Biol Toxicol 26(1):57–68. doi:10.1007/s10565-009-9133-y
26. Lu CH, Lin KJ, Chiu HY, Chen CY, Yen TC, Hwang SM, Chang YH, Hu YC (2012) Improved chondrogenesis and engineered cartilage formation from TGF-beta3-expressing adipose-derived stem cells cultured in the rotating-shaft bioreactor. Tissue Eng Part A 18(19–20):2114–2124. doi:10.1089/ten.TEA.2012.0010
27. Wang W, Li W, Ma N, Steinhoff G (2013) Non-viral gene delivery methods. Curr Pharm Biotechnol 14(1):46–60
28. Kaestner L, Scholz A, Lipp P (2015) Conceptual and technical aspects of transfection and gene delivery. Bioorg Med Chem Lett 25(6):1171–1176. doi:10.1016/j.bmcl.2015.01.018
29. Yalvac ME, Ramazanoglu M, Gumru OZ, Sahin F, Palotas A, Rizvanov AA (2009) Comparison and optimisation of transfection of human dental follicle cells, a novel source of stem cells, with different chemical methods and electro-poration. Neurochem Res 34(7):1272–1277. doi:10.1007/s11064-008-9905-4
30. Yalvac ME, Rizvanov AA, Kilic E, Sahin F, Mukhamedyarov MA, Islamov RR, Palotas A (2009) Potential role of dental stem cells in the cellular therapy of cerebral ischemia. Curr Pharm Des 15(33):3908–3916
31. Nakashima M, Iohara K, Ishikawa M, Ito M, Tomokiyo A, Tanaka T, Akamine A (2004) Stimulation of reparative dentin formation by ex vivo gene therapy using dental pulp stem cells electrotransfected with growth/differentiation factor 11 (Gdf11). Hum Gene Ther 15(11):1045–1053
32. Tomizawa M, Shinozaki F, Motoyoshi Y, Sugiyama T, Yamamoto S, Sueishi M (2013) Sonoporation: gene transfer using ultrasound. World J Methodol 3(4):39–44. doi:10.5662/wjm.v3.i4.39
33. Ramamoorth M, Narvekar A (2015) Non viral vectors in gene therapy - an overview. J Clin Diagn Res 9(1):GE01–GE06. doi:10.7860/JCDR/2015/10443.5394
34. Solovyeva VV, Kudryashova NV, Rizvanov AA (2011) Transfer of recombinant nucleic acids into cells (transfection) by means of histones and other nuclear proteins. Cell Transpl Tiss Eng 6(3):29–40
35. Gheisari Y, Soleimani M, Azadmanesh K, Zeinali S (2008) Multipotent mesenchymal stromal cells: optimization and comparison of five cationic polymer-based gene delivery methods. Cytotherapy 10(8):815–823. doi:10.1080/14653240802474307
36. Chesnoy S, Huang L (2000) Structure and function of lipid-DNA complexes for gene delivery. Annu Rev Biophys Biomol Struct 29:27–47
37. Tang F, Hughes JA (1998) Introduction of a disulfide bond into a cationic lipid enhances transgene expression of plasmid DNA. Biochem Biophys Res Commun 242(1):141–145
38. Rao NM, Gopal V (2006) Cell biological and biophysical aspects of lipid-mediated gene delivery. Biosci Rep 26(4):301–324
39. Madeira C, Mendes RD, Ribeiro SC, Boura JS, Aires-Barros MR, da Silva CL, Cabral JMS (2010) Nonviral gene delivery to mesenchymal stem cells using cationic liposomes for gene and cell therapy. J Biomed Biotechnol. doi:10.1155/2010/735349
40. Rodier F, Campisi J (2011) Four faces of cellular senescence. J Cell Biol 192(4):547–556. doi:10.1083/jcb.201009094
41. Burkhart DL, Sage J (2008) Cellular mechanisms of tumour suppression by the retinoblastoma gene. Nat Rev Cancer 8(9):671–682. doi:10.1038/nrc2399
42. Ramboer E, De Craene B, De Kock J, Vanhaecke T, Berx G, Rogiers V, Vinken M (2014) Strategies for immortalization of primary hepatocytes. J Hepatol 61(4):925–943. doi:10.1016/j.jhep.2014.05.046
43. Jha KK, Banga S, Palejwala V, Ozer HL (1998) SV40-mediated immortalization. Exp Cell Res 245(1):1–7
44. Yalvac ME, Yilmaz A, Mercan D, Aydin S, Dogan A, Arslan A, Demir Z, Salafutdinov II, Shafigullina AK, Sahin F, Rizvanov AA, Palotas A (2011) Differentiation and neuro-protective

properties of immortalized human tooth germ stem cells. Neurochem Res 36(12):2227–2235. doi:10.1007/s11064-011-0546-7
45. Egbuniwe O, Grant AD, Renton T, Di Silvio L (2013) Phenotype-independent effects of retroviral transduction in human dental pulp stem cells. Macromol Biosci 13(7):851–859. doi:10.1002/mabi.201300020
46. Wang J, Zhang H, Zhang W, Huang E, Wang N, Wu N, Wen S, Chen X, Liao Z, Deng F, Yin L, Zhang J, Zhang Q, Yan Z, Liu W, Zhang Z, Ye J, Deng Y, Luu HH, Haydon RC, He TC (2014) Bone morphogenetic protein-9 effectively induces osteo/odontoblastic differentiation of the reversibly immortalized stem cells of dental apical papilla. Stem Cells Dev 23(12):1405–1416. doi:10.1089/scd.2013.0580
47. Yokoi T, Saito M, Kiyono T, Iseki S, Kosaka K, Nishida E, Tsubakimoto T, Harada H, Eto K, Noguchi T, Teranaka T (2007) Establishment of immortalized dental follicle cells for generating periodontal ligament in vivo. Cell Tissue Res 327(2):301–311
48. Carreira AC, Alves GG, Zambuzzi WF, Sogayar MC, Granjeiro JM (2014) Bone morphogenetic proteins: structure, biological function and therapeutic applications. Arch Biochem Biophys 561:64–73. doi:10.1016/j.abb.2014.07.011
49. Tasli PN, Aydin S, Yalvac ME, Sahin F (2014) Bmp 2 and bmp 7 induce odonto- and osteogenesis of human tooth germ stem cells. Appl Biochem Biotechnol 172(6):3016–3025. doi:10.1007/s12010-013-0706-0
50. Yang X, van der Kraan PM, Bian Z, Fan M, Walboomers XF, Jansen JA (2009) Mineralized tissue formation by BMP2-transfected pulp stem cells. J Dent Res 88(11):1020–1025. doi:10.1177/0022034509346258
51. Yang X, van der Kraan PM, van den Dolder J, Walboomers XF, Bian Z, Fan M, Jansen JA (2007) STRO-1 selected rat dental pulp stem cells transfected with adenoviral-mediated human bone morphogenetic protein 2 gene show enhanced odontogenic differentiation. Tissue Eng 13(11):2803–2812
52. Yang X, Walboomers XF, van den Dolder J, Yang F, Bian Z, Fan M, Jansen JA (2008) Non-viral bone morphogenetic protein 2 transfection of rat dental pulp stem cells using calcium phosphate nanoparticles as carriers. Tissue Eng Part A 14(1):71–81. doi:10.1089/ten.a.2007.0102
53. Zhang W, Zhang X, Ling J, Liu W, Ma J, Zheng J (2014) Proliferation and odontogenic differentiation of BMP2 gene-transfected stem cells from human tooth apical papilla: an in vitro study. Int J Mol Med 34(4):1004–1012. doi:10.3892/ijmm.2014.1862
54. Yang X, Han G, Pang X, Fan M (2012) Chitosan/collagen scaffold containing bone morphogenetic protein-7 DNA supports dental pulp stem cell differentiation in vitro and in vivo. J Biomed Mater Res A. doi:10.1002/jbm.a.34064
55. Nakashima M, Tachibana K, Iohara K, Ito M, Ishikawa M, Akamine A (2003) Induction of reparative dentin formation by ultrasound-mediated gene delivery of growth/differentiation factor 11. Hum Gene Ther 14(6):591–597
56. Nakashima M, Mizunuma K, Murakami T, Akamine A (2002) Induction of dental pulp stem cell differentiation into odontoblasts by electroporation-mediated gene delivery of growth/differentiation factor 11 (Gdf11). Gene Ther 9(12):814–818
57. Pan K, Sun Q, Zhang J, Ge S, Li S, Zhao Y, Yang P (2010) Multilineage differentiation of dental follicle cells and the roles of Runx2 over-expression in enhancing osteoblast/cementoblast-related gene expression in dental follicle cells. Cell Prolif 43(3):219–228. doi:10.1111/j.1365-2184.2010.00670.x
58. Vimalraj S, Arumugam B, Miranda PJ, Selvamurugan N (2015) Runx2: structure, function, and phosphorylation in osteoblast differentiation. Int J Biol Macromol 78:202–208. doi:10.1016/j.ijbiomac.2015.04.008
59. Morsczeck C (2006) Gene expression of runx2, Osterix, c-fos, DLX-3, DLX-5, and MSX-2 in dental follicle cells during osteogenic differentiation in vitro. Calcif Tissue Int 78(2):98–102
60. Viale-Bouroncle S, Felthaus O, Schmalz G, Brockhoff G, Reichert TE, Morsczeck C (2012) The transcription factor DLX3 regulates the osteogenic differentiation of human dental follicle precursor cells. Stem Cells Dev 21(11):1936–1947. doi:10.1089/scd.2011.0422

61. Press T, Viale-Bouroncle S, Felthaus O, Gosau M, Morsczeck C (2015) EGR1 supports the osteogenic differentiation of dental stem cells. Int Endod J 48(2):185–192. doi:10.1111/iej.12299
62. Li Y, Lu Y, Maciejewska I, Galler KM, Cavender A, D'Souza RN (2011) TWIST1 promotes the odontoblast-like differentiation of dental stem cells. Adv Dent Res 23(3):280–284. doi:10.1177/0022034511405387
63. Scheller EL, Chang J, Wang CY (2008) Wnt/beta-catenin inhibits dental pulp stem cell differentiation. J Dent Res 87(2):126–130
64. Patan S (2004) Vasculogenesis and angiogenesis. Cancer Treat Res 117:3–32
65. Ribatti D, Nico B, Crivellato E (2011) The role of pericytes in angiogenesis. Int J Dev Biol 55(3):261–268. doi:10.1387/ijdb.103167dr
66. Bergers G, Song S (2005) The role of pericytes in blood-vessel formation and maintenance. Neuro Oncol 7(4):452–464
67. Sieveking DP, Ng MK (2009) Cell therapies for therapeutic angiogenesis: back to the bench. Vasc Med 14(2):153–166. doi:10.1177/1358863X08098698
68. Dissanayaka WL, Zhan X, Zhang C, Hargreaves KM, Jin L, Tong EH (2012) Coculture of dental pulp stem cells with endothelial cells enhances osteo-/odontogenic and angiogenic potential in vitro. J Endod 38(4):454–463. doi:10.1016/j.joen.2011.12.024
69. Gandia C, Arminan A, Garcia-Verdugo JM, Lledo E, Ruiz A, Minana MD, Sanchez-Torrijos J, Paya R, Mirabet V, Carbonell-Uberos F, Llop M, Montero JA, Sepulveda P (2008) Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells 26(3):638–645
70. Janebodin K, Zeng Y, Buranaphatthana W, Ieronimakis N, Reyes M (2013) VEGFR2-dependent angiogenic capacity of pericyte-like dental pulp stem cells. J Dent Res 92(6):524–531
71. Ahmad S, Hewett PW, Al-Ani B, Sissaoui S, Fujisawa T, Cudmore MJ, Ahmed A (2011) Autocrine activity of soluble Flt-1 controls endothelial cell function and angiogenesis. Vasc Cell 3(1):15. doi:10.1186/2045-824X-3-15
72. Storkebaum E, Lambrechts D, Carmeliet P (2004) VEGF: once regarded as a specific angiogenic factor, now implicated in neuroprotection. Bioessays 26(9):943–954
73. Sun Y, Jin K, Xie L, Childs J, Mao XO, Logvinova A, Greenberg DA (2003) VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. J Clin Invest 111(12):1843–1851
74. Jin K, Zhu Y, Sun Y, Mao XO, Xie L, Greenberg DA (2002) Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. Proc Natl Acad Sci U S A 99(18):11946–11950
75. Solovyeva VV, Blatt NL, Shafigullina AK, Rizvanov AA (2012) Endogenous secretion of vascular endothelial growth factor by multipotent mesenchymal stromal cells derived from human third molar dental follicles. Cell Transpl Tiss Eng 7(3):155–158

Chapter 6
Dental Stem Cells *vs*. Other Mesenchymal Stem Cells: Their Pluripotency and Role in Regenerative Medicine

Selami Demirci, Ayşegül Doğan, and Fikrettin Şahin

6.1 Introduction

MSCs residing in dental tissues are self-renewing progenitors which could help regeneration process of other tooth components such as dentin, pulp or periodontal ligament. Up to date, several kinds of stem cell populations from mature or immature tooth have been isolated and characterized for their stem cell characteristics in terms of self-renewal and differentiation capacities [1]. Gronthos and his colleagues isolated the first stem cell type from dental pulp tissue in 2000 and named as dental pulp stem cells (DPSCs) [2]. Stem cells from exfoliated deciduous teeth (SHEDs) [3], periodontal ligament stem cells (PDLSCs) [4], dental follicle stem cells (DFSCs) [5] and stem cells from apical papilla (SCAPs) [6] have been presented at the following years. DSCs are identified according to the procedures established for MSC stem cell characterization, specifically for adult stem cells (ASCs) of bone marrow. Colony-forming capacity and differentiation potential of DSCs into multiple cell types (osteo-, chondro-, adipo- and neuro-genic lineages) make them potential candidates to be used in regenerative medicine [1]. Because dental-derived stem cells have mainly neuroectodermal origin [7], they are defined as attractive sources for stem cell-based regenerative treatments, particularly for neuroregeneration.

Different stem cell types residing in specialized dental tissues have distinct features and comprise a great heterogeneity, leading to significant differentiation potential. Understanding the possible mechanisms underlying pluripotency, plasticity and self-renewal capacity of DSCs could allow researchers to maintain stem cells in culture, obtain enough number of cells required for clinical applications, direct stem cell fate for a certain treatment choice and create a promising stem cell therapy option for ASC-based approaches. Moreover, regulation of DSC

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differentiation for a special tissue engineering practice such as bone, tooth and neuronal regeneration, and controlling the expansion of genetically stable stem cells are crucial for medicinal applications [8]. This chapter will review the pluripotency of DSCs and comparison of their regenerative potential with other famous MSC sources in detail.

6.2 Pluripotency of DSCs

6.2.1 Pluripotency: Definition, Assessment and Markers

Apart from tissue-specific ASCs having capacity of differentiation into limited cell lineages, pluripotent stem cells can give rise to cells of all three embryonic germ layers (ectoderm, endoderm and mesoderm). Pluripotency of a cell can be simply defined as the potential of generating an entire organism and all types of cells of an adult body with broad range of functions [9]. First studies investigating cell pluripotency were started in late 1800s, and Driesch and co-workers managed to obtain two sea urchins from cells of the early blastocyst [10]. After further progresses in embryonic cell (ES) studies in the 1980s and 1990s, pluripotent cells were successfully isolated from mouse [11] and human [12] blastocysts. Understanding the potential of ES cells to differentiate into a limitless number of cell types have given an excellent opportunity for stem cell research. Some critical problems including immune rejections after cell transplantation, teratoma formation and ethical issues have been faced in ES cell studies. These challenges have been partly overcome by the induced pluripotent stem cells (iPSC) breakthrough of Takahashi and Yamanaka who have made the pluripotent stem cell studies more popular and attractive research of area [13].

There are some established criteria for assessment of pluripotency. Programmed pluripotent cells should have colonies similar to ES cells, reactivate the telomerase gene expression and express a set of genes related to pluripotency including octamer-binding transcription factor 4 (OCT4), SRY (sex determining region Y)-box 2 (SOX2) and Nanog. Although reliability of alkaline phosphatase (ALP) assay is doubtful, it is generally used to prove pluripotent state of stem cells. Providing these features, programmed cells behave just like ES cells and could differentiate into a wide range of cell types [9]. In addition, fully reprogrammed cells form embryoid bodies and create teratoma when injected to immunodeficient mice. However, there are still vital challenges with iPSC technology including less efficiency, safety problem and teratoma formation, which direct scientist to search for safer, efficient and pluripotent stem cells to be used in regenerative approaches. Although development of pluripotent cells from any adult cell or stem cell is theoretically possible in culture conditions using multiple cloning techniques, it would be a powerful approach to isolate stem cells from adult body with their own pluripotency and differentiation capacity. The idea of “whether pluripotent stem

cells reside in the adult body and distribute into several body parts, they can be used after differentiation into desired lineage to restore tissue function instead of ES cells or iPSCs" have attracted scientists to investigate ASCs in terms of pluripotency [14]. To this end, identification of new ASC types or defining the pluripotent characteristics of known stem cells could be a promising approach for stem cell-based regenerative medicine applications.

6.2.2 Pluripotency of MSCs

Although adult MSCs have been defined in a vast amount of human tissues, and they have displayed a limited differentiation and self-renewal potential, recent studies have proven their remarkable multipotent differentiation capacity [14]. The stem cell plasticity comprises self-renewal potential, and in vitro and in vivo multilineage differentiation capacity. To address the plasticity and pluripotency concept, bone marrow mesenchymal stem cells (BMMSCs) have generally been the subject of many researches. Differentiation of BMMSCs into mesoderm, neuroectoderm and endodermal lineages has been reported in previous studies [15]. Moreover, ES cell-like phenotype of BMMSCs has been pointed by showing OCT4, Nanog, SOX2, ALP and stage specific embryonic antigen-4 (SSEA-4) expressions. Apart from BMMSCs, adipose, dermal and heart tissue-derived MSCs have also expressed ES cell specific markers [16]. In addition, Wharton jelly-derived MSCs have surprisingly demonstrated higher pluripotent/stem cell marker expression levels compared to BMMSCs even at high passage numbers in standard in vitro culture conditions [17]. These and other similar reports have concluded that although BMMSCs are the most popular and well-established source for MSC isolation, there could be more suitable alternative sites in the adult body for pluripotent progenitor cell isolation including Wharton jelly, cord blood, adipose tissue, dermis and tendon [17–19]. Therefore, stem cells without exerting any ethical problems, and being highly proliferative, multipotent, easily accessible and cultured in vitro are necessary for regenerative therapy approaches. In this line, DSCs comprising many advantages exist as an alternative pluripotent stem cell source for future researches and applications.

6.2.3 Pluripotent Characteristics of DSCs

As dental tissues, particularly tooth germs, are developed by ectomesodermal interactions, these cells are able to differentiate into several cell lineages. Multipotent stem cells residing in dental tissues remain quiescent until late ages and have been successfully transformed into osteo-, chondro-, adipo-cytes and neurons. DSCs are easily isolated from teeth and surgical procedure of dental tissues is relatively easy compared to bone marrow or other sources. Teeth are waste materials of dental

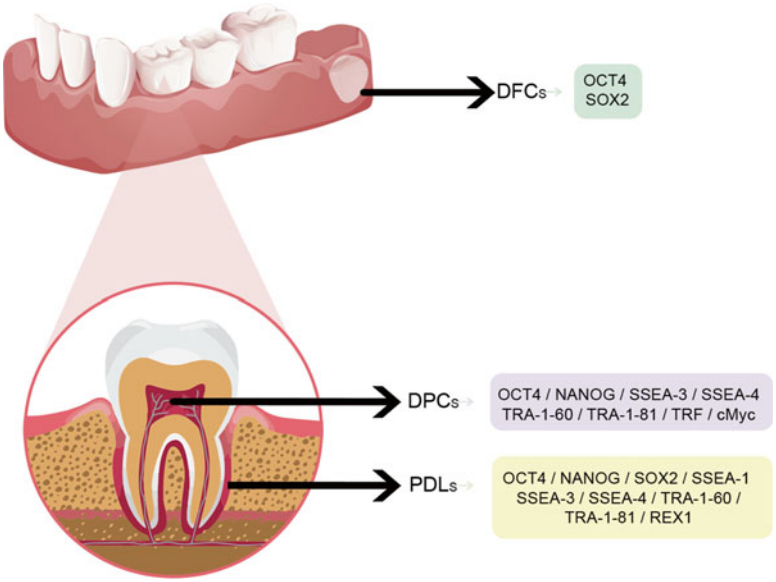

Fig. 6.1 Embryonic stem cells marker expression profile of dental stem cells. DFSCs: Dental follicle stem cells, DPSCs: Dental pulp stem cells, PDLSCs: Periodontal ligament derived stem cells

procedures and do not cause any ethical problems for stem cell isolation [7]. Therefore, isolation and characterization of pluripotent stem cells from dental tissues might be a promising approach for clinical studies. DSCs have been used in vitro and in vivo to evaluate their restorative potential for regenerative medicine and have started to be used in clinical studies in recent years. Researchers have identified ES cell-like characteristics of DSCs in many studies to determine their pluripotency (Fig. 6.1). In this line, studies have aimed to develop new culture methods that could prevent spontaneous differentiation and protect pluripotency of dental-derived stem cells are currently of great interest.

Fetal bovine serum (FBS) is generally used as an important supplement in culture media to provide cell growth but might cause sensitivity reactions and prion transmission [20]. Although FBS is required for stem cell isolation from dental tissues as well as from other adult tissues, it includes animal proteins and other components which might cause immune reactions or undesirable spontaneous transformation, which is the main obstacle for multilineage differentiation. Using autologous human serum (AHS) instead of FBS in cell culture medium could be a solution for immune reactions and contamination. However, required amount of blood collection from particular patients for clinical applications might not be feasible every time. Therefore, development of defined media and culture conditions is the first step for maintenance of DSC pluripotency. Optimum serum free medium supplemented with 1 % insulin-transferrin-selenium-X (ITS-X) and 100 mg/mL of embryotrophic factor for deciduous and wisdom tooth pulp stem cells have provided desired proliferation, survival and stem cell marker expressions [21].

Similarly, basic fibroblast growth factor (bFGF or FGF2) supplementation to the cell culture medium (either containing FBS or serum-free) has protected the pluripotency of DPSCs, and increased the expression of stem cell marker, STRO-1 level. These stem cells have protected self-renewal capacity and differentiation potential, and formed bone, cartilage or adipose-like structures when transplanted to immunocompromised mice [22].

Although culture conditions and provided environment are crucial for maintenance of stem cell characteristics, cell source itself is the main determinant to obtain stem cells with high pluripotency. Stem cells isolated from healthy periodontal ligament (PDL) tissues have been shown for ectodermal, endodermal and mesodermal differentiation capacities, indicating remarkable regenerative potential not only limited to dental tissues. In addition, SSEA-4 as a definitive marker of ES cells has also been suggested as a PDLSC marker. Stem cells isolated from periodontal tissues of permanent teeth have been shown for their fibroblastic cell morphology, multilineage differentiation capacity towards all three germ layers (ectodermal, endodermal and mesodermal) and expressions of ES cell markers (SSEA-1, SSEA-3, SSEA-4, TRA-1-60, TRA-1-81, OCT4, Nanog, SOX2, and REX1) [23]. These observations have also been confirmed by showing that stem cells either isolated from healthy or defected periodontal ligament tissues have exerted ES cell characteristics proven by Nanog, OCT4, REX-1 and SOX2 gene expressions and have been successfully differentiated into osteogenic lineage in vitro [23]. In another interesting study, inflammatory granulation tissue removed from periodontal tissue during surgery has been used for progenitor cell isolation, and it has been proposed that pluripotent subpopulation found in periodontal tissue might favor tissue regeneration. ES cell markers including OCT4, REX-1, Nanog and SOX2 were expressed in inflamed PLDSCs at high levels, suggesting the contribution of pluripotent PLDSCs to healing process of damaged tissues [24].

As the oral cavity and dental tissues are rich sources for stem cell isolation, lots of dental tissue parts have been investigated for pluripotent stem cell isolation and ES cell-like properties. SHEDs were found to express ES cell markers such as OCT4, Nanog, SSEA-3, SSEA-4, TRA-1-60 and TRA-1-81, display high growth kinetics, clonogenic capacity up to passage 25, and form dense structures when injected to immunocompromised mice [25]. Similarly, Ferro and his colleagues have investigated the expression of ES cell marker (OCT4, Nanog, KLF4 and c-Myc) expressions in undifferentiated and differentiated DPSCs derived from human deciduous teeth [26]. Both ES cell and stem cell markers (CD10, CD29 and CD117) have reduced after induction of differentiation into osteocytes, myocytes, neurons and hepatocytes. Particularly, expression of OCT4, a key transcription factor for pluripotency of ES cells, has down-regulated about 50 % after differentiation process and changed localization, indicating the importance of this transcription factor for stem cell phenotype and maintenance.

While DSC isolation from various dental tissue parts has been successfully conducted for years, and valuable cell types have been derived, search for potential of these cells and characterization of pluripotent properties to fully understand regenerative potential are still on progress. Tooth germs of third molars, generated by

ectomesodermal interactions, might be used as a new pluripotent stem cell source due to remaining undifferentiated state until the age of six, and starting organogenesis process after birth. Pluripotent characteristics of these cells and differentiation capacity to several lineages have been shown in several reports [27, 28]. In 2009, Yalvac and co-workers showed MSC properties of human tooth germ stem cells (hTGSCs) by surface marker expression and multiple differentiation potential. While OCT4 expression has been detected in early passages, expression level decreased in late passages and differentiated cells. In addition to OCT4 expression, SOX2, KLF4, Nanog and c-Myc expressions have also been observed in hTGSCs similar to ES cells [7]. Similar findings were reported in a recent study pointing the role of OCT4 in maintenance of pluripotency and multilineage potential of DPSCs obtained from tooth germs. Using an inducible OCT4 expressing system has been proposed to keep DSCs in a pluripotent state to be used in regenerative medicine [29]. Other than genetic modifications, some growth factors are included in the culture media to maintain pluripotency of stem cells. Progenitor cells isolated from pulp tissues of human tooth germs cultured in a specific media containing leukemia inhibitory factor (LIF), epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) showed an embryonic phenotype, formed embryoid bodies, and were positive for ALP staining and embryonic markers including Nanog, SOX2, OCT3/4 and c-Myc [30]. The same group published a further study in 2012 showing that hTGSCs have exhibited ES cell phenotype by forming embryoid bodies in vitro and teratoma-like structures in nude mice, and differentiated into cell types derived from ectoderm, endoderm and mesoderm [31].

All of the available research comparing the ES cell-like characteristics of DSCs has demonstrated that pluripotency and differentiation capacity of stem cells are dependent on the originated tissue. Distinct stem cell populations reside in the various teeth and dental parts of the oral cavity. Detailed characterization studies of various DSCs and their pluripotency could be useful for therapeutic interventions.

6.3 DSCs *vs.* Other MSCs

Comparison of pluripotency and multilineage differentiation capacity for MSC types might allow scientist to determine proper seed cells for a specific therapeutic application in which multi-differentiation capability is required, such as neuroregeneration approaches.

After the discovery of BMMSCs, scientists are searching for alternative MSCs from other tissues displaying higher regeneration ability. In general, there are at least three accepted criteria for a cell population to be considered as MSC: (i) cells must display plastic-adherent properties in cell culture applications, (ii) cells must express mesenchymal cell surface markers including CD73, CD90 and CD105 but not hematopoietic cell surface markers such as CD14, CD19, CD34, CD45 and HLA-DR, and (iii) cells must have the ability to differentiate into osteo-, adipo- and chondro-genic cell lineages [32]. However, as MSCs display different properties

according to their origin of tissues, it is quite essential to be aware of variations between MSCs to apply the most suitable cell type in regeneration approaches. During the characterization of newly identified MSCs, their properties are generally compared with well-known and widely studied MSC type, BMMSCs, or other famous MSCs such as adipose derived stem cells (ADSCs) or umbilical cord stem cells (UCSCs). Comparison articles so far have proved that MSCs derived from different tissues might display variable behavior in vitro and in vivo conditions in terms of proliferation, expansion and differentiation characteristics. Therefore, while MSCs can be isolated from almost all parts of the body, selecting the most appropriate candidate for a specific tissue regeneration application is the most critical step in MSC-based tissue engineering approaches [33]. These disparities in MSC are mainly originated from donor and tissue which cells are isolated, isolation technique and cell culture applications. Firstly, as MSCs from different sources are isolated using different techniques such as applying various enzymes for digestion or explant growth, their proliferation and differentiation properties varies. Even a stem cell type derived from the same donor isolated by applying different techniques might display varying characteristics and differentiation potential [34, 35]. Therefore, using different enzymes for tissue digestion, incubation periods along with having different source of origin result in variable cell behavior in cell culture and in vivo studies. In this sense, dental tissue derived stem cells including SHEDs, SCAPs, PDLSCs, DFSCs and DPSCs exhibit comparable peculiarities with regard to their differentiation, proliferation, in vitro expansion and immunosuppression capacities which mainly determines their potential uses in regenerative medicine (Fig. 6.2).

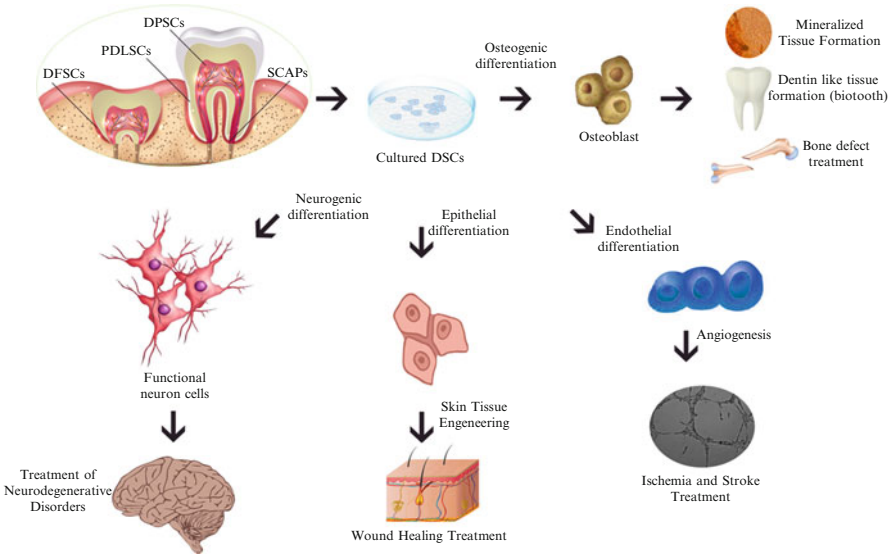

Fig. 6.2 Potential uses of dental stem cells (DSCs) in regenerative medicine. DFSCs: Dental follicle stem cells, DPSCs: Dental pulp stem cells, PDLSCs: Periodontal ligament derived stem cells, SCAPs: Stem cells from the apical papilla

The time required to reach full confluency for DPSCs was relatively higher with respect to BMMSCs and ADSCs immediately after the isolation, but cell viability rate of DPSCs were significantly higher than BMMSCs and not different from ADSCs after a 14-days cryopreservation period [36]. Moreover, colony forming ability and proliferation rate of DPSC were superior to BMMSCs in later passages possibly because DPSCs isolated from unerupted third molar are in later stage of development in comparison with BMMSCs [2]. Apart from their behavior in cell culture, MSC surface protein profiles which determines the pluripotency of stem cells, differ from each other.

Cell behavior, proteins expression and differentiation potential of stem cells are regulated by their basal gene expression profiles. Early microarray studies have indicated that DPSCs and BMMSCs share similar pattern of gene expression levels for known 4,000 human genes except few differences. Undifferentiated DPSCs expressed high levels of insulin-like growth factor-2, collagen type XVIII α1, discordin domain tyrosine kinase 2 and cyclin-dependent kinase 6, and lower levels of insulin-like growth factor binding protein-7 and collagen type I α2 compared to BMMSCs [37]. In the following surveys, DPSCs have been compared with BMMSCs for broad range of gene sets. Yamada and co-workers have shown that there are several upregulated and down-regulated genes in DPSCs compared to BMMSCs, which take place in vital cell progresses including proliferation, migration, cell adhesion, growth factor production and differentiation [38]. These variances in basal gene expression profiles are critical factors determining the differentiation capacity of MSCs towards various cell lineages. The most prominent and well-studied transformation capacity of DSCs is towards osteo-/odonto-genic cell types [8], most probably due to their natural microenvironments. All DSC types display varying degree of osteo-/odonto-genic potentials confirmed by several in vitro and in vivo studies [39]. As DPSCs are the most studied and well-elucidated DSC type, they are commonly used in MSC comparison studies. DPSCs transplanted into immunocompromised mice have formed dentin-like structure, whereas BMMSCs formed lamellar bone consisting of osteocytes and osteoblasts [2]. In addition, DPSCs co-cultured with apical bud cells (ABCs) mineralized faster and expressed higher levels of tooth specific proteins and genes compared to BMMSCs/ABCs co-culture, indicating the superior odontogenic differentiation potential of DPSCs over BMMSCs [40]. Davies and co-workers have compared mineralization and dentinogenic potential of MSCs derived from dental pulp, bone marrow and adipose tissue [41]. Their results have revealed that both mineralized matrix and dentinogenesis were significantly elevated in DPSCs with respect to ADSCs and BMMSCs. Similarly, DPSCs have displayed significantly higher alkaline phosphatase (ALP) activity and osteogenic differentiation than ADSCs [42]. While several other studies have claimed better osteo-/odonto-genic capacity of DPSCs over BMMSCs and ADSCs, there are also a few conflicting reports. Hung and co-workers have published that ADSCs and DPSCs used in tooth regeneration rabbit model exhibited similar gene expression profiles and regeneration potentials but as ADSCs had higher growth rate and senescence resistance, they were proposed to be suitable candidate for tissue regeneration approaches [43].

Another important DSC type, PDLSCs, have also been compared with BMMSCs in an immunodeficient rat calvarium critical size defect model [44]. Although both stem cells incorporated into Bio-Oss scaffolds have increased the regeneration efficiency compared to scaffold alone group, PDLSCs have been claimed to be more effective. In addition, colony forming ability, proliferation rate and mineralization matrix formation capacity of PDLSCs were higher compared to BMMSCs in in vitro conditions [45]. However, Liu and co-workers have proposed that although PDLSCs and BMMSCs have similar proliferation rate and cell surface protein profiles, PDLSCs had lower osteogenic differentiation capacity determined by observing mineralization deposition areas stained by alizarin red and, Runx2 and ALP protein expressions compared to BMMSCs [46]. In addition, they have shown that osteogenic lineage differentiation potential of PDLSCs significantly inhibited by main inflammatory cytokine, tumor necrosis factor alpha (TNF-α), stimulation via canonical Wnt signaling pathway. DFSCs have also been compared with BMMSCs and skin derived MSCs (SMSCs) for their osteogenic capacity in in vitro and in vivo conditions using demineralized bone matrix and fibrin glue scaffolds [47]. The findings of the study have indicated that while SMSCs exhibited the weakest osteogenic transformation ability, DFSCs possessed similar differentiation potential with BMMSCs in vitro, but displayed higher osteocalcin intensity and calcium deposition along with similar radiologic intensities in vivo. Superior osteogenesis of DFSCs could be explained by having osteoblast progenitor cells and cementoblasts along with expressing higher and stable levels of osteoblast specific genes before and after differentiation [48].

While osteo-/odonto-genic transformation capacity of DSCs are comparable with other MSCs, adipogenic lineage differentiation ability of DSCs relatively remains weaker. Lipid droplets in DPSCs after adipogenic medium treatment were significantly lower than UCSCs [33] and BMMSCs [2, 49] but higher than SMSCs [47]. Although these reports indicate potential insufficiency of DSCs in fat tissue replacement therapies, there are promising and encouraging attempts to increase adipogenesis potential of DSCs. Exogenous growth factor applications [50], gene overexpression [51] or knock-down approaches [52], and engineered surfaces [53] have been used to enhance adipogenesis capacity of various dental tissue-derived stem cells. Similar to adipogenesis of DSCs, chondrogenesis potential of DSCs remains relatively unsatisfactory in comparison with other MSCs. While BMMSCs could differentiate into chondrocyte-like cells and express elevated levels of chondrogenesis-related proteins, chondrogenic differentiation ability of DPSCs was insufficient to be used in regenerative approaches [54, 55]. In contrast, PDLSCs displayed better chondrogenesis in comparison with MSCs derived from Wharton's Jelly of the umbilical cord [56]. To modify and enhance chondrogenesis capacity of DSCs, researches have focus on additional applications including use of tissue engineered scaffold systems [57], exogenous growth factors [58] and gene modifications [52].

It is undeniable that current knowledge of DSCs offers limited options for chondrogenic and adipogenic regeneration potential, but their neurogenic potential is much more promising, most probably due to their neural-crest origin. Undifferentiated

DPSCs and PDLSCs expressed higher levels of vital neuronal markers including nestin with respect to ADSCs and SMSCs [59]. Neuronal differentiation ability of DSCs was evident not only by cellular morphology but also expression of neuron specific markers under appropriate cell culture conditions [60]. Neuron-like cell formation potential of DPSCs was higher than BMMSCs determined at both protein and gene levels [61]. However, while neuronal progenitors cells could be obtained from DPSCs in in vitro conditions, they do not further differentiate into mature functional neurons, indicating more researches are strictly required to optimize culture conditions to initiate neuronal functionality [62]. One promising attempt has been performed by Király and co-workers, mentioning that activation of protein kinase C and the cyclic adenosine monophosphate pathways, and maturation with important neurotropic factors and other supplements resulted in functional neuronal derivation from DPSCs [63]. Most of the in vivo studies, on the other hand, indicate positive contribution of DSCs to neuroregeneration process by expressing important neurotropic agents and anti-inflammatory cytokines rather than regenerating the lost neuronal network [64]. Additional advances in controlling and managing neurogenesis of DSCs would increase regeneration capacity and realize their use in clinical applications.

Similar to neurogenic potential of DSCs, angiogenic activity of DSCs has also been investigated in several studies. Dental pulp is a highly vascularized tissue with an ability of healing after various injuries [65]. Therefore, the idea of "endothelial progenitor cells must have been inside the pulp tissue to reorganize lost vascular network and provide a complete healing" has been examined. As expected, DPSCs, SHED and PDLSCs cells have been successfully differentiated into endothelial cells [66–68]. After a proper stimuli and culture conditions, DPSCs formed tube-like structures and expressed high levels of endothelial cell-specific markers including FMS-like tyrosine kinase 1 (Flt1), kinase insert domain receptor (KDR), intercellular adhesion molecule 1 (ICAM-1) and von willebrand factor (vWF) [69]. Apart from differentiating into endothelial-like cells, DSCs, mainly DPSCs and SHED cells, promote angiogenesis by secreting a vast amount of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), FGF-2 and PDGF [70]. PDLSCs also produced more VEGF proteins in comparison with BMMSCs but SHED cells secreted relatively low levels of VEGF while their vessel formation inducing effects were similar [71]. SCAPs, DPSCs and DFSCs were found to express more VEGF proteins with respect to gingival stem cells, and angiogenesis in SCAPs' and DPSCs' condition medium treated chorioallantoic membranes significantly augmented [72]. Similarly, $CD31^-$ side population of DPSCs expressed higher levels of pro-angiogenic stimulators such as VEGFA and granulocyte macrophage colony-stimulating factor (GM-CSF) with respect to $CD31^-$ side populations of ADSCs and BMMSCs isolated from same donors [73]. In the same study, authors have claimed that while conditioned medium of BMMSCs and DPSCs resulted in better proliferation rates compared to conditioned medium of ADSCs, migratory and tube-like structure formation promoting effects were found to be highest in DPSCs' conditioned medium applied group. They also proved that DPSC application to mouse hindlimb ischemia model provided better blood flow and

denser capillary network than other two MSC types. In line with this study, co-culturing endothelial cells with DPSCs resulted in more tube-like structure formation compared to BMMSCs in vitro, and more blood vessels in vivo matrigel plug assay in a VEGFR-2 dependent pathway, showing superior angiogenic activity [74].

Epithelial cell-like transformation of tooth germ stem cells was shown [75] and epithelial stem cell properties of DPSCs was found to be higher than BMMSCs [61]. The study have proved that DPSCs expressed significantly higher levels of cytokeratin-18 and cytokeratin-19 with respect to BMMSCs as expected due to having epithelial stem cell fraction in the pulp tissue [76]. MSCs derived from dental tissues have also been shown to differentiate into neural crest derived melanocytes [77], pancreatic cells [78, 79] and hepatic cells [80]. Only hepatic differentiation capacity of SHEDs has been compared with BMMSCs [81]. $CD117^{+}$ fraction of SHEDs, which maintained their remarkable stem cells properties up to 50 passages, exhibited better differentiation potential than BMMSCs and differentiation was further promoted by hydrogen sulfide treatment.

6.4 Conclusion

DSCs have been the subject of various reports mentioning superior self-renewal and differentiation capacities along with their pluripotency. As every MCS type possesses varying differentiation capacities, determining the best MSC option for a specific regeneration approach would be powerful. DSCs, in this regards, have not been widely investigated. Although researchers have investigated other MSCs to compare differentiation capacities of DSCs towards specific lineages, there is not any systemic comparison studies for DSCs with other MSCs to elucidate advantages and limitations in cell-based regeneration approaches. Additional works should strictly be carried out to explore full-potential and realize use of DSCs in clinics.

References

1. Estrela C, Alencar AHG, Kitten GT, Vencio EF, Gava E (2011) Mesenchymal stem cells in the dental tissues: perspectives for tissue regeneration. Braz Dent J 22(2):91–98
2. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97(25):13625–13630
3. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100(10):5807–5812
4. Seo B-M, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. The Lancet 364(9429):149–155
5. Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, Sippel C, Hoffmann K (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 24(2):155–165

6. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo B-M, Zhang C, Liu H, Gronthos S, Wang C-Y, Shi S (2006) Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One 1(1):e79
7. Yalvac M, Ramazanoglu M, Rizvanov A, Sahin F, Bayrak O, Salli U, Palotas A, Kose G (2010) Isolation and characterization of stem cells derived from human third molar tooth germs of young adults: implications in neo-vascularization, osteo-, adipo-and neurogenesis. Pharmacogenomics J 10(2):105–113
8. Huang G-J, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 88(9):792–806
9. Robinton DA, Daley GQ (2012) The promise of induced pluripotent stem cells in research and therapy. Nature 481(7381):295–305
10. Dreisch H (1891) Entwicklungsmechanische Studien I. Der Wert der ersten beiden Furchungszellen in der Echinodermenentwickelung. Experimentelle Erzeugung von Teil und Doppelbildungen. Z Wiss Zool 53:160–183
11. Evans MJ, Kaufman MH (1981) Establishment in culture of pluripotential cells from mouse embryos. Nature 292(5819):154–156
12. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391): 1145–1147
13. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676
14. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M (2002) Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 418(6893):41–49
15. Verfaillie CM (2002) Adult stem cells: assessing the case for pluripotency. Trends Cell Biol 12(11):502–508
16. Riekstina U, Cakstina I, Parfejevs V, Hoogduijn M, Jankovskis G, Muiznieks I, Muceniece R, Ancans J (2009) Embryonic stem cell marker expression pattern in human mesenchymal stem cells derived from bone marrow, adipose tissue, heart and dermis. Stem Cell Rev 5(4):378–386
17. Nekanti U, Rao VB, Bahirvani AG, Jan M, Totey S, Ta M (2010) Long-term expansion and pluripotent marker array analysis of Wharton's jelly-derived mesenchymal stem cells. Stem Cells Dev 19(1):117–130
18. Kögler G, Sensken S, Wernet P (2006) Comparative generation and characterization of pluripotent unrestricted somatic stem cells with mesenchymal stem cells from human cord blood. Exp Hematol 34(11):1589–1595
19. Salingcarnboriboon R, Yoshitake H, Tsuji K, Obinata M, Amagasa T, Nifuji A, Noda M (2003) Establishment of tendon-derived cell lines exhibiting pluripotent mesenchymal stem cell-like property. Exp Cell Res 287(2):289–300
20. Kuznetsov SA, Mankani MH, Robey PG (2000) Effect of serum on human bone marrow stromal cells: ex vivo expansion and in vivo bone formation. Transplantation 70(12):1780–1787
21. Hirata TM, Ishkitiev N, Yaegaki K, Calenic B, Ishikawa H, Nakahara T, Mitev V, Tanaka T, Haapasalo M (2010) Expression of multiple stem cell markers in dental pulp cells cultured in serum-free media. J Endod 36(7):1139–1144
22. Morito A, Kida Y, Suzuki K, Inoue K, Kuroda N, Gomi K, Arai T, Sato T (2009) Effects of basic fibroblast growth factor on the development of the stem cell properties of human dental pulp cells. Arch Histol Cytol 72(1):51–64
23. Kawanabe N, Murata S, Fukushima H, Ishihara Y, Yanagita T, Yanagita E, Ono M, Kurosaka H, Kamioka H, Itoh T (2012) Stage-specific embryonic antigen-4 identifies human dental pulp stem cells. Exp Cell Res 318(5):453–463
24. Ronay V, Belibasakis G, Schmidlin P, Bostanci N (2012) Infected periodontal granulation tissue contains cells expressing embryonic stem cell markers. A pilot study. Schweiz Monatsschr Zahnmed 123(1):12–16

25. Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi S, Pereira LV, Caplan AI, Cerruti HF (2006) Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 184(3–4):105–116
26. Ferro F, Spelat R, D'Aurizio F, Puppato E, Pandolfi M, Beltrami AP, Cesselli D, Falini G, Beltrami CA, Curcio F (2012) Dental pulp stem cells differentiation reveals new insights in Oct4A dynamics. PLoS One 7(7):e41774
27. Ikeda E, Yagi K, Kojima M, Yagyuu T, Ohshima A, Sobajima S, Tadokoro M, Katsube Y, Isoda K, Kondoh M (2008) Multipotent cells from the human third molar: feasibility of cell-based therapy for liver disease. Differentiation 76(5):495–505
28. Koyama N, Okubo Y, Nakao K, Bessho K (2009) Evaluation of pluripotency in human dental pulp cells. J Oral Maxillofac Surg 67(3):501–506
29. Liu L, Wu L, Wei X, Ling J (2014) Induced overexpression of Oct4A in human dental pulp cells enhances pluripotency and multilineage differentiation capability. Stem Cells Dev 24(8):962–972
30. Atari M, Barajas M, Hernández-Alfaro F, Gil C, Fabregat M, Ferres Padro E, Giner L, Casals N (2011) Isolation of pluripotent stem cells from human third molar dental pulp. Histol Histopathol 26(7):1057–1070
31. Atari M, Gil-Recio C, Fabregat M, García-Fernández D, Barajas M, Carrasco MA, Jung H-S, Alfaro FH, Casals N, Prosper F (2012) Dental pulp of the third molar: a new source of pluripotent-like stem cells. J Cell Sci 125(14):3343–3356
32. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4):315–317
33. Cui X, Chen L, Xue T, Yu J, Liu J, Ji Y, Cheng L (2015) Human umbilical cord and dental pulp-derived mesenchymal stem cells: Biological characteristics and potential roles in vitro and in vivo. Mol Med Rep 11(5):3269–3278
34. Hilkens P, Gervois P, Fanton Y, Vanormelingen J, Martens W, Struys T, Politis C, Lambrichts I, Bronckaers A (2013) Effect of isolation methodology on stem cell properties and multilineage differentiation potential of human dental pulp stem cells. Cell Tissue Res 353(1):65–78
35. Karamzadeh R, Eslaminejad MB, Aflatoonian R (2012) Isolation, characterization and comparative differentiation of human dental pulp stem cells derived from permanent teeth by using two different methods. J Vis Exp 69:e4372
36. Davies O, Smith A, Cooper P, Shelton R, Scheven B (2014) The effects of cryopreservation on cells isolated from adipose, bone marrow and dental pulp tissues. Cryobiology 69(2): 342–347
37. Shi S, Robey P, Gronthos S (2001) Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. Bone 29(6):532–539
38. Yamada Y, Fujimoto A, Ito A, Yoshimi R, Ueda M (2006) Cluster analysis and gene expression profiles: a cDNA microarray system-based comparison between human dental pulp stem cells (hDPSCs) and human mesenchymal stem cells (hMSCs) for tissue engineering cell therapy. Biomaterials 27(20):3766–3781
39. Ramamoorthi M, Bakkar M, Jordan J, Tran SD (2015) Osteogenic potential of dental mesenchymal stem cells in preclinical studies: a systematic review using modified ARRIVE and CONSORT guidelines. Stem Cells Int 2015:1–28
40. Yu J, Wang Y, Deng Z, Tang L, Li Y, Shi J, Jin Y (2007) Odontogenic capability: bone marrow stromal stem cells versus dental pulp stem cells. Biol Cell 99(8):465–474
41. Davies O, Cooper P, Shelton R, Smith A, Scheven B (2014) A comparison of the in vitro mineralisation and dentinogenic potential of mesenchymal stem cells derived from adipose tissue, bone marrow and dental pulp. J Bone Miner Metab. doi:10.1007/s00774-014-0601-y
42. Lee SH, Ryu J-S, Lee J-W, Kwak DH, Ko K, Choo Y-K (2010) Comparison of ganglioside expression between human adipose-and dental pulp-derived stem cell differentiation into osteoblasts. Arch Pharm Res 33(4):585–591

43. Hung C-N, Mar K, Chang H-C, Chiang Y-L, Hu H-Y, Lai C-C, Chu R-M, Ma CM (2011) A comparison between adipose tissue and dental pulp as sources of MSCs for tooth regeneration. Biomaterials 32(29):6995–7005
44. Yu B-H, Zhou Q, Wang Z-L (2014) Periodontal ligament versus bone marrow mesenchymal stem cells in combination with Bio-Oss scaffolds for ectopic and in situ bone formation: a comparative study in the rat. J Biomater Appl 29(2):243–253
45. Park S-Y, Park J-C, Kim M-S, Lee S-E, Kim K-J, Jung B-J, Park W, Jeon D-W, Cho K-S, Kim C-S (2014) Differential effect of water-soluble chitin on collagen synthesis of human bone marrow stem cells and human periodontal ligament stem cells. Tissue Eng Part A. doi:10.1089/ten.tea.2014.0156
46. Liu W, Konermann A, Guo T, Jäger A, Zhang L, Jin Y (2014) Canonical Wnt signaling differently modulates osteogenic differentiation of mesenchymal stem cells derived from bone marrow and from periodontal ligament under inflammatory conditions. Biochim Biophys Acta 1840(3):1125–1134
47. Park B-W, Kang E-J, Byun J-H, Son M-G, Kim H-J, Hah Y-S, Kim T-H, Kumar BM, Ock S-A, Rho G-J (2012) In vitro and in vivo osteogenesis of human mesenchymal stem cells derived from skin, bone marrow and dental follicle tissues. Differentiation 83(5):249–259
48. Morsczeck C, Schmalz G, Reichert TE, Völlner F, Saugspier M, Viale-Bouroncle S, Driemel O (2009) Gene expression profiles of dental follicle cells before and after osteogenic differentiation in vitro. Clin Oral Investig 13(4):383–391
49. Ponnaiyan D, Jegadeesan V (2014) Comparison of phenotype and differentiation marker gene expression profiles in human dental pulp and bone marrow mesenchymal stem cells. Eur J Dent 8(3):307–313
50. Song DS, Park JC, Jung IH, Choi SH, Cho KS, Kim CK, Kim CS (2011) Enhanced adipogenic differentiation and reduced collagen synthesis induced by human periodontal ligament stem cells might underlie the negative effect of recombinant human bone morphogenetic protein-2 on periodontal regeneration. J Periodontal Res 46(2):193–203
51. Lee Y-M, Shin S-Y, Jue S-S, Kwon I-K, Cho E-H, Cho E-S, Park S-H, Kim E-C (2013) The role of PIN1 on odontogenic and adipogenic differentiation in human dental pulp stem cells. Stem Cells Dev 23(6):618–630
52. Dong R, Yao R, Du J, Wang S, Fan Z (2013) Depletion of histone demethylase KDM2A enhanced the adipogenic and chondrogenic differentiation potentials of stem cells from apical papilla. Exp Cell Res 319(18):2874–2882
53. Kim D, Kim J, Hyun H, Kim K, Roh S (2014) A nanoscale ridge/groove pattern arrayed surface enhances adipogenic differentiation of human supernumerary tooth-derived dental pulp stem cells in vitro. Arch Oral Biol 59(8):765–774
54. Alge DL, Zhou D, Adams LL, Wyss BK, Shadday MD, Woods EJ, Chu G, Goebel WS (2010) Donor-matched comparison of dental pulp stem cells and bone marrow-derived mesenchymal stem cells in a rat model. J Tissue Eng Regen Med 4(1):73–81
55. Pierdomenico L, Bonsi L, Calvitti M, Rondelli D, Arpinati M, Chirumbolo G, Becchetti E, Marchionni C, Alviano F, Fossati V (2005) Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. Transplantation 80(6):836–842
56. Yu S, Long J, Yu J, Du J, Ma P, Ma Y, Yang D, Fan Z (2013) Analysis of differentiation potentials and gene expression profiles of mesenchymal stem cells derived from periodontal ligament and Wharton's jelly of the umbilical cord. Cells Tissues Organs 197(3):209–223
57. Nemeth CL, Janebodin K, Yuan AE, Dennis JE, Reyes M, Kim D-H (2014) Enhanced chondrogenic differentiation of dental pulp stem cells using nanopatterned PEG-GelMA-HA hydrogels. Tissue Eng Part A 20(21–22):2817–2829
58. Choi S, Cho T-J, Kwon S-K, Lee G, Cho J (2013) Chondrogenesis of periodontal ligament stem cells by transforming growth factor-β 3 and bone morphogenetic protein-6 in a normal healthy impacted third molar. Int J Oral Sci 5(1):7–13
59. Foudah D, Monfrini M, Donzelli E, Niada S, Brini AT, Orciani M, Tredici G, Miloso M (2014) Expression of neural markers by undifferentiated mesenchymal-like stem cells from different sources. J Immunol Res 2014(1–16)

60. Xiao L, Tsutsui T (2013) Human dental mesenchymal stem cells and neural regeneration. Hum Cell 26(3):91–96
61. Karaöz E, Demircan PC, Sağlam Ö, Aksoy A, Kaymaz F, Duruksu G (2011) Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow-derived mesenchymal stem cells. Histochem Cell Biol 136(4):455–473
62. Aanismaa R, Hautala J, Vuorinen A, Miettinen S, Narkilahti S (2012) Human dental pulp stem cells differentiate into neural precursors but not into mature functional neurons. Stem Cell Discov 2(3):85–91
63. Kiraly M, Porcsalmy B, Pataki A, Kadar K, Jelitai M, Molnar B, Hermann P, Gera I, Grimm W-D, Ganss B (2009) Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons. Neurochem Int 55(5):323–332
64. Song M, Jue SS, Cho YA, Kim EC (2015) Comparison of the effects of human dental pulp stem cells and human bone marrow-derived mesenchymal stem cells on ischemic human astrocytes in vitro. J Neurosci Res 93(6):973–983
65. Kim J-J, Kim S-J, Kim Y-S, Kim S-Y, Park S-H, Kim E-C (2012) The role of SIRT1 on angiogenic and odontogenic potential in human dental pulp cells. J Endod 38(7):899–906
66. Amin HD, Olsen I, Knowles J, Dard M, Donos N (2014) A tyrosine-rich amelogenin peptide promotes neovasculogenesis in vitro and ex vivo. Acta Biomater 10(5):1930–1939
67. d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, Papaccio G (2007) Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ 14(6):1162–1171
68. Sakai V, Zhang Z, Dong Z, Neiva K, Machado M, Shi S, Santos C, Nör J (2010) SHED differentiate into functional odontoblasts and endothelium. J Dent Res 89(8):791–796
69. Marchionni C, Bonsi L, Alviano F, Lanzoni G, Di Tullio A, Costa R, Montanari M, Tazzari P, Ricci F, Pasquinelli G (2009) Angiogenic potential of human dental pulp stromal (stem) cells. Int J Immunopathol Pharmacol 22(3):699–706
70. Saghiri MA, Asatourian A, Sorenson CM, Sheibani N (2015) Role of angiogenesis in endodontics: contributions of stem cells and proangiogenic and antiangiogenic factors to dental pulp regeneration. J Endod. doi:10.1016/j.joen.2014.12.019
71. Yeasmin S, Ceccarelli J, Vigen M, Carrion B, Putnam AJ, Tarle SA, Kaigler D (2013) Stem cells derived from tooth periodontal ligament enhance functional angiogenesis by endothelial cells. Tissue Eng Part A 20(7–8):1188–1196
72. Hilkens P, Fanton Y, Martens W, Gervois P, Struys T, Politis C, Lambrichts I, Bronckaers A (2014) Pro-angiogenic impact of dental stem cells in vitro and in vivo. Stem Cell Res 12(3):778–790
73. Ishizaka R, Hayashi Y, Iohara K, Sugiyama M, Murakami M, Yamamoto T, Fukuta O, Nakashima M (2013) Stimulation of angiogenesis, neurogenesis and regeneration by side population cells from dental pulp. Biomaterials 34(8):1888–1897
74. Janebodin K, Zeng Y, Buranaphatthana W, Ieronimakis N, Reyes M (2013) VEGFR2-dependent angiogenic capacity of pericyte-like dental pulp stem cells. J Dent Res 92(6):524–531
75. Doğan A, Demirci S, Şahin F (2015) In vitro differentiation of human tooth germ stem cells into endothelial-and epithelial-like cells. Cell Biol Int 39(1):94–103
76. Nam H, Lee G (2009) Identification of novel epithelial stem cell-like cells in human deciduous dental pulp. Biochem Biophys Res Commun 386(1):135–139
77. Stevens A, Zuliani T, Olejnik C, LeRoy H, Obriot H, Kerr-Conte J, Formstecher P, Bailliez Y, Polakowska RR (2008) Human dental pulp stem cells differentiate into neural crest-derived melanocytes and have label-retaining and sphere-forming abilities. Stem Cells Dev 17(6): 1175–1184
78. Ishkitiev N, Yaegaki K, Kozhuharova A, Tanaka T, Okada M, Mitev V, Fukuda M, Imai T (2013) Pancreatic differentiation of human dental pulp CD117+ stem cells. Regen Med 8(5):597–612
79. Lee JS, An SY, Kwon IK, Heo JS (2014) Transdifferentiation of human periodontal ligament stem cells into pancreatic cell lineage. Cell Biochem Funct 32(7):605–611

80. Ishkitiev N, Yaegaki K, Imai T, Tanaka T, Fushimi N, Mitev V, Okada M, Tominaga N, Ono S, Ishikawa H (2014) Novel management of acute or secondary biliary liver conditions using hepatically differentiated human dental pulp cells. Tissue Eng Part A 31(3–4):586–593
81. Ishkitiev N, Calenic B, Aoyama I, Ii H, Yaegaki K, Imai T (2012) Hydrogen sulfide increases hepatic differentiation in tooth-pulp stem cells. J Breath Res 6(1):1142–1150

Chapter 7
Induced Pluripotent Stem Cells Derived from Dental Stem Cells: A New Tool for Cellular Therapy

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7.1 Introduction

Currently, fetal, neonatal, or adult fibroblasts are the main cell types used for reprogramming of human somatic cells into the pluripotent state. These cell sources seem to be highly accessible for generating human iPSCs and already show great promise in future clinical applications. However, dermal fibroblasts, as well as foreskin or hair keratinocytes and, as a consequence, adult stem cells derived from these sources, are strongly exposed to environmental factors, which can compromise their use as genetic models and therapeutic tools [1, 2]. The question then becomes "which source of donor cells is the best for iPSCs generation". The biological and functional characteristics of donor cells, their ability to produce iPSCs and to differentiate efficiently into cell types of interest in therapeutics, as well as safety and tolerance concerns after transplantation, initially as determined in preclinical models, will help us to answer this question.

Findings demonstrate that successful generation of iPSCs may be easier if these iPSCs originate from actively dividing cells rather than from slow or non-dividing cells [3]. Dental tissues (DTs) contain mesenchymal stem cells (MSCs), which are multipotent cells of neural crest origin similar to dermal fibroblasts, and foreskin or hair keratinocytes [4, 5]. These cells rapidly proliferate in vitro and are able to differentiate into mesenchymal tissues such as bone, cartilage, muscle, ligament, tendon and adipose tissue [6]. Additionally, they are able to produce ectopic dentin and related pulp tissues, and neural cells. A variety of SC sources derived from DTs have been studied. SCs from deciduous teeth are especially useful because they are young and mainly healthy cells, which is good for clinical use, plus they can be used to study pediatric diseases [6–8].

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7.2 DTs as a Source of Stem Cells (SCs)

The embryonic counterparts of adult DSCs are cranial neural crest derived multipotent dental MSCs. These cells, after neurulation, migrate away from the neural tube into developing craniofacial tissues. Following the developmental courses of determination and differentiation, they give rise to all structures of the tooth and its supporting tissues, except enamel. They also robustly contribute to central nervous system (CNS) formation. Thus, DTs as well as CNS both derive from the embryonic ectoderm [9].

Primary (baby) teeth start their development during prenatal life, and are formed between the 6–8th week of fetal development. Nonetheless, SCs derived from pulp of deciduous teeth, due to ethical considerations, can only be isolated when baby teeth start to spontaneously fall out, which usually happens when children are between 5–10 years old, within a relatively short time interval in comparison to the human normal life span. Baby teeth are habitually discarded, thus dental pulp derived from these teeth represents a healthy, available source of SCs, which is free from the ethical considerations associated with human embryonic stem (ES) cells isolation [6].

The process known as exfoliation occurs when the last primary tooth falls out and permanent teeth start to form, usually at 11 to 12 years of age. Extraction of permanent teeth is ethically more problematic then that of baby teeth, and can happen only if such procedure is necessary for oral health, as determined by the dentist. The extraction of permanent teeth is uncomfortable for donors and requires medical attention. Generally, dental pulp is extracted from vital teeth of healthy adults; however, the majority of these teeth are extracted due to severe periodontal disease, the need to fabrication complete dentures, etc. All of the above is also true in respect to third molars (also called "wisdom teeth"), which are a type of permanent teeth frequently extracted because of decay, pain or impaction. Impaction occurs when an "impacted" tooth has failed to fully emerge in its expected position and needs to be extracted.

Dental follicles, periodontal ligament tissue, gingiva and apical papilla are additional DT sources that can be used for stem cell isolation. The periodontal ligament (PDL), the supporting tooth structure, is differentiated from the dental follicle (sac containing the developing tooth) and consists of the cementum, periodontal ligaments, gingiva and alveolar bone. PDL stem cells (PDLSCs) can be collected from the root surface after permanent, deciduous or third molar tooth extraction, while SCs from gingival tissues can be obtained from remnant or discarded tissue following routine dental procedures from human donors with relatively healthy periodontium and without previous history of periodontal disease. Finally, SCs can be isolated from the apical papilla (SCAPs), which contributes to tooth formation. This tissue is known as "apical" because as the root continues to develop after the bell stage (fetal stage of tooth development), the dental papilla localization is apical to the pulp tissue (reviewed by [10, 11]).

As mentioned before, SCs are usually extracted from vital DT of healthy adults, nevertheless the majority of the teeth themselves are extracted due to medical indication and severe inflammation occurring in DT, which can impair the quality of isolated SCs, debilitating their differentiation capacity into bone, for

example [12]. The age of the donor of DT can also be important for the quality of SCs, as previously reported [13]. Although every type of DSCs can be used for iPSCs generation, to date, the SCs isolated are from apical papilla-SCAPs, from pulp tissue of primary deciduous teeth, such as SHEDs (human exfoliated deciduous teeth) and iDPSCs (immature dental pulp stem cells), as well as pulp tissues from permanent (DPSCs) and from wisdom teeth (TGSCs) were used [14–23].

7.3 Expression of Pluripotent Markers in DSCs

Pluripotent transcription factors, such as, KLF4, a member of the Krüppel-like factor (KLF) family, OCT-4 (octamer-binding transcription factor 4) also known as POU5F1 (POU domain, class 5, transcription factor 1) and SOX-2 (SRY (sex determining region Y)-box 2) are highly expressed in ES cells, thus regulating the developmental signaling network necessary for ES cell pluripotency. The overexpression of these factors induces reprogramming of both mouse and human somatic cells into the embryonic stage [24]. The expression of PSC markers such as OCT-4, NANOG, SOX-2 and STAT-3 is observed during different fetal stages of tooth germ development [25, 26]. During adulthood, in situ expression of OCT-4 has been found in the dental pulp of deciduous teeth in the cell-rich zone which contains fibroblasts and undifferentiated mesenchymal cells, and also in the cell-free zone, which is rich in both capillaries and nerve networks [27]. Recently, this finding was confirmed by another group [10]. Interestingly, OCT-4 expression is also maintained in a small amount of iDPSCs ($\geq$10–20 %) after isolation and in vitro cultivation. The OCT-4 transcription factor is critical for pluripotency and multilineage differentiation potential of SCs and its expression, even at low levels, in DSCs may play a critical role in reprogramming [5, 6, 10].

7.4 DSC Reprogramming

Freshly isolated DSCs are plastic-adherent, present common fibroblast-like morphology (Fig. 7.1a), are clonogenic and express a set of markers, which, as the scientific community has determined, is typical of MSCs. Furthermore, these cells are able to differentiate into osteoblasts, adipocytes and chondroblasts in vitro [28]. The majority of SCs isolated from dental pulp seems to be of multipotent MSCs rather than of multipotent neural crest cells. Such multipotent MSCs derived from DTs preserve their main characteristics, such as immunophenotype, proliferation rate and differentiation potentials, unchanged over several passages (up to 25) of in vitro cultivation [5]. However, culture characteristics certainly depend on method of isolation, enzymatic digestion or explant culture, and cultivation-different culture media can be used supplemented, or not, with different growth factors [5].

The first study on iPSCs generation was focused on SHEDs and SCAPs. For reprogramming, a lentiviral vector carrying C-MYC (Myc proto-oncogene

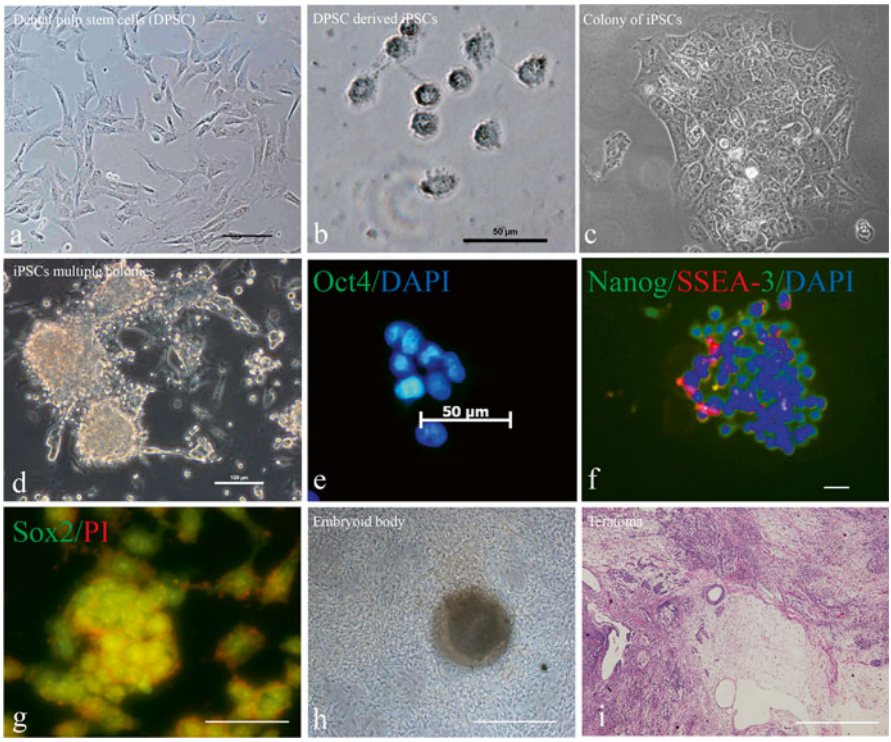

Fig. 7.1 Immature dental pulp derived stem cell (iDPSC)-derived induced pluripotent stem cells (iPSCs). This figure depicts (**a**) iDPSCs before reprogramming; (**b**) Morphological changes observed in iDPSCs after reprogramming; (**c**) A colony of DSC-derived iPSCs; (**d**) Multiple iDPSC-derived iPSC colonies. (**e–g**) Expression of transcription factors, such as, OCT-4 (**e**), NANOG (**f**) and SOX-2 (**g**) in the nuclei (*green*) and cytoplasm (*red*) SSEA-4 (**f**), of iDPSC-derived iPSCs. (**h**) In vitro differentiation, through embryoid body (EB) formation, of iDPSC-derived iPSCs. Differentiated cells can be observed around EB. (**i**) Haematoxylin- and Eosin-stained teratoma sections obtained 3 months after intramuscular injection of iDPSC-derived iPSCs into nude mice. (**a–d**, **h**, **i**) Light microscopy and Phase contrast (except of **i**). (**e–g**) Epifluorescence. Scale bars: (**a**, **b**, **e**, **g**) = 50 µm; (**f**) = 10 µm; (**c**, **d**, **h**) = 100 µm; (**i**) = 200 µm. PI; Propidium iodide (*red*) and DAPI; 4′,6-diamidino-2-phenylindole (*blue*), DNA dyes. Methodology described in Beltrao-Braga et al. [14]

protein), KLF4, OCT-4 and SOX-2 was used. However, this vector failed to reprogram these cells. Next, another lentiviral vector carrying four factors, this time LIN28 (LIN-28 homolog A), NANOG, OCT-4, and SOX-2, was constructed to obtain iPSCs, but the efficiency of colony generation from SHEDs and SCAPs was still very low. To improve reprogramming efficiency, a retroviral vector carrying the same genes initially studied: C-MYC, KLF4, OCT-4, and SOX-2, was employed and, after a second round of transduction, iPSCs colonies were finally produced. Nevertheless, human fibroblasts, used as a control in this study, were not able to undergo reprogramming under such conditions [22].

Other authors reported reprogramming of DSCs from deciduous teeth iDPSCs, which are different from SHEDs due to method of isolation [6], using a retroviral vector expressing four of Yamanaka's factors (KLF4, OCT-4, C-MYC and SOX-2) [24]. The derived cells so far express a low level of OCT-4 and NANOG [5]. The successful reprogramming of these cells occurs after one round of transduction, with satisfying efficiency: 0.1–1 % or even higher [14] (Fig. 7.1a–i). More recently, an alternative polycistronic lentiviral vector encoding OCT-4, SOX-2, KLF4, and C-MYC [29] with addition of the dTomato reporter gene that allows real-time monitoring of transduction efficiency and silencing of transgenes, was used for successful reprogramming DSCs from deciduous teeth [16].

Reprogramming of DSCs from wisdom teeth, deciduous teeth and human dermal fibroblasts (HDFs) has also been carried out using retroviruses expressing four (OCT-4, SOX-2, KLF4, and C-MYC) or three (without C-MYC) factors [15, 18, 21]. In order to avoid an integration of an RNA virus into the host genome, a vector based on Sendai virus [30] was constructed to generate iPSCs that express the transcription factors encoded by OCT-4, SOX-2, KLF4, and C-MYC from permanent teeth-derived DSCs [19].

7.5 Factors Relevant in Reprogramming

In general, reprogramming requires the use of mouse embryonic fibroblasts (MEF) as a feeder layer for iPSC growth. MEF have been employed in all studies with DSCs reprogramming except one, which succeeded in establishing and propagating iPSCs under feeder-free conditions on matrigel-coated dishes (Fig. 7.1b–d) [14], thus avoiding the contamination of human cells with zoonoses derived from mice cells. This is an essential step in iPSC technology development, especially when iPSCs are obtained for their potential use in cell therapy.

Fetal bovine serum (FBS) is also considered an undesirable component of culture media to obtain SCs for therapeutic use. Although currently FBS can be purchased from companies whose product is originated from FDA-approved regions, where production is followed by extensive inspection and rigorous quality control (www.pan-biotech.com), chemically-defined media protocols that avoid the use of FBS, have already been developed: first, for the isolation and growth of DSCs used for iPSC generation and, second, for generation, growth and differentiation of iPSCs [19]. From a practical point of view, the great disadvantage of this protocol is that, even before iPSC generation, DSCs cultured under chemically-defined conditions show delay in growth dynamics and generate significantly lower number of primary colonies than those obtained in the presence of FBS. Most importantly, under FBS-free conditions, growth of these cells is strongly donor-dependent, and the use of DNA array analyses demonstrates that gene expression patterns are robustly altered in DSCs grown under chemically-defined conditions in comparison with the cells grown in FBS. Another disadvantage of the protocol used is that despite all the care that was taken to avoid FBS use, the iPSCs obtained in this study, before they

were transferred onto matrigel, were grown on SNL Feeder Cells, which are clonally derived from a mouse fibroblast STO cell line. Therefore, potential contamination with mouse-derived zoonoses can still occur by using this protocol [19].

Next, as hypoxia enhances the reprogramming efficiency of HDFs into iPSCs [31], DSCs were also submitted to early and transient hypoxia (3 % O_2) during reprogramming, and under such conditions, the transition of SCs to iPSCs was 3.3- to 5.1-fold higher as compared to that of cells cultured in normoxia (21 % O_2). Interestingly, in contrast to what is observed during HDF reprogramming to iPSCs, when DSC-derived iPSCs are treated with 3 % O_2 during the later stage of reprogramming (from day 6 to day 21), the generation of iPSCs under such conditions is strongly inhibited. There is still no rational explanation for such phenomenon and the authors speculate that metabolic changes may be involved [17].

The process of iPSC isolation is still very costly, largely because of low reprogramming efficiency. It seems that less differentiated somatic cells can be reprogrammed more efficiently than terminally differentiated cells, and even require fewer viruses than fibroblasts for efficient reprogramming [32–34]. Additional factors that may influence reprogramming efficiency are the age of cell donor, cell type and number of transcription factors used [34, 35]. Thus, the efficiency of reprogramming of young DSCs is higher than those obtained from their adult counterparts as well as than that of HDFs and human primary gingival fibroblasts. Additionally, a comparative study between immature and mature teeth derived DSCs converted into pluripotent states has been carried out in order to understand the low reprogramming efficiency of mature human iPSC. This study has shown that only two factors, OCT-4 and SOX-2, are needed for immature teeth SC reprogramming and these factors are not sufficient to convert mature teeth DSCs to iPSCs [14, 16, 18, 21, 22]. The comparison of gene expression profiles between these two DSC groups (immature and mature) unveiled a new transcript factor, distal-less homeobox 4 (DLX4), which is highly expressed in immature teeth DSCs in comparison to mature ones. The suppression of this gene by transforming growth factor beta (TGF-β) impairs iPSC generation. This gene may be the first candidate that can substitute already known transcription factors (*e.g.,* C-MYC oncogene) and make this process safer due to lower cancer risk [20].

Another issue that must be considered when deriving iPSC from DSCs is the system used for gene delivery. The use of lentivirus and retrovirus is efficient; but this technique is not safe since these viruses can integrate into the host DNA, potentially altering gene expression and leading to cancer. Adenoviral delivery of these genes is safer because adenoviral DNA does not integrate into the genome [21]. Experimental approaches, however, have demonstrated that this is not a rule and that retroviral vectors can be transcriptionally silent in iPSCs [18] and that transgene expression from retroviral vectors can even be lost for some reason during reprogramming [14].

Overall, iPSCs derived from DSCs show stable karyotype after reprogramming, although the methylation status of cytosine guanine dinucleotides (CpG) in these cells remains to be clarified due to controversies found in the literature and to the

few data available for analysis [14, 21–23]. Another important marker of undifferentiated PSCs is telomerase activity, which is found to be restored in iPSCs in comparison with the original cells used for iPSC generation [18, 22].

7.6 Transcription Factor Expression in DSC-Derived iPSCs

A very important issue for the potential clinical applications of iPSCs, which has been less studied, is that of transcription factor expression in iPSCs after reprogramming in comparison with naturally developed human ES cells derived from human embryos. Two recent studies reported that transcription factor expression in iPSCs is similar to that of human ES cells [17, 20]. Nevertheless, we have previously demonstrated that although immunofluorescence analyses reveal expression of OCT-4, NANOG and SOX-2 in iPSCs derived from iDPSCs of deciduous teeth (Fig. 7.1e–g), molecular analysis shows a low level of expression of all factors (OCT-4, NANOG and SOX-2), and, especially, of NANOG (Fig. 7.1f) in iPSCs [14]. This low level of expression of PSC factors apparently does not affect their ability to form embryoid bodies (EBs, spherical structures with cystic cavities resembling early embryos, albeit, chaotically organized) and teratomas upon in vivo implantation-a "gold standard" to test pluripotency of ES cells. In spite of the lower expression of transcription factors, as compared with previous studies [17, 20], these iPSCs demonstrate very robust differentiation within teratomas and strong neuronal commitment in vitro and in vivo [14].

7.7 Differentiation Potential

The differentiation potential of iPSCs derived from DSCs has been studied using conventional models: in vitro formation and differentiation of EB (Fig. 7.1h); and in vivo teratoma generation (Fig. 7.1i). All authors have reported the capacity of these cells to produce the wide spectrum of cells derived of the three germ layers (mesoderm, endoderm and ectoderm) (Fig. 7.1i); moreover, neuronal differentiation is a "trademark" of DSCs derived iPSCs [14, 15].

The HOX proteins participate in many common developmental processes during normal embryogenesis and the vertebrate nervous system is a major site of HOX gene expression and function. Furthermore, they play a key role in extending our understanding of the CNS development [36]. Additional evidence that DSC-derived iPSCs have predominantly a neuronal fate was recently provided. Expression profiling of HOX genes by neurons differentiated from DSC-derived iPSCs and HDF-iPSCs was compared and showed a high degree of correlation for the two sources of neurons; nevertheless, they differ in the expression of some important genes, especially in several members of the HOX gene families. Lower levels of expression for genes involved in hindbrain development are

observed in the neurons differentiated from DSC-derived iPSCs as compared with HDF-iPSCs. In contrast, several transcription factors involved in the forebrain development are considerably increased, such as FOXP2 (Forkhead box 2 encoded by the FOXP2 gene, also known as CAGH44, SPCH1 or TNRC10, and required for proper development of speech and language), OTX1 (orthodenticlehomeobox 1, which encodes a member of the bicoid sub-family of homeodomain-containing transcription factors and may play a role in brain and sensory organ development), and LHX2 (LIM/homeoboxprotein that specifies cortical identity and suppresses hippocampal organization fate). These transcription factors are involved in the development of communicative and linguistic neural networks [37–41]. Such difference might influence the decision to use DSC-or HDF-derived iPSCs in neuropsychiatric disorder studies and treatments, such as in schizophrenia and autism spectrum disorders [42].

Interestingly, spontaneous (without the use of specific differentiation inducing agents in culture medium) robust neuronal and endothelial differentiations of DSC-derived iPSCs have been demonstrated [16]. Furthermore, when a chemically-defined protocol to isolate DSC-iPSCs focusing on their safe establishment was used, significantly less primary colony formation was observed with respect to the protocols using FBS. Although the DNA array analyses indicate that the culture conditions robustly alter DSCs gene expression patterns, DSC-derived iPSCs grown under defined conditions show a donor-dependent growth capacity but the differentiation capacity of these cells is not changed in comparison to that of DSC-derived iPSCs grown with FBS [19, 43].

Regarding cell differentiation to improve vascularization, two factors, OCT-4 and SOX-2, have been used to produce iPSCs (2 factors (2F)–iPSCs). After the addition of basic fibroblast growth factor (bFGF) and vascular endothelial growth factor A (VEGF A) to the culture medium, the effective in vitro differentiation of DSC-derived iPSCs into functional endothelial progenitor cells (EPCs) and smooth muscle cells has been shown. The global transcriptomic analysis of iPSC-derived EPCs and endothelial cells (ECs) demonstrates limited variations in gene expression similar to those of EPCs and ECs derived from human ES cells. However, evaluation of the expression of CD31 in iPSCs-derived EPCs and ECs suggests that they are highly heterogeneous in respect to the presence of the cell populations: arterial, venous, and lymphatic cell subtypes. Such heterogeneity is genetically controlled by the multistep regulatory system associated with key signaling pathways and transcription factors before circulation begins [43]. Therefore, to validate this method, it should be determined whether iPSC-derived EPCs cultured in vitro are associated with the same signaling pathways that control cells during early embryonic development in vivo. On the other hand, in order to understand heterogeneity, we should determine functional benefits of each subtype of arterial, venous, and lymphatic endothelial cells to establish the protocol for optimal differentiation of iPSC-derived EPCs. The heterogeneity aforementioned also strongly indicates the immature state of iPSC-derived EPCs, which increases the possibility of teratoma formation or even tumor development. Regarding endothelial cell differentiation, it is also worth mentioning the matrigel plug angiogenesis

assay, which is a simple in vivo technique to detect newly formed blood vessels in transplanted gel plugs in nude mice. This assay was used and confirmed the angiogenic and neovasculogenic capacities of 2F-hEPCs [23].

7.8 DSC-Derived iPSCs and Disease Modeling

Neurodegenerative diseases combine a wide range of pathogeneses which affect neurons in the human brain and spinal cord. When neurons become damaged or die, they cannot be replaced rapidly by natural sources of SCs in the human body. Such diseases result in progressive degeneration, which causes problems with movement (ataxias), or mental functioning (dementias). The list of such diseases includes Parkinson's, Alzheimer's, and Huntington's diseases. They are incurable and the lack of effective treatments for various neurodegenerative disorders has placed an enormous burden on society. iPSC technology has emerged as a powerful tool for in vitro modeling of neurodegenerative diseases, the study of their cellular and molecular mechanisms, and drug screening and cell therapy for their treatment (Fig. 7.2). Before iPSC technology became a reality, researchers used post-mortem tissues, which often are not available and frequently obtained at the last stage of disease, or, alternatively, from transgenic animals. Both approaches are not able to fully reproduce the course of human disease or development of cells with neural phenotypes [44, 45]. The establishment of iPSC lines from patients has been an essential and novel step in medicine and biotechnology (Fig. 7.2). Thus, in 2008, the first iPSCs derived from patients with genetic diseases, including Parkinson's and Huntington's diseases, were obtained. The majority of iPSC lines were able to maintain the patient genotype and phenotype in vitro, while, for other diseases, further phenotypic confirmation is needed [37, 46–48]. Moreover, because of the non-invasive method of DT isolation, DSCs are important source of iPSC for modeling and investigating pediatric diseases [49].

DSCs present clear advantages over commonly used skin fibroblasts and other somatic cell types because of the easy access to DT with minimum discomfort for the patient, their rapid cell proliferating, young donor age, and lower exposure to environmental factors such as ultraviolet irradiation [6]. Clonal variation among pluripotent SCs is also a very important factor, which seems to be less problematic in iPSCs from DSCs [23]. Furthermore, normal ES cells derived from human blastocysts and iPSCs derived from fibroblasts of the same donor show the variable neuronal differentiation potentials [50, 51]. In vitro differentiation of iPSCs derived from DSCs occurs practically spontaneously [14, 15]. Despite high neuronal commitment and great potential in research in neurological conditions and disorders, only one study used DSCs to generate patient-specific iPSCs for modeling of non-syndromic autism and to investigate the impact of TRPC6 (transient receptor potential cation channel, subfamily C, member 6) disruption in human neurons [52]. This group identified the disruption of the TRPC6 gene by a balanced de novo

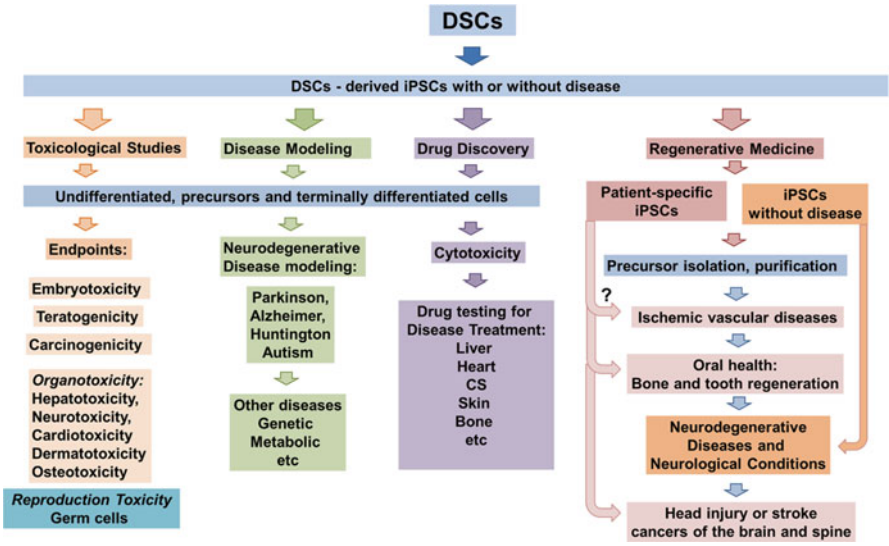

Fig. 7.2 Near and Not-So-Near Future of dental stem cell (DSC)-Derived induced pluripotent stem cells (iPSCs). *Near*: This figure depicts that DSC-derived iPSCs can originate from healthy or diseased donors. Both these cell types can be used in toxicological studies, disease modeling and drug discovery. The great advantage of iPSCs is that they can be used undifferentiated and as precursors of cell lineages of many types upon differentiation. In toxicological studies, multiple endpoints can be evaluated in two dimension culture systems (organotoxicity) or 3D systems (embryotoxicity, embryoid body models), other cell culture models and even germ cells, which can be produced in vitro; iPSCs derived from donors with diseases provide unique tools to study molecular and cellular mechanisms of diseases of interest, which help to understand the etiology of this disease and forms of treatment. To study neurodegenerative diseases, DSCs-derived iPSCs are particularly important tools, since the original cells tend to be committed to neural differentiation. DSC-derived iPSCs are potentially important in drug discovery and can be used for cytotoxic endpoint assays, as well as to test a variety of drugs in both undifferentiated and differentiated cells, and especially to evaluate the drug effect on differentiation pathways throughout the daisy chain of intermediates. *Not-So-Near Future:* DSCs-derived iPSCs without disease are of great interest in regenerative medicine. However, many questions still need to be answered. Patient-specific iPSCs can be used in autologous treatments, however when genetic disease issues need to be addressed, the use of autologous cells is not welcome and therefore, allogenic iPSCs without disease are recommended. In addition, patients with a family history of ischemic vascular disease should likewise avoid the use of autologous iPSCs. In both cases, need an additional in vitro iPSCs manipulation in order to produce more mature and safe precursors. Finally, we would like to emphasize once again that DSCs-derived iPSCs use is highly recommended to treat neurological diseases

translocation in a non-syndromic autism spectrum disorder (ASD) individual. This gene is involved in the regulation of axonal guidance, dendritic spine growth and excitatory synapse formation [53, 54]. Generation of DSC-derived iPSCs from the ASD individual allowed this group to explore the functional consequences of TRPC6 disruption in human neuronal cells. Overall, they demonstrated that patient-specific iPSC-derived neurons can be used to associate novel variants to

ASD patients to study the etiology of these disorders [52]. Finally, the importance of DSC-derived iPSCs for neuropsychiatric disorders, such as schizophrenia and ASD, has also been suggested [42].

7.9 Drug Discovery and Cytotoxicity Studies

Drug discovery today has been very unsuccessful considering time and capital investment, with several drugs failing in the clinical trials phases due to lack of efficiency or safety. This should not be happening, considering the major progress associated with chemical synthesis technologies, the large amount of data derived from "omics" initiatives (genomics, epigenomics, transcriptomics, proteomics, metabolomics, among others), the advances in analytical sciences and the possibility of high-throughput screening. However, the absence of adequate models of human disease for drug screening, which include cell lines, is probably one of the reasons for the high failure rate in this field.

Traditional drug discovery uses animal and human cell lines established long ago that poorly reflect the in vivo biology. Drug screening and toxicological studies often require cells from highly differentiated tissues or from tissues where there is no cell proliferation, such as neurons, cardiomyocytes and some gland cells. Toxicological studies, which are carried out in parallel with drug discovery to weed out highly toxic drug candidates, require, for instance, hepatocytes and cardiomyocytes. Liver cells are important since they receive high quantities of drugs in the first pass stage of distribution, and also because they carry out drug metabolism, which may lead to the generation of highly toxic compounds. Hepatocytes are known to be highly capable of proliferation. However, hepatocyte-derived cell lines rarely maintain all metabolic routes necessary for drug metabolism and safety screening. As for cardiomyocytes, these cells typically do not proliferate in vivo after development, which is why it has been hard to establish cardiomyocyte models. However, they are of major importance in drug discovery and toxicity studies, given the many forms of heart disease on one hand, and the fact that heart damage by drugs being a reason for abandonment of drug development projects. Likewise, drug screening and toxicity studies lack good models of neuronal cells, which are also hard to establish in spite of their need in the discovery of drugs that are used to treat central nervous system disorders and in toxicological protocols to prevent neurotoxicity of new drugs.

Most of the above considerations can be addressed using patient-specific iPSCs. iPSCs with genetic disorders are important models which allow, upon differentiation, studying the effect of genetic mutations on cell function and drug discovery screening on these diseased models (Fig. 7.2). In particular, drug discovery can profit from iPSCs and differentiated cells derived from diseased individuals, whereby the total diversity of genetic background of the individual can be considered (Fig. 7.2). This is particularly true nowadays, when whole genomic sequencing, or, at least, polymorphism screening, are straightforward. Most common diseases are associated with combinations of genetic polymorphisms [55], and the development of cell lines

obtained from many patients can be useful in drug discovery and toxicity by addressing this complex genetic background in vitro (Fig. 7.2). It is likely that such cell lines can improve drug screening success by indicating which subpopulations will respond to drugs and which will be unresponsive or even not tolerate the medication due to toxic side effects. These studies are still in their "infancy" and need to be further improved. Thus, Muotri's group provides insights supporting the testing of novel drugs in patient-specific ASD DSC-derived iPSCs such as hyperforin, a drug that specifically activates TRPC6, or insulin growth factor-1 (IGF-1), which is expected to increase not only TRPC6 protein levels but also other synaptic components [52].

7.10 Regenerative Medicine and Cell Therapy

The main goal of regenerative medicine is to obtain unlimited numbers of a specific cell type, which can be achieved by reprogramming of DSCs into pluripotent state, and establishment of differentiation protocols, which allow direct differentiation of DSC-derived iPSCs into "pure" populations of precursors, which, when introduced back into the organism, are able to produce therapeutically significant numbers of mature differentiated cells with functional capacities that allow total restoration of lost cells, tissues and organs function. However, it seems that, currently, researchers are mainly focused on the methods of iPSCs derivation and cultivation, and less on differentiation potential of these cells, which needs to be more extensively studied from the perspective of iPSC future applications in regenerative medicine.

The neural precursor is a cell type consistently obtained from iPSCs and great progress has been made in the area of neuronal lineage specification, which is highly dependent on imitating in vitro the early patterning signals that convey axial coordinates during neural development. However, in vivo replacement of nerve cells in traumatic or degenerative disorders of the central nervous system (CNS) is still at early stages of development. Over the years, embryoid body formation, co-culture on neural inducing feeders and direct neural induction have been used in the field of directed neural differentiation, which are still complex, long lasting and time consuming [56]. Therefore, the finding that DSC-derived iPSCs are able to undergo spontaneous differentiation into neurons and endothelial cells is of great importance. From a practical point of view, developing protocols for purification of neural and endothelial precursors obtained as a result of spontaneous differentiation of DSC-derived iPSCs may be much more interesting than in vitro induced differentiation. However, this would be nice, if not for a small detail, that we don't know how immature or mature these precursors are. The study, which demonstrates the derivation of EPCs from DSC-derived iPSCs, is an alert for the scientists that we still need to study a lot about iPSC differentiation. These cells have a very strong differentiation potential which is abundant in different cell types, when compared with DSC-derived MSCs from post-natal tissues. Therapeutic use of iPSCs expects that their differentiation occurs not in a "dish", but in particular tissue and organ, and under the complete control of an organism. Hence, teratogenicity, as well as, short- and

long-term tumorigenicity of iPSC-derived precursors must be thoroughly evaluated before any clinical application (Fig. 7.2).

Another popular field of regenerative medicine regards treatment of peripheral arterial disease (PAD). All over the world, PAD affects many people; only in the United States, there are about 10 million individuals who suffer from PAD. The murine hind-limb ischemia preparation is a model of PAD, and is useful for testing new therapies. The advantages of this model are the ease of access to the femoral artery and the low mortality rates. It has been shown that 2F hEPC-iPSCs have a strong ability to produce angiogenic and vasculogenic EPCs. The therapeutic effects of 2F hEPC-iPSC transplantation were verified in mouse models of hind-limb ischemia and myocardial infarction. The 2F-EPCs efficiently incorporated into newly formed vascular structures and enhanced neovascularization in both experimental models. This study recommends a follow up investigation of the use of EPC derived from iPSCs in patient-specific therapies, especially in ischemic vascular diseases [23] (Fig. 7.2).

The origin of cell types is an important factor, which can influence the molecular and functional properties of SCs. Thus, upon reprogramming, iPSCs generally gain new characteristics, but they habitually hold a 'footprint' of the tissue of origin [57–59]. The use of SCs in regenerative medicine and cell therapies, therefore, may dependent on SC origin, which could have significant effects on the outcome, for example, in efficient differentiation and functional properties of cells. DSCs can differentiate into odontoblasts, osteoblasts, endotheliocytes, smooth muscle cells, adipocytes and chondrocytes [5], however, due to their neural crest origin, they may also be a very important source of SCs to be used to repair spinal cord injuries and to prevent or even treat patients with neurological disorders [42] (Fig. 7.2). The dental tissue origin also suggests that DSC-derived iPSCs can potentially have a major impact on oral health [60, 61]. Regeneration of the tooth structure may avoid or delay the loss of the whole tooth. A preclinical study has already focused on tissue engineering of tooth-like structures, although it was developed using non-odontogenic SCs as a cell source [62]. On the other hand, this study is interesting and important, and will allow the comparison of the capacity of non-odontogenic SCs and odontogenic SCs to regenerate tooth structures (Fig. 7.2).

7.11 Near and Not-So-Near Future

The advantage of iPSCs as compared to ES cell lines includes ethical issues-the first are harvested from discarded teeth, without harm to the donors, whereas the later come from non-implanted embryos, which sometimes can be associated with social, ethical and legal complications. The risk aspect is the teratogenic potential of both these cell types as well as the efficiency of inducing differentiation into definite cell types, which can be isolated as pure progenitor populations that will not produce cells with negative features, such as hyperproliferation, tumorigenicity, and ectopic tissue formation. All SCs which are potentially tumorigenic should be eliminated in order to prevent recurrence of malignancies. The safety of iPSCs and iPSC-derived

progenitors can be, for example, evaluated by genome and epigenome analyses, and this may minimize the risk to a level acceptable for clinical trials, but nobody can confirm how the cells will respond until clinical trials are completed.

The iPSCs can be relatively rapidly obtained from DSCs, and this process seems to have good reproducibility, which means a high variety of cell lines will soon be available representing human genetic diversity. Since the human donor is known and alive, the cell lines can be associated with many phenotypic traits that can be useful additional data. Whereas human ES cells can also represent genetic diversity, one cannot have further information on the "donor", since it is destroyed in the process of cell line establishment. Furthermore, iPSCs derived from the DSCs of patients, or close relatives, can differentiate to tissues associated with the disorder (*i.e.* lung tissue, Langerhans cells, cardiomyocytes, etc.) and can be used for therapeutic purposes as well as to screen for effective drugs for that given patient. Currently, such approach is too complex and expensive for the general public. Nevertheless, automation and protocol standardization will probably soon follow, rendering this form of drug "pre-screening" more viable in order to find effective and low-toxicity treatments, especially for very fragile patients.

References

1. Boehnke K, Falkowska-Hansen B, Stark HJ, Boukamp P (2012) Stem cells of the human epidermis and their niche: composition and function in epidermal regeneration and carcinogenesis. Carcinogenesis 33(7):1247–1258
2. Mouret S, Forestier A, Douki T (2012) The specificity of UVA-induced DNA damage in human melanocytes. Photochem Photobiol Sci 11(1):155–162
3. Haase A, Olmer R, Schwanke K et al (2009) Generation of induced pluripotent stem cells from human cord blood. Cell Stem Cell 5(4):434–441
4. Achilleos A, Trainor PA (2012) Neural crest stem cells: discovery, properties and potential for therapy. Cell Res 22(2):288–304
5. Kerkis I, Kerkis A, Dozortsev D et al (2006) Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 184(3–4):105–116
6. Kerkis I, Caplan AI (2012) Stem cells in dental pulp of deciduous teeth. Tissue Eng Part B Rev 18(2):129–138
7. Kerkis I, Kerkis A, Lizier NF, Wenceslau CV (2015) Dental stem cells: risk and responsibilities. In: Bhattacharya N, Stubblefield FG (eds) Regenerative medicine using non-fetal sources of stem cells. Springer, London, pp 171–175
8. Lizier NF, Kerkis I, Wenceslau CV (2013) Generation of induced pluripotent stem cells from dental pulp somatic cells, pluripotent stem cells. In: Bhartiya D (ed) Pluripotent stem cells. inTech, Rijeka. doi:10.5772/55856
9. Shakhova O, Sommer L (2008) Neural crest-derived stem cells. In: Gage F, Watt F (eds) StemBook. The Stem Cell Research Community, Boston, http://www.ncbi.nlm.nih.gov/books/NBK44752/
10. Liu J, Yu F, Sun Y et al (2015) Concise reviews: characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells 33(3):627–638
11. Ponnaiyan D (2014) Do dental stem cells depict distinct characteristics? - Establishing their "phenotypic fingerprint". Dent Res J (Isfahan) 11(2):163–172

12. Park JC, Kim JM, Jung IH et al (2011) Isolation and characterization of human periodontal ligament (PDL) stem cells (PDLSCs) from the inflamed PDL tissue: in vitro and in vivo evaluations. J Clin Periodontol 38(8):721–731
13. Zhang J, An Y, Gao LN, Zhang YJ, Jin Y, Chen FM (2012) The effect of aging on the pluripotential capacity and regenerative potential of human periodontal ligament stem cells. Biomaterials 33(29):6974–6986
14. Beltrao-Braga PC, Pignatari GC, Maiorka PC et al (2011) Feeder-free derivation of induced pluripotent stem cells from human immature dental pulp stem cells. Cell Transplant 20(11–12):1707–1719
15. Chang YC, Li WC, Twu NF et al (2014) Induction of dental pulp-derived induced pluripotent stem cells in the absence of c-Myc for differentiation into neuron-like cells. J Chin Med Assoc 77(12):618–625
16. Dambrot C, van de Pas S, van Zijl L et al (2013) Polycistronic lentivirus induced pluripotent stem cells from skin biopsies after long term storage, blood outgrowth endothelial cells and cells from milk teeth. Differentiation 85(3):101–109
17. Iida K, Takeda-Kawaguchi T, Hada M et al (2013) Hypoxia-enhanced derivation of iPSCs from human dental pulp cells. J Dent Res 92(10):905–910
18. Oda Y, Yoshimura Y, Ohnishi H et al (2010) Induction of pluripotent stem cells from human third molar mesenchymal stromal cells. J Biol Chem 285(38):29270–29278
19. Takeda-Kawaguchi T, Sugiyama K, Chikusa S et al (2014) Derivation of iPSCs after culture of human dental pulp cells under defined conditions. PLoS One 9(12):e115392. doi:10.1371/journal.pone.0115392
20. Tamaoki N, Takahashi K, Aoki H et al (2014) The homeobox gene DLX4 promotes generation of human induced pluripotent stem cells. Sci Rep 4:7283. doi:10.1038/srep07283
21. Tamaoki N, Takahashi K, Tanaka T et al (2010) Dental pulp cells for induced pluripotent stem cell banking. J Dent Res 89(8):773–778
22. Yan X, Qin H, Qu C, Tuan RS, Shi S, Huang GT (2010) iPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. Stem Cells Dev 19(4):469–480
23. Yoo CH, Na HJ, Lee DS et al (2013) Endothelial progenitor cells from human dental pulp-derived iPS cells as a therapeutic target for ischemic vascular diseases. Biomaterials 34(33):8149–8160
24. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676
25. da Cunha JM, da Costa-Neves A, Kerkis I, da Silva MC (2013) Pluripotent stem cell transcription factors during human odontogenesis. Cell Tissue Res 353(3):435–441
26. Tompkins K (2006) Molecular mechanisms of cytodifferentiation in mammalian tooth development. Connective tissue research 47(3):111–118
27. Lizier NF, Kerkis A, Gomes CM et al (2012) Scaling-up of dental pulp stem cells isolated from multiple niches. Plos One 7(6):e39885. doi:10.1371/journal.pone.0039885
28. Dominici M, Le Blanc K, Mueller I et al (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4):315–317
29. Warlich E, Kuehle J, Cantz T et al (2011) Lentiviral vector design and imaging approaches to visualize the early stages of cellular reprogramming. Mol Ther 19(4):782–789
30. Fusaki N, Ban H, Nishiyama A, Saeki K, Hasegawa M (2009) Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci 85(8):348–362
31. Yoshida Y, Takahashi K, Okita K, Ichisaka T, Yamanaka S (2009) Hypoxia enhances the generation of induced pluripotent stem cells. Cell Stem Cell 5(3):237–241
32. Muchkaeva IA, Dashinimaev EB, Terskikh VV, Sukhanov YV, Vasiliev AV (2012) Molecular mechanisms of induced pluripotency. Acta Naturae 4(1):12–22
33. Patel M, Yang S (2010) Advances in reprogramming somatic cells to induced pluripotent stem cells. Stem Cell Rev 6(3):367–680
34. Zouboulis CC, Adjaye J, Akamatsu H, Moe-Behrens G, Niemann C (2008) Human skin stem cells and the ageing process. Exp Gerontol 43(11):986–997

35. Banito A, Rashid ST, Acosta JC et al (2009) Senescence impairs successful reprogramming to pluripotent stem cells. Genes Dev 23(18):2134–2139
36. Nolte C, Krumlauf R (2000) Expression of Hox genes in the nervous system of vertebrates. Madame Curie Bioscience Database [Internet]. Landes Bioscience, Austin (TX)
37. Larsen KB, Lutterodt MC, Mollgard K, Moller M (2010) Expression of the homeobox genes OTX2 and OTX1 in the early developing human brain. J Histochem Cytochem 58(7):669–678
38. Salvatierra J, Lee DA, Zibetti C et al (2014) The LIM homeodomain factor Lhx2 is required for hypothalamic tanycyte specification and differentiation. J Neurosci 34(50):16809–16820
39. Vargha-Khadem F, Gadian DG, Copp A, Mishkin M (2005) FOXP2 and the neuroanatomy of speech and language. Nat Rev Neurosci 6(2):131–138
40. Zembrzycki A, Perez-Garcia CG, Wang CF, Chou SJ, O'Leary DD (2015) Postmitotic regulation of sensory area patterning in the mammalian neocortex by Lhx2. Proc Natl Acad Sci U S A 112(21):6736–6741
41. Zhang YF, Liu LX, Cao HT et al (2015) Otx1 promotes basal dendritic growth and regulates intrinsic electrophysiological and synaptic properties of layer V pyramidal neurons in mouse motor cortex. Neuroscience 285:139–154
42. Chen J, Lin M, Foxe JJ et al (2013) Transcriptome comparison of human neurons generated using induced pluripotent stem cells derived from dental pulp and skin fibroblasts. PLoS One 8(10):e75682. doi:10.1371/journal.pone.0075682
43. Kume T (2010) Specification of arterial, venous, and lymphatic endothelial cells during embryonic development. Histol Histopathol 25(5):637–646
44. Phillips W, Michell A, Pruess H, Barker RA (2009) Animal models of neurodegenerative diseases. Methods Mol Biol 549:137–155
45. Young AB (2009) Four decades of neurodegenerative disease research: how far we have come! J Neurosci 29(41):12722–12728
46. Park IH, Arora N, Huo H et al (2008) Disease-specific induced pluripotent stem cells. Cell 134(5):877–886
47. Soldner F, Hockemeyer D, Beard C et al (2009) Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors. Cell 136(5):964–977
48. Yagi T, Ito D, Okada Y et al (2011) Modeling familial Alzheimer's disease with induced pluripotent stem cells. Hum Mol Genet 20(23):4530–4539
49. Zheng GP, Ge MH, Shu Q, Rojas M, Xu J (2013) Mesenchymal stem cells in the treatment of pediatric diseases. World J Pediatr 9(3):197–211
50. Hu BY, Weick JP, Yu J et al (2010) Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. Proc Natl Acad Sci U S A 107(9):4335–4340
51. Osafune K, Caron L, Borowiak M et al (2008) Marked differences in differentiation propensity among human embryonic stem cell lines. Nat Biotechnol 26(3):313–315
52. Griesi-Oliveira K, Acab A, Gupta AR et al (2014) Modeling non-syndromic autism and the impact of TRPC6 disruption in human neurons. Mol Psychiatry. doi:10.1038/mp.2014.141
53. Li Y, Jia YC, Cui K et al (2005) Essential role of TRPC channels in the guidance of nerve growth cones by brain-derived neurotrophic factor. Nature 434(7035):894–898
54. Zhou J, Du W, Zhou K et al (2008) Critical role of TRPC6 channels in the formation of excitatory synapses. Nat Neurosci 11(7):741–743
55. Altshuler D, Daly MJ, Lander ES (2008) Genetic mapping in human disease. Science 322(5903):881–888
56. Tabar V, Studer L (2014) Pluripotent stem cells in regenerative medicine: challenges and recent progress. Nat Rev Genet 15(2):82–92
57. Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P et al (2010) Epigenetic memory in induced pluripotent stem cells. Nature 467(7313):285–290
58. Polo JM, Liu S, Figueroa ME, Kulalert W, Eminli S, Tan KY et al (2010) Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. Nat Biotechnol 28(8):848–855

59. Kuijk EW, Chuva de Sousa Lopes SM, Geijsen N, Macklon N, Roelen BA (2011) The different shades of mammalian pluripotent stem cells. Hum Reprod Update 17(2):254–271
60. Estrela C, Alencar AH, Kitten GT, Vencio EF, Gava E (2011) Mesenchymal stem cells in the dental tissues: perspectives for tissue regeneration. Braz Dent J 22(2):91–98
61. Machado E, Fernandes MH, Gomes Pde S (2012) Dental stem cells for craniofacial tissue engineering. Oral Surg Oral Med Oral Pathol Oral Radiol 113(6):728–733
62. Wen Y, Wang F, Zhang W et al (2012) Application of induced pluripotent stem cells in generation of a tissue-engineered tooth-like structure. Tissue Eng Part A 18(15–16):1677–1685

Chapter 8
Dental Stem Cells in Oral, Maxillofacial and Craniofacial Regeneration

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8.1 Overview

Craniofacial defects are caused by various conditions, including trauma, tumor/cyst resection, degenerative diseases and congenital/developmental anomalies, bringing serious functional, aesthetical and psychological outcomes for patients. Conventional approaches toward the treatment of craniofacial defects include recruitment of diverse techniques and materials; autogenous bone graft, allogenic materials, guided bone regeneration [1–7] and distraction osteogenesis [1], to list a few. Despite significant success rate of these methods, they possess several disadvantages which hamper their application in several cases [8, 9]. As an example, due to their osteoinductive and osteoconductive properties [10], autogenous bone grafts has long been recognized as the gold standard in regeneration of skeletal defects [11]. However, their utilization has several drawbacks such as source and shape limitations, as well as possibility of creating donor site morbidities [3, 12]. On this account, regenerative medicine has been developed to overcome these shortcomings. Accordingly, this cell-based approach is termed the "platinum standard", and has provided the chance to deliver personalized autologous bone grafts to the patients [13].

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In recent years, the axiom of regenerative medicine was built on three elements; cells, scaffolds and growth factors [4, 14]. The aim of regenerative medicine is to reiterate the developmental steps in the primary formation of the tissues to perform the ideal repair. This necessitates the propagation and differentiation of stem cell/progenitor cells towards the favorable lineages [15]. All human tissues arise from pluripotent embryonic stem cells (ESCs) [16], and these cells remain in adult tissues as multipotent adult stem cells (ASCs) which are in charge of hemostasis and repairing of the injuries through the life [17]**.** ASCs are obtained from various tissues, namely adipose tissue [18], bone marrow [19], skin [20], dental tissues [21, 22], etc. Compared to ESCs, ASCs are easily accessible, immunocompatible and they lack the ethical issues associated with ESCs [23]. It is assumed that ASCs have the capability to migrate to the site of injury and differentiate into the tissue-specific cells, and take part in tissue repair process [24, 25]. The current chapter provides information regarding potential application of ASCs derived from dental tissues in oral, maxillofacial and craniofacial regeneration.

8.2 Mesenchymal Stem Cells (MSCs)

MSCs are ASCs characterized by their fibroblast-like morphology, plastic adherence, pluripotentcy, colony-forming properties, and considerable proliferation rate [26]. Under proper stimuli, they possess the potential to differentiate into specific mesenchymal cells including adipocytes, chondrocytes, and osteoblasts [27]. Taking into consideration their multipotency, accessibility and predictable behavior in cell cultures; MSCs have provided a great source in regenerative medicine [28]. These cells were first detected in bone marrow aspirates, named as bone marrow mesenchymal stem cells (BMMSCs) [26]. BMMSCs have been widely used due to their promising osteogenic capability [29, 30]. However, the procedure of BMMSCs harvesting is invasive and accompanied with morbidity and post-surgical discomforts [31]. An ideal criteria for application of cell sources in regenerative medicine includes being easily accessed and isolated, leading to least patient morbidity. In addition, it should have the feasibility to further reproduce them in several clinical cases. Hence, harvesting MSCs from other sources including dental tissues has been in the spotlight.

8.3 Dental Stem Cells (DSCs)

Dental stem cells (DSCs) possess significant advantages over BMMSCs. They have been an interesting topic for researchers, since these cells can be obtained from medical waste [32]. In addition, they face no ethical issues compared to recruitment of ESCs [33]. DSCs are originated from neural crest, while BMMSCs are derived from mesoderm. Accordingly, DSCs possess superior properties for regenerating neural crest-derived tissues [34], namely defects in periodontal and craniofacial

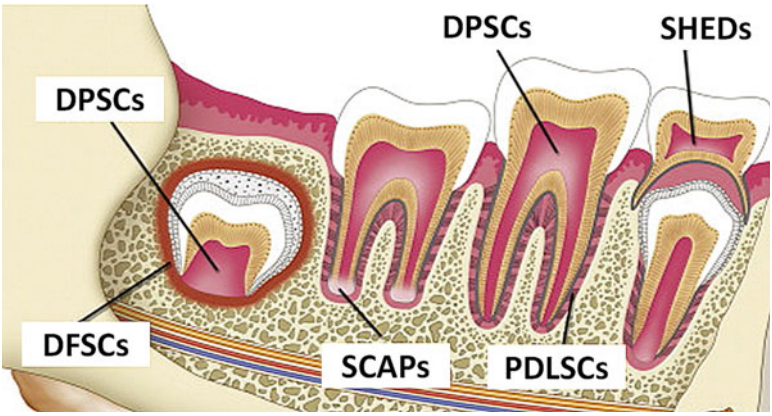

Fig. 8.1 Types of dental stem cells (DSCs). DPSCs: Dental pulp stem cells; SCAPs: stem cells from apical papilla; SHEDs: stem cells from exfoliated deciduous teeth; DFSCs: dental follicle stem cells; PDLSCs: stem cells isolated from PDL. Figure is reprinted from a published paper by Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K. Stem cells in dentistry-part I: stem cell sources. J Prosthodont Res. 2012;56(3):151–165. With permission granted from ELSEVIER publisher

regions [32]. Recent studies have isolated stem cells from various dental and oral tissues, including dental pulp [21], human exfoliated deciduous teeth [35], apical papillae [36], periodontal ligament [37] and dental follicle [38] (Fig. 8.1). Based on the ability to form dentin-pulp complex or periodontium, DSCs are classified into two categories. Dental pulp stem cells (DPSCs), stem cells from apical papillae (SCAPs) and stem cells obtained from human exfoliated deciduous teeth (SHEDs) are recognized in the first group, and they have the capability to differentiate into dentin, pulp or dentin-pulp complex, while dental follicle stem cells (DFSCs) and periodontal ligament stem cells (PDLSCs) are allocated to the second group, and they are related to formation of periodontium elements [39–43].

8.3.1 DPSCs

The presence of DPSCs was first suggested by Fitzgerald et al. in 1990 [44], and were first isolated by Gronthos et al. in 2000 [21]. They described DPSCs as clonogenic, rapidly proliferating cells capable of producing mineralized tissue both in vitro and in vivo [45].

DPSC niches are frequently quiescent, and are activated following injuries and stimuli [46]. Comparing to BMMSCs, they possess 30% higher proliferation and growth rates [47]. These findings are attributed to cell cycling mediators such as cyclin-dependent kinase 6 and insulin-like growth factors [48]. Several studies have demonstrated that this cell population express various perivascular markers including trypsin-resistance cell surface antigen (STRO-1), vascular cell adhesion molecule 1

(VCAM-1/CD106), melanoma cell adhesion molecule (MCAM/MUC-18/CD146) and α-smooth muscle actin, indicating that they consist of heterogeneous mesenchymal cell population [21, 49].

The STRO-1 positive cells isolated from dental pulp demonstrated capability of differentiating into chondrogenic, myogenic, adipogenic and neurogenic lineages [50] in addition to their odontogenic potential [51]. It is noteworthy that odontoblast differentiation was exclusively allocated to STRO-1 positive cells, and STRO-1 negative cells were not capable of differentiating into odontoblasts [52].

8.3.2 SHEDs

SHED were first isolated from the dental pulp of exfoliated deciduous teeth by Miura et al. in 2003 [35]. Compared to DPSCs, SHED population presents higher proliferation rate. They form sphere-like cell clusters, and possess in vivo osteoinductive potential. However, SHEDs fail to generate a complete dentin-pulp like complex [35, 53]. Furthermore, they seem to be more immature than DPSCs [54], and have the potential to differentiate into neural cells, adipocytes, osteoblasts and odontoblasts [35].

8.3.3 SCAPs

During tooth development, an epithelial sheath is created by fusion of the inner and outer enamel epithelium at the bottom of the crown cervical level. This sheath plays a pivotal role in root development [55]. During root development, the dental papilla moves more apical, and a dense cell rich zone is detected between the pulp and apical papilla that can be easily removed. It is known that primary odontoblasts are originated from developing dental papillae, while reparative and reactionary dentins are synthesized by the replacement odontoblast originating from the dental pulp [56]. Thus, it is hypothesized that DPSCs produce secondary odontoblasts while SCAPs are probably the source of primary odontoblasts [36, 40]. Furthermore, SCAPs had a greater bromodeoxyuridine (BrdU) uptake rate, population doublings and regeneration capacity, as well as higher number of STRO-1-positive cells in comparison with DPSCs. SCAPs expressed higher levels of survivin (anti-apoptotic protein), and they were positive for hTERT (human telomerase reverse transcriptase) which maintains the telomerase length. This marker has been associated with ESCs and it is frequently absent in MSCs [57].

Since SCAPs are obtained from a developing tissue, and possess an early population of stem cell, it is postulated that SCAPs may be a superior reservoir for tissue regeneration. Hence, it may be concluded that stem cells obtained from developing tissue may be distinct from those obtained from mature tissues [40].

8.3.4 PDLSCs

Periodontium is composed of cementum, periodontal ligament (PDL) and alveolar bone which support the tooth [58]. PDL is a specialized connective tissue originating from the dental follicle which stems from neural crest cells [59, 60]. The presence of progenitor/stem cells in mice PDL was first reported by McCulloch and co-workers in 1985 [61], and later PDLSCs were isolated from human PDL in 2004 [37]. Limited regeneration following periodontitis is an evidence for existence of these stem cells [62]. As PDLSCs were cultured in conditions described for DPSCs and BMMSCs; clonogenic and adherent cells were generated, and the rate of fibroblastic colony-forming units were greater comparing to aforementioned cells [37]. It is noted that proliferation and osteogenic potentials of BMMSCs are influenced by donor age [63], while this trend was not reported for PDLSCs [64]. PDLSCs are distinct from other MSCs due to their differentiation potential towards the cementoblast lineage [37].

8.3.5 DFSCs

Dental follicle (DF) is originated from ectomesenchyme, and presents as a loose connective tissue surrounding the unerupted tooth [65]. It is hypothesized that DF controls tooth eruption via regulating osteoclastogenesis and osteogenesis by producing growth factors and cytokines [66]. DF is separated from dentin by Hertwig's sheet [67, 68], and following disintegration of this sheet, the DFSCs differentiate into periodontium [69, 70]. This function proposed the presence of DFSCs. Several studies have isolated stem cells population from DF in various developmental stages [38, 71, 72]. DFSCs have shown to differentiate mainly toward the osteoblast but not cementoblast and odontoblast lineages [22].

8.4 DSC Niches

The microenvironment where the stem cell resides is termed "niche". Niches determine stem cells fate and lineage differentiation by providing specific signals [73]**.** Cell proliferation, fate and death are regulated by elements found in the niche [74–76]. The interaction between transcription factors such as Oct-4, Sox-2, Nanog and Stat-3 results in stem cell niche formation [77]. By evaluating BrdU uptake of proliferating cells in the pulp tissue, DPSC niches were claimed to reside in perivascular regions [78]. However, researches have also proposed various niches other than perivascular niche. Evaluation of notch signaling in pulp injury has demonstrated that these cells could be found in odontoblast-sub-odontoblast layer and stroma of the pulp, in addition to the perivascular structures [79].

8.5 DSC Isolation and Characterization

Isolation of DPSCs from dental pulp has been established by Gronthos et al. [21]. Since then, other types of DSCs have been isolated using similar procedures. Detailed procedure of isolating DPSCs used in our laboratory is provided as follow: Tooth surface is cleaned by several washes in sterile phosphate buffer solution (PBS), followed by immersion in 1% povidone-iodine solution for 2 min and 0.1% sodium thiosulfate for 1 min. Then, the tooth is washed again in sterile PBS. The root of cleaned tooth is separated from crown. The pulp tissue is isolated from the pulp chamber with sterile forceps. Dental pulp is digested with 0.075% collagenase Type I for 1 hour at 37 °C. Digested cells are centrifuged to collect cell pellet. Cells are re-suspended in the medium containing DMEM + 10% fetal bovine serum (FBS) + 1% Penicillin-Streptomycin (10,000 u/ml). Cell suspension is immediately plated in a T-25 flask, and placed at 37 °C and 5% CO_2, and non-adherent cells are removed by fresh medium following day. At this stage, cells are called passage 0 (P0). The adherent cells are cultured until they reach 80-90% confluency. Medium is changed every 4 days. In this method, no selection of stem cells is administered; instead selective culture is used for stem cell enrichment. Cells are passaged using 0.25% trypsin-EDTA at a ratio of 1:3. Primary culture of DPSCs is illustrated in Fig. 8.2.

Extensive studies have been carried out to identify specific marker genes for MSC characterization. Procedures such as cell migration, proliferation and differentiation are highly controlled by stem cell markers. Furthermore, expression of these genes is recruited as a biomarker for identification, isolation and determination for their differentiation potential [80]. There is no single, generally acceptable stem cell marker. However, International Society of Stem Therapy (ISCT) has proposed three primary markers for identification of MSCs; CD73 (membrane-bound ecto-5'-nucleotidase), CD90 (Thy1) and CD105 (endoglin) markers [81]. STRO-1 is also another important marker widely used for characterization and isolation of DSCs [49, 50, 54, 57]. This surface antigen is proposed to regulate cell migration [80]. Other studies have also reported several markers highly expressed in DSCs [32, 39–43] (Table 8.1).

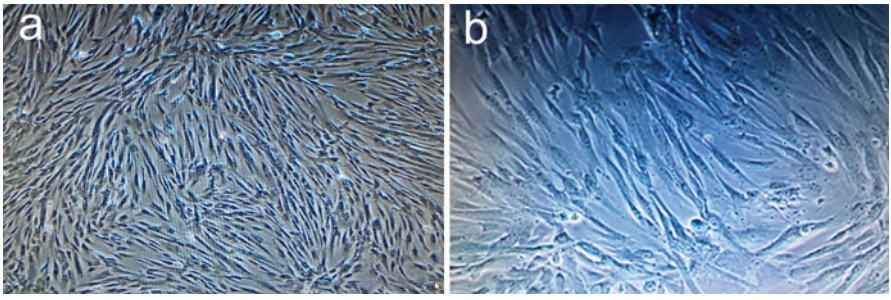

Fig. 8.2 The primary culture of dental pulp stem cells (DPSCs) at early passage. (**a**) DPSCs passage 3 at lower magnification (5×). (**b**) DPSCs passage 3 at higher magnification (10×)

Table 8.1 Types of dental stem cells (DSCs) and specific surface markers [32, 39–43]

Type of DSCs	Surface marker
DPSCs	STRO-1, CD13, CD29, CD44, CD59, CD73, CD90, D105, CD146, NESTIN
SHEDs	STRO-1, CD13, CD29, CD44, CD73, CD90, CD105, CD146, CD166
SCAPs	STRO-1, CD13, CD24, CD29, CD44, CD73, CD90, D105, CD106, CD146, NESTIN
PDLSCs	STRO-1, CD13, CD29, CD44, CD59, CD73, CD90, CD105
DFSCs	STRO-1, CD13, CD29, CD44, CD59, CD73, CD90, D105, CD146, NOTCH1, NESTIN

8.6 Dentin/Pulp Regeneration

Maintenance of dental pulp tissue is critical for integrity of tooth shape and function. A layer of odontoblast cells lies outside the pulp tissue and its functions include secretion and mineralization of extracellular matrix of dentin [82, 83]. In case of mild stimulus, existing odontoblasts produce increased rates of matrix known as reactionary dentin. However, in case of strong stimulus and death of odontoblasts, new odontoblasts are differentiated from undifferentiated MSCs found in pulp tissue, leading to formation of reparative dentin [84]. However, when dental pulp tissue is infected, it is difficult for the immune system to eradicate the infection; therefore, removing of the infected pulp is necessary. Demand for pulp regeneration technique is more evident in cases of pulp exposure in young immature teeth. Immature teeth possess incomplete root and large pulp chambers and thin walls, making these teeth susceptible to fracture. The current practice of replacing infected pulp with artificial material results in a nonvital tooth with a questionable prognosis [85].

Among the different types of DSCs mentioned previously, the potential of DPSCs, SHEDs and SCAPs to regenerate dentin/pulp tissue has been investigated. PDLSCs have been shown to regenerate cementum/PDL-like structure, indicating their potential role in PDL tissue regeneration [37]. The large body of the literature have shown that in vivo transplantation of DPSCs seeded in ceramic phosphates can form ectopic dentin-pulp like complexes in immunocompromised mice [21, 45, 86, 87]. Unlike DPSCs, subcutaneous transplantation of SHEDs did not show complete dentin-pulp-like complexes, whereas formation of odontoblast-like cells was observed [35]. In a later study, regenerative potential of DPSCs and SHEDs to form dentin-pulp-like complexes with vascularized tissue using tooth root fragments with empty root canal space was demonstrated. In this study, the root canal was filled with a poly-D,L-lactide/glycolide (PLG) scaffold seeded with DPSCs [88]. Similarly, formation of dentin-pulp like complex has been shown by odontogenically differentiated DPSCs seeded on nanofibrous poly(L-lactic acid) (PLLA) scaffolds in immunocompromised mice [89]. Transplantation of DPSCs seeded on polyglycolic acid (PGA) scaffold in immunocompromised mice subcutaneously demonstrated extracellular matrix production, secretion of type 1 collagen, fibronectin and invasion of blood vessels into cell-PGA complex after 3 weeks of implantation [90]. In addition, placing DPSCs in combination with collagen scaffold and dentin matrix protein-1 (DMP-1) in the simulated perforation sites in dentin slices, and transplanting the

construct subcutaneously into immunocompromised mice showed a well-organized pulp-like tissue in the perforation site [91]. Transplantation of SHED seeded on PLLA scaffold within human tooth slices not only have the potential to form dental pulp-like structures, but also can form endothelial-like cells [92]. It is hypothesized that directing the natural process of progenitor cell recruitment to the defect site may mimic repair process [54]. In this context, some researchers investigated this hypothesis by transplanting bone morphogenetic protein-2 (BMP-2) treated DPSCs [93] and growth/differentiation factor 11(Gdf11)-electrotransfected DPSCs [94] to the amputated pulps. The primary generated tissue expressed osteo-dentin like structure resembling atubular reparative dentin. It is noteworthy that generating atubular dentin may be even more beneficial, since it is more resistant to carious attacks [54]. Similar to DPSCs, SCAPs have also exhibited the potential to generate dentin-pulp like complex in vivo. A pulp-like tissue with well-established vascularity and a layer of odontoblast-like cells was also observed when SCAPs were seeded into tooth fragments and then transplanted to immunodeficient mice [56].

One of the challenging aspects of tooth regeneration is promoting angiogenesis and vascular ingrowth. When cell-scaffold systems, just nourished by apical blood supply, are seeded in canals, the vitality of cells may be jeopardized [36]. Accordingly, efforts have been made to modify scaffolds in order to overcome this obstacle and enhance vascularization [95–97]. One of the approaches is enriching the scaffold with vascular endothelial growth factor (VEGF) and/or platelet-derived growth factor (PDGF) or seeding the stem cells together with endothelial cells [36]. Nör and co-workers seeded DPSCs and SHEDs together with human dermal microvascular endothelial cells subcutaneously to the immunocompromised mice. The results demonstrated a dental pulp generation similar to normal pulp tissue structure [85]. It is also demonstrated that dental pulp-derived $CD31^{-}/CD146^{-}$ side population cells can induce a strong vasculogenic response in amputated pulps [98]. Similarly, a study conducted by Iohara et al. showed complete pulp regeneration with neurogenesis and vasculogenesis when autogenous dental pulp $CD105^{+}$ side population cells in combination with stromal cell-derived factor-1 (SDF-1) transplanted to a canine model of pulpectomy [99].

8.7 Bone Regeneration

Similar to other repair mechanisms, inflammation is the first phase of bone repair. Following the inflammatory phase, the endogenous stem/progenitor cells are recruited from local and distant sources to the site of injury [30, 100]. Subsequent to proliferation, the stem/progenitor cells differentiate into chondrocytes or osteoblasts. Osteoblasts carry out intramembranous ossification by direct deposition of bone; likewise, chondrocytes perform endochondral ossification by multiplying, getting hypertrophied and mineralized, and finally the deposition of new bone on the cartilaginous matrix. This process outlines the significant role of stem/progenitor cells as the precursor of osteoblasts and chondrocytes as well as the modulator of healing response [30]. However, endogenous repair would not be sufficient in

severe injuries and an exogenous source of stem cell is required to have satisfied bone regeneration.

BMMSCs are well studied source of stem cells in tissue engineering. Our laboratory as well as others have extensively studied the potential application of BMMSCs for treatment of craniofacial skeletal defects [2–5, 11, 29, 101–104]. However, due to difficulty of harvesting a sufficient amount of cells as well as discomfort during the harvesting procedures, researchers have been exploring other source of MSCs. DSCs express osteogenic markers, and they have shown to properly respond to the signal/inductors of osteogenic differentiation. Subjecting DSCs to the defined culture conditions differentiates cells towards the osteoblast lineage [40].

In early studies about osteogenic capacity of DFSCs, bone formation was observed following subcutaneous transplantation of DFSCs with ceramic phosphate [105, 106]. Later studies have also confirmed their potential in healing critical-sized defects in immunocompromised animal models [107–109]. Several studies have demonstrated that subcutaneous transplantation of DPSCs in immunocompromised animals can form dentin-pulp complex while no bone-like structures were observed [21, 86]. However, Carinci and co-workers have shown that a subpopulation of stem cells from human dental pulp named osteoblasts derived from human pulpar stem cells (ODHPSCs) have osteogenic potential forming bone-like tissue in vivo [110]. A similar study demonstrated that subcutaneous transplantation of $CD34^+$ cells obtained from dental pulp tissues transplanted into immunocompromised rats can form nodules of bone [111]. Furthermore, the osteogenic potential of osteo-induced DPSCs seeded on fibroin scaffold resulted in healing of calvarial critical-sized defects [112]. Similarly, DPSCs treated with osteogenic medium and seeded into granular deproteinized bovine bone (GDPB) or beta-tricalcium phosphate (b-TCP) were transplanted into a rat calvarial critical-sized defect. Increased bone mineralization was observed in DPSCs in conjunction with GDPB [113]. Later clinical study demonstrated that autogenous DPSCs seeded on collagen sponge biocomplex can completely repair human mandibular bone defect [114]. Similarly, seeding human DPSCs from extracted third molars on collagen scaffold followed by transplantation of the construct into the extraction sites demonstrated a rich vascularized lamellar bone for three years [115]. Miura and co-workers have proven that SHED cells were unable to directly differentiate into osteoblasts but they could induce bone formation by forming an osteoinductive template which recruited host osteogenic cells [35]. On the other hand, it has also been demonstrated that oste-induced SHEDs were potent in repairing critical-sized calvarial defects in mice with substantial bone formation [37]. In addition, high regenerative potential of canine DPSCs and SHED in combination with platelet rich plasma (PRP) was noted in treatment of canine mandibular defect [116].

PDLSCs have typically formed cementum/PDL-like structures following subcutaneous transplantation [40]. However, it is observed that transplantation of human PDLSCs into the periodontal defects of immunocompromised mice generates trabecular bone next to PDL-like structure, indicating their potential role in alveolar bone regeneration [37]. Furthermore, transplantation of PDLSCs to the rat periodontal defects resulted in bone regeneration [117] and PDLSCs encapsulated in

arginine-glycine-aspartate (RGD)-modified alginate microspheres are able to promote mineralization in rat calvarial critical-sized defects [118]. Some of the studies have compared bone formation potential of PDLSCs and BMMSCs. Placing canine PDLSCs and BMMSCs around the surgically created peri-implant saddle-like defects in canine models demonstrated that BMMSCs have a greater potential in bone regeneration in alveolar ridge than PDLSCs [119]. Another study has also confirmed that PDLSCs have less osteogenic potential compared to BMMSCs [120]. PDLSC matrix mineralizes at a slower rate with respect to BMMSCs which may be attributed to presence of terminally-differentiated cells in PDLSCs [121]. In contrast, some studies have reported similar osteogenic differentiation potential for BMMSCs and PDLSCs [122]. Comparison of osteogenic differentiation of human PDLSCs and SHEDs has suggested that PDLSCs present superior osteogenic stem cell source [123, 124]. Similar to DPSCs, typical dentin-pulp like structures are formed when SCAPs are transplanted into immunocompromised mice [40]. However, BMP-9 immortalized SCAP cells exhibit mature mineralized trabecular bone in ectopic site [125].

8.8 Neural Tissue Regeneration

Neural stem cells (NSCs) are the most ideal cell source for neural regeneration. However, their harvesting is complicated and limited cell quantities can be obtained [126]. DSCs provide a convenient source of autogenous stem cells, and they are more accessible than NSCs. In addition, they are originated from neural crest which makes them more potent for neurogenesis compared to mesoderm-derived BMMSCs [40, 126].

DSCs express immature neural cell markers including nestin, neurofilament even in their undifferentiated state [35, 56, 126–128]. Differentiation towards the neural lineage can be induced using epidermal growth factor (EGF), fibroblast growth factor (FGF) and retinoic acid (RA) [129, 130]. Upon induction, expression of mature neural markers increases in DSCs [35, 56, 126–128]. The neurogenesis potential is not only evaluated by expression of neural markers but also following criteria should be assessed: 1) neural morphology, including polarized cells with axon and multiple dendrites; 2) neural functionality confirmed by evaluating the voltage-dependent channels; and 3) presence of synapse and neurotransmitters required for the communication between neurons [126].

The large body of literature has evaluated the neurogenic potential of DPSCs and SHEDs. Limited in vitro studies are available on neurogenesis of DFSCs, PDLSCs and SCAPs [126]. A study conducted by Kiraley et al. showed neural-induced DPSCs expressed mature neural markers including neurogenin-2, neuron-specific enolase, neurofilament-M and glial fibrillary acidic protein (GFAP). In addition, they revealed the functional activity of both voltage-dependent sodium and potassium channels [131]. A latter in vivo study conducted by the same group on cortical lesion of postnatal rat brain showed that differentiated/transplanted DPSCs expressed neural markers, and they exhibited voltage-dependent sodium and potassium channels [132]. However, they did not observe any synapse or network connection between

neural cells. Several studies have also shown the ability of DPSCs to secret many neurotropic growth factors including brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), fibroblast growth factor (FGF), glial cell-derived growth factor (GDNF), nerve growth factor (NGF), and VEGF. These factors are essential for cell survival, differentiation, maturation, and promote neurite outgrowth and axon guidance [129, 133–135]. Potential role of DPSCs in recovery of central nervous system damages are extensively investigated in animal models. A study performed by Huang et al. showed that undifferentiated DPSC transplantation into the hippocampus of immunocompromised mice stimulated neural cell proliferation, and also resulted in recruitment and maturation of endogenous neural cells to the site of the graft [136]. In another study, DPSCs were detected in cortical lesion of a rat model following transplantation into the cerebro-spinal fluid [132].

The investigation of regenerative potential of DSCs for peripheral nerve injuries in maxillofacial region, including facial nerve and inferior alveolar nerve injuries, are also crucial. The only study in this regard performed by Sasali et al. evaluated the neural regeneration potential of DPSCs in facial nerve injury of a rat model and they observed axon regeneration of facial nerve after transplantation of DPSCs loaded into degradable polyglycolic acid (PLGA)-collagen tubes [137]. In addition to neurogenesis, angiogenesis is also essential for pulp regeneration. Study of Nakashima and Iohara demonstrated complete pulp regeneration with neural processes as well as proper vascularization following autogenous transplantation of DPSCs side-population of $CD31^-/CD146^-$ cells and $CD105^+$ cells in a rat model of amputated pulp [138].

8.9 Vascular Tissue Regeneration

Angiogenesis plays a pivotal role in differentiation and maintenance of transplanted cells in tissue engineering [139]. The principal strategy utilized in tissue engineering is ingrowth and extension of vascularity from the host. Any impairment or delay in this process may lead to damage or loss of implanted cells; subsequently lack of tissue regeneration [140]. On this account, the angiogenic potential of DSCs has been studied by several research groups.

DPSCs have demonstrated capability of forming tube-like structures on matrigel coated tissue culture plates upon induction in endothelial growth medium. Expression of CD31, the endothelial progenitor cell marker, also confirmed differentiation toward the endothelial lineage [141]. Comparison of two side population cells from human dental pulp; $CD31^-$/CD 146^- and $CD31^+/CD146^-$ suggested that $CD31^-/CD146^-$ cells generated extensive cord networks and tube-like structures on matrigel. $CD31^-/CD146^-$ cells exhibited 13-fold higher capillary density and greater blood flow, and signs of neovascularization in a hind limb ischemia model compared to $CD31^+/CD146^-$ cell population [142]. It is noteworthy that this side population can give rise to osteoblasts as well as endotheliocytes, and transplantation of this side population in immunocompromised rats resulted in vascularized adult bone formation [143]. It is

proposed that low concentration of growth factors secreted by human DPSCs or presence of anti-angiogenic molecules may be the reason for lack of significant effect of DPSCs on human microvascular endothelial and mouse brain endothelial cell proliferation. However, significant angiogenesis is detected following incubation of human DPSC matrigel with chicken chorioallantonic membrane [144].

Some studies have investigated the mechanisms associated with endothelial differentiation of DSCs. As mentioned earlier, CD31 and VEGF receptor-2 (VEGFR-2) are detected in vascular endothelial cells [145] .VEGFR-1 is normally present in normal and malignant tissues [146] and controls the angiogenic potential of cells when exposed to VEGF [147]. Treatment of SHED cells with VEGF generated vessels connected to the host vasculature, and detection of vascular markers including VEGFR-2, platelet endothelial cell adhesion molecule 1 and vascular endothelial cadherin confirmed this finding [148]. Furthermore, it is demonstrated that SHEDs normally express VEGFR-1 [148] and less angiogenic potential is observed in vivo when VEGFR-1 is silenced; hence, it was postulated that VEGFR-1 plays a pivotal role in differentiation of DPSCs into endothelial cells [149].

Co-culture of human DPSCs and endothelial cells promotes vasculogenesis compared to single cultures of endothelial cells [150]. The co-culture of human umbilical vein endothelial cells (HUVECs) with DPSCs demonstrated an increase in odonto-/osteo-genic potential of DPSCs as well as the vasculogenic potential of HUVECs, furthermore, DPSCs contributed to longer stabilization of vessel-like structures generated by HUVECs [140]. In addition, DPSCs led to improvement in cardiac function correlating with infarct size reduction, greater vascular density, and greater wall thickness in infracted myocardium. Despite of having no differentiation toward cardiac, smooth muscle or endothelial cell lineages, greater number of cardiomyocyte bundles and myofibroblasts were observed, which may be attributed to paracrine factors secreted by DPSCs [151].

8.10 Muscle Tissue Regeneration

Tissue-resident muscle stem cells, known as satellite cells, are responsible for muscle growth, and regeneration. These cells have been utilized for replacement of damaged muscle cells for a long time [152, 153]. While some studies have evaluated the myogenic potential of DSCs, majority of them have focused on myocardial differentiation or myocardial infarction models [154, 155]. The first report on differentiation of DPSCs into cardiomyocyte-like cells was demonstrated by in vitro co-cultivation of DPSCs with neonatal cardiomyocytes [154]. Serum-free condition is an efficient technique for differentiating myoblasts to skeletal myogenic cells. Likewise, DPSCs were incapable of expressing any myogenic markers in this culture condition [156]. The literature has proposed that skeletal myogenic differentiation might be regulated by DNA methylation [157, 158]. Treating DPSCs by 5-Aza-20-deoxycytidine (5-Aza) demonstrated formation of myotube like structures as well as expression of myosin heavy chain, myogenic differentiation 1 (Myod1), myogenic factor 4

(myogenin) and Pax7 (a transcription factor that plays a role in myogenesis) [156]. It was observed that PDLSCs also displayed myotube-like structures and desmin expression, a subunit of intermediate filaments in skeletal muscle after 5-Aza treatment [159]. Injection of DPSCs in cardiotoxin induced injury in tibialis anterior muscles of dogs demonstrated that cells significantly engrafted in the muscles and expressed higher levels of dystrophin and myosin heavy chain [155].

8.11 Cartilage Tissue Regeneration

Cartilage possesses limited regenerative potential, and its low metabolic rate and avascularity should be taken into consideration prior to any regenerative measurements [160]. Stem cells, chondrocytes and osteoblasts as well as endothelial cells are essentials for articular condylogenesis [60, 161]. Several studies focused on the regeneration of temporomandibular joint (TMJ) using MSCs. However, some challenges such as facilitating remodeling potential of the cartilage and enhancing mechanical properties still exist [162, 163]. Studies on TMJ engineering was mainly based on stem cells obtained from disc [164–166] and hyaline cartilage [167, 168]. Considering the fact that both DSCs and TMJ cartilage originate from neural crest [168], utilization of DSCs may be a potential candidate for regeneration of TMJ [118].

Chondrogenic differentiation potential of DSCs has already been demonstrated by several studies [40, 169]. It has been proven that the early passages of STRO-1^+ DPSCs are able to generate cartilage [170]. Comparable results were also obtained following transplantation of bFGF treated DPSCs in immunocompromised mice [171]. Culturing SHEDs and DPSCs with BMP-2 displayed chondrocyte formation along with expression of type X collagen and increase in alkaline phosphatase level. However, expressions of chondrogenic markers, including type II and X collagen, and SOX9 were significantly lower in DPSCs in comparison with SHEDs [172].

The TGF-β loaded alginate system demonstrated that PDLSCs expressed greater levels of collagen type II in comparison with gingival MSCs and human BMMSCs [118]. Furthermore, the chondrogenic differentiation of DPSCs seems to be enhanced by ultrasound application [141].

8.12 Adipose Tissue Regeneration

Reconstruction of adipose tissue is essential, because it has considerable impact on facial features. MSCs including DSCs can differentiate into adipocytes in vitro upon induction in the specific medium containing dexamethasone, isobutylmethylxanthine, indomethacin, and anti-inflammatory drugs [27, 173]. Both BMMSCs and adipose-derived stem cells (ADSCs) have been used for regeneration of adipose tissues for cosmetic purposes [174, 175]. Despite the in vitro adipogenic differentiation of DSCs, there is almost no literature using them for adipose tissue regeneration. The

only available study conducted by Soe et al. indicated formation of adipocytes by transplantation of PDLSCs into immunocompromised rodent [37].

In summary, regenerative medicine is the promising treatment approach of the era that may revolutionize the traditional therapies in coming years. Neural crest-derived DSCs as a medical waste can be promising in craniofacial regeneration. The application of DSCs for craniofacial regeneration is still in its infancy. Stem cells from dental pulp is the most studied DSCs in craniofacial regeneration. Despite the well-established hard tissue formation potential of DSCs, very few studies have begun to address the potential application of these cells in regeneration of craniofacial soft tissues. The further large scale human studies, particularly investigation of their potential for soft tissue reconstruction, would pave the way for clinical application of this novel cell source in future.

References

1. Behnia H, Homayoun S, Qaranizade K, Morad G, Khojasteh A (2013) Multidisciplinary reconstruction of a palatomaxillary defect with nonvascularized fibula bone graft and distraction osteogenesis. J Craniofac Surg 24(2):e186–e190
2. Khojasteh A, Behnia H, Shayesteh YS, Morad G, Alikhasi M (2011) Localized bone augmentation with cortical bone blocks tented over different particulate bone substitutes: a retrospective study. Int J Oral Maxillofac Implants 27(6):1481–1493
3. Khojasteh A, Eslaminejad MB, Nazarian H, Morad G, Dashti SG, Behnia H, Stevens M (2013) Vertical bone augmentation with simultaneous implant placement using particulate mineralized bone and mesenchymal stem cells: a preliminary study in rabbit. J Oral Implantol 39(1):3–13
4. Khojasteh A, Ghahremani MH, Ostad SN, Eslami M, Motahhary P, Morad G, Shidfar S (2013) The effect of deproteinized bovine bone mineral on Saos-2 cell proliferation. Iran Endod J 8(3):118–122
5. Khojasteh A, Sadr SJ, Saboury A, Shidfar S (2014) Onlay bone augmentation and bilateral open sinus lifting with simultaneous implant placement in a cherubic patient. J Craniofac Surg 25(2):e193–e196
6. Morad G, Khojasteh A (2013) Cortical tenting technique versus onlay layered technique for vertical augmentation of atrophic posterior mandibles: a split-mouth pilot study. Implant Dent 22(6):566–571
7. Hassani A, Khojasteh A, Shamsabad AN (2005) The anterior palate as a donor site in maxillofacial bone grafting: a quantitative anatomic study. J Oral Maxillofac Surg 63(8):1196–1200
8. McAllister BS, Haghighat K (2007) Bone augmentation techniques. J Periodontol 78(3):377–396. doi:10.1902/jop.2007.060048
9. Rocchietta I, Fontana F, Simion M (2008) Clinical outcomes of vertical bone augmentation to enable dental implant placement: a systematic review. J Clin Periodontol 35(8 Suppl):203–215. doi:10.1111/j.1600-051X.2008.01271.x
10. Montazem A, Valauri DV, St-Hilaire H, Buchbinder D (2000) The mandibular symphysis as a donor site in maxillofacial bone grafting: a quantitative anatomic study. J Oral Maxillofac Surg 58(12):1368–1371. doi:10.1053/joms.2000.18268
11. Khojasteh A, Behnia H, Naghdi N, Esmaeelinejad M, Alikhassy Z, Stevens M (2013) Effects of different growth factors and carriers on bone regeneration: a systematic review. Oral Surg Oral Med Oral Pathol Oral Radiol 116(6):e405–423. doi:10.1016/j.oooo.2012.01.044
12. Marx RE (2008) Application of tissue engineering principles to clinical practice. In: Lynch SE, Genco RJ, Marx RE (eds) Tissue engineering: application in oral and maxillofacial surgery and periodontics, 2nd edn. Quintessence Publishing, Chicago, IL, pp 54–57

13. Bhumiratana S, Vunjak-Novakovic G (2012) Concise review: personalized human bone grafts for reconstructing head and face. Stem Cells Transl Med 1(1):64–69. doi:10.5966/sctm.2011-0020
14. Jimi E, Hirata S, Osawa K, Terashita M, Kitamura C, Fukushima H (2012) The current and future therapies of bone regeneration to repair bone defects. Int J Dent 2012:1–7. doi:10.1155/2012/148261
15. Buxton P, Cobourne M (2007) Regenerative approaches in the craniofacial region: manipulating cellular progenitors for oro-facial repair. Oral Dis 13(5):452–460
16. Behjati S, Huch M, van Boxtel R, Karthaus W, Wedge DC, Tamuri AU, Martincorena I, Petljak M, Alexandrov LB, Gundem G, Tarpey PS, Roerink S, Blokker J, Maddison M, Mudie L, Robinson B, Nik-Zainal S, Campbell P, Goldman N, van de Wetering M, Cuppen E, Clevers H, Stratton MR (2014) Genome sequencing of normal cells reveals developmental lineages and mutational processes. Nature 513(7518):422–425. doi:10.1038/nature13448
17. Korbling M, Estrov Z (2003) Adult stem cells for tissue repair - a new therapeutic concept? N Engl J Med 349(6):570–582. doi:10.1056/NEJMra022361
18. Chen L, Song J, Cui J, Hou J, Zheng X, Li C, Liu L (2013) microRNAs regulate adipocyte differentiation. Cell Biol Int 37(6):533–546. doi:10.1002/cbin.10063
19. Ding L, Morrison SJ (2013) Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. Nature 495(7440):231–235. doi:10.1038/nature11885
20. Blanpain C, Fuchs E (2009) Epidermal homeostasis: a balancing act of stem cells in the skin. Nat Rev Mol Cell Biol 10(3):207–217. doi:10.1038/nrm2636
21. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97(25):13625–13630. doi:10.1073/pnas.240309797
22. Rezai Rad M, Liu D, He H, Brooks H, Xiao M, Wise GE, Yao S (2015) The role of dentin matrix protein 1 (DMP1) in regulation of osteogenic differentiation of rat dental follicle stem cells (DFSCs). Arch Oral Biol 60(4):546–556. doi:10.1016/j.archoralbio.2014.12.013
23. Han J, Menicanin D, Gronthos S, Bartold PM (2014) Stem cells, tissue engineering and periodontal regeneration. Aust Dent J 59(Suppl 1):117–130. doi:10.1111/adj.12100
24. Leeb C, Jurga M, McGuckin C, Moriggl R, Kenner L (2010) Promising new sources for pluripotent stem cells. Stem Cell Rev 6(1):15–26. doi:10.1007/s12015-009-9102-0
25. McKay R (2000) Stem cells--hype and hope. Nature 406(6794):361–364. doi:10.1038/35019186
26. Friedenstein AJ, Piatetzky S II, Petrakova KV (1966) Osteogenesis in transplants of bone marrow cells. J Embryol Exp Morphol 16(3):381–390
27. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284(5411):143–147
28. Hynes K, Menicanin D, Gronthos S, Bartold PM (2012) Clinical utility of stem cells for periodontal regeneration. Periodontol 2000 59(1):203–227. doi:10.1111/j.1600-0757.2012.00443.x
29. Khojasteh A, Behnia H, Dashti SG, Stevens M (2012) Current trends in mesenchymal stem cell application in bone augmentation: a review of the literature. J Oral Maxillofac Surg 70(4):972–982
30. Knight MN, Hankenson KD (2013) Mesenchymal stem cells in bone regeneration. Adv Wound Care (New Rochelle) 2(6):306–316. doi:10.1089/wound.2012.0420
31. Arvidson K, Abdallah BM, Applegate LA, Baldini N, Cenni E, Gomez-Barrena E, Granchi D, Kassem M, Konttinen YT, Mustafa K, Pioletti DP, Sillat T, Finne-Wistrand A (2011) Bone regeneration and stem cells. J Cell Mol Med 15(4):718–746. doi:10.1111/j.1582-4934.2010.01224.x
32. Rad MR (2014) Characteristics of dental follicle stem cells and their potential application for treatment of craniofacial defects. Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in The Interdepartmental Program in Veterinary Medical Science through The Department of Comparative Biomedical Sciences by Maryam Rezai Rad DDS, Tehran University of Medical Sciences
33. Jaquery A (2007) Implantation of dental pulp stem cells in a biodegradable scaffold for dental pulp tissue engineering. University of Michigan, Ann Arbor, MI

34. Yu J, Wang Y, Deng Z, Tang L, Li Y, Shi J, Jin Y (2007) Odontogenic capability: bone marrow stromal stem cells versus dental pulp stem cells. Biol Cell 99(8):465–474. doi:10.1042/bc20070013
35. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100(10):5807–5812. doi:10.1073/pnas.0937635100
36. Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S (2008) The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod 34(6):645–651. doi:10.1016/j.joen.2008.03.001
37. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364(9429):149–155. doi:10.1016/s0140-6736(04)16627-0
38. Morsczeck C, Gotz W, Schierholz J, Zeilhofer F, Kuhn U, Mohl C, Sippel C, Hoffmann KH (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 24(2):155–165. doi:10.1016/j.matbio.2004.12.004
39. Estrela C, Alencar AH, Kitten GT, Vencio EF, Gava E (2011) Mesenchymal stem cells in the dental tissues: perspectives for tissue regeneration. Braz Dent J 22(2):91–98
40. Huang GT, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 88(9):792–806. doi:10.1177/0022034509340867
41. Mori G, Ballini A, Carbone C, Oranger A, Brunetti G, Di Benedetto A, Rapone B, Cantore S, Di Comite M, Colucci S, Grano M, Grassi FR (2012) Osteogenic differentiation of dental follicle stem cells. Int J Med Sci 9(6):480–487. doi:10.7150/ijms.4583
42. Morsczeck C, Schmalz G, Reichert TE, Vollner F, Galler K, Driemel O (2008) Somatic stem cells for regenerative dentistry. Clin Oral Investig 12(2):113–118. doi:10.1007/s00784-007-0170-8
43. Rodriguez-Lozano FJ, Bueno C, Insausti CL, Meseguer L, Ramirez MC, Blanquer M, Marin N, Martinez S, Moraleda JM (2011) Mesenchymal stem cells derived from dental tissues. Int Endod J 44(9):800–806. doi:10.1111/j.1365-2591.2011.01877.x
44. Fitzgerald M, Chiego DJ Jr, Heys DR (1990) Autoradiographic analysis of odontoblast replacement following pulp exposure in primate teeth. Arch Oral Biol 35(9):707–715
45. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S (2002) Stem cell properties of human dental pulp stem cells. J Dent Res 81(8):531–535
46. Waddington RJ, Youde SJ, Lee CP, Sloan AJ (2009) Isolation of distinct progenitor stem cell populations from dental pulp. Cells Tissues Organs 189(1–4):268–274. doi:10.1159/000151447
47. Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S (2005) The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthod Craniofac Res 8(3):191–199. doi:10.1111/j.1601-6343.2005.00331.x
48. Shi S, Robey PG, Gronthos S (2001) Comparison of human dental pulp and bone marrow stromal stem cells by cDNA microarray analysis. Bone 29(6):532–539
49. Shi S, Gronthos S (2003) Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. J Bone Miner Res 18(4):696–704. doi:10.1359/jbmr.2003.18.4.696
50. Yang X, Zhang W, van den Dolder J, Walboomers XF, Bian Z, Fan M, Jansen JA (2007) Multilineage potential of STRO-1+ rat dental pulp cells in vitro. J Tissue Eng Regen Med 1(2):128–135. doi:10.1002/term.13
51. Yang X, Walboomers XF, van den Beucken JJ, Bian Z, Fan M, Jansen JA (2009) Hard tissue formation of STRO-1-selected rat dental pulp stem cells in vivo. Tissue Eng Part A 15(2):367–375. doi:10.1089/ten.tea.2008.0133
52. Yang X, van den Dolder J, Walboomers XF, Zhang W, Bian Z, Fan M, Jansen JA (2007) The odontogenic potential of STRO-1 sorted rat dental pulp stem cells in vitro. J Tissue Eng Regen Med 1(1):66–73. doi:10.1002/term.16
53. Nakamura S, Yamada Y, Katagiri W, Sugito T, Ito K, Ueda M (2009) Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. J Endod 35(11):1536–1542. doi:10.1016/j.joen.2009.07.024

54. Sloan AJ, Waddington RJ (2009) Dental pulp stem cells: what, where, how? Int J Paediatr Dent 19(1):61–70. doi:10.1111/j.1365-263X.2008.00964.x
55. Zeichner-David M, Oishi K, Su Z, Zakartchenko V, Chen LS, Arzate H, Bringas P Jr (2003) Role of Hertwig's epithelial root sheath cells in tooth root development. Dev Dyn 228(4):651–663. doi:10.1002/dvdy.10404
56. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT (2008) Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 34(2):166–171. doi:10.1016/j.joen.2007.11.021
57. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Wang S, Shi S (2006) Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One 1(1):1–8. doi:10.1371/journal.pone.0000079
58. Hoffman RL (1966) Bone formation and resorption around developing teeth transplanted into the femur. Am J Anat 118(1):91–102. doi:10.1002/aja.1001180106
59. Coura GS, Garcez RC, de Aguiar CB, Alvarez-Silva M, Magini RS, Trentin AG (2008) Human periodontal ligament: a niche of neural crest stem cells. J Periodontal Res 43(5):531–536. doi:10.1111/j.1600-0765.2007.01065.x
60. Mao JJ, Giannobile WV, Helms JA, Hollister SJ, Krebsbach PH, Longaker MT, Shi S (2006) Craniofacial tissue engineering by stem cells. J Dent Res 85(11):966–979
61. McCulloch CA, Nemeth E, Lowenberg B, Melcher AH (1987) Paravascular cells in endosteal spaces of alveolar bone contribute to periodontal ligament cell populations. Anat Rec 219(3):233–242. doi:10.1002/ar.1092190304
62. Bartold PM, Shi S, Gronthos S (2006) Stem cells and periodontal regeneration. Periodontol 2000 40:164–172. doi:10.1111/j.1600-0757.2005.00139.x
63. Zhou S, Greenberger JS, Epperly MW, Goff JP, Adler C, Leboff MS, Glowacki J (2008) Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell 7(3):335–343. doi:10.1111/j.1474-9726.2008.00377.x
64. Xia L, Zhang Z, Chen L, Zhang W, Zeng D, Zhang X, Chang J, Jiang X (2011) Proliferation and osteogenic differentiation of human periodontal ligament cells on akermanite and beta-TCP bioceramics. Eur Cell Mater 22:68–82
65. Ten Cate AR (1997) The development of the periodontium--a largely ectomesenchymally derived unit. Periodontol 2000 13:9–19
66. Wise GE, King GJ (2008) Mechanisms of tooth eruption and orthodontic tooth movement. J Dent Res 87(5):414–434
67. MacNeil RL, Thomas HF (1993) Development of the murine periodontium. II. Role of the epithelial root sheath in formation of the periodontal attachment. J Periodontol 64(4):285–291. doi:10.1902/jop.1993.64.4.285
68. Spouge JD (1980) A new look at the rests of Malassez. A review of their embryological origin, anatomy, and possible role in periodontal health and disease. J Periodontol 51(8):437–444. doi:10.1902/jop.1980.51.8.437
69. Diekwisch TG (2001) The developmental biology of cementum. Int J Dev Biol 45(5–6):695–706
70. Ten Cate AR, Sharpe PT, Roy S, Nanci A (2003) Ten Cate's oral histology: development, structure, and function. Elsevier Health Sciences, St Louis
71. Honda MJ, Imaizumi M, Tsuchiya S, Morsczeck C (2010) Dental follicle stem cells and tissue engineering. J Oral Sci 52(4):541–552
72. Yao S, Pan F, Prpic V, Wise GE (2008) Differentiation of stem cells in the dental follicle. J Dent Res 87(8):767–771
73. Watt FM, Hogan B (2000) Out of Eden: stem cells and their niches. Science 287(5457):1427–1430
74. Jones DL, Wagers AJ (2008) No place like home: anatomy and function of the stem cell niche. Nat Rev Mol Cell Biol 9(1):11–21. doi:10.1038/nrm2319
75. Mitsiadis TA, Feki A, Papaccio G, Caton J (2011) Dental pulp stem cells, niches, and notch signaling in tooth injury. Adv Dent Res 23(3):275–279. doi:10.1177/0022034511405386
76. Scadden DT (2006) The stem-cell niche as an entity of action. Nature 441(7097):1075–1079

77. da Cunha JM, da Costa-Neves A, Kerkis I, da Silva MC (2013) Pluripotent stem cell transcription factors during human odontogenesis. Cell Tissue Res 353(3):435–441. doi:10.1007/s00441-013-1658-y
78. Tecles O, Laurent P, Zygouritsas S, Burger AS, Camps J, Dejou J, About I (2005) Activation of human dental pulp progenitor/stem cells in response to odontoblast injury. Arch Oral Biol 50(2):103–108. doi:10.1016/j.archoralbio.2004.11.009
79. Lovschall H, Tummers M, Thesleff I, Fuchtbauer EM, Poulsen K (2005) Activation of the Notch signaling pathway in response to pulp capping of rat molars. Eur J Oral Sci 113(4):312–317. doi:10.1111/j.1600-0722.2005.00221.x
80. Boxall SA, Jones E (2012) Markers for characterization of bone marrow multipotential stromal cells. Stem Cells Int 2012:1–12. doi:10.1155/2012/975871
81. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4):315–317. doi:10.1080/14653240600855905
82. Couve E (1986) Ultrastructural changes during the life cycle of human odontoblasts. Arch Oral Biol 31(10):643–651
83. Romagnoli P, Mancini G, Galeotti F, Francini E, Pierleoni P (1990) The crown odontoblasts of rat molars from primary dentinogenesis to complete eruption. J Dent Res 69(12):1857–1862
84. Smith AJ, Cassidy N, Perry H, Begue-Kirn C, Ruch JV, Lesot H (1995) Reactionary dentinogenesis. Int J Dev Biol 39(1):273–280
85. Nor JE (2006) Tooth regeneration in operative dentistry. Oper Dent 31(6):633–642. doi:10.2341/06-000
86. Batouli S, Miura M, Brahim J, Tsutsui TW, Fisher LW, Gronthos S, Robey PG, Shi S (2003) Comparison of stem-cell-mediated osteogenesis and dentinogenesis. J Dent Res 82(12):976–981
87. Takeda T, Tezuka Y, Horiuchi M, Hosono K, Iida K, Hatakeyama D, Miyaki S, Kunisada T, Shibata T, Tezuka K (2008) Characterization of dental pulp stem cells of human tooth germs. J Dent Res 87(7):676–681
88. Huang GT, Yamaza T, Shea LD, Djouad F, Kuhn NZ, Tuan RS, Shi S (2010) Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng Part A 16(2):605–615. doi:10.1089/ten.TEA.2009.0518
89. Wang J, Ma H, Jin X, Hu J, Liu X, Ni L, Ma PX (2011) The effect of scaffold architecture on odontogenic differentiation of human dental pulp stem cells. Biomaterials 32(31):7822–7830. doi:10.1016/j.biomaterials.2011.04.034
90. Buurma B, Gu K, Rutherford RB (1999) Transplantation of human pulpal and gingival fibroblasts attached to synthetic scaffolds. Eur J Oral Sci 107(4):282–289
91. Prescott RS, Alsanea R, Fayad MI, Johnson BR, Wenckus CS, Hao J, John AS, George A (2008) In vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice. J Endod 34(4):421–426. doi:10.1016/j.joen.2008.02.005
92. Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, Smith AJ, Nor JE (2008) Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 34(8):962–969. doi:10.1016/j.joen.2008.04.009
93. Iohara K, Zheng L, Ito M, Tomokiyo A, Matsushita K, Nakashima M (2006) Side population cells isolated from porcine dental pulp tissue with self-renewal and multipotency for dentinogenesis, chondrogenesis, adipogenesis, and neurogenesis. Stem Cells 24(11):2493–2503. doi:10.1634/stemcells.2006-0161
94. Nakashima M, Iohara K, Ishikawa M, Ito M, Tomokiyo A, Tanaka T, Akamine A (2004) Stimulation of reparative dentin formation by ex vivo gene therapy using dental pulp stem cells electrotransfected with growth/differentiation factor 11 (Gdf11). Hum Gene Ther 15(11):1045–1053. doi:10.1089/hum.2004.15.1045
95. Peters MC, Polverini PJ, Mooney DJ (2002) Engineering vascular networks in porous polymer matrices. J Biomed Mater Res 60(4):668–678

96. Stiver SI, Tan X, Brown LF, Hedley-Whyte ET, Dvorak HF (2004) VEGF-A angiogenesis induces a stable neovasculature in adult murine brain. J Neuropathol Exp Neurol 63(8):841–855
97. Sun Q, Chen RR, Shen Y, Mooney DJ, Rajagopalan S, Grossman PM (2005) Sustained vascular endothelial growth factor delivery enhances angiogenesis and perfusion in ischemic hind limb. Pharm Res 22(7):1110–1116. doi:10.1007/s11095-005-5644-2
98. Iohara K, Zheng L, Ito M, Ishizaka R, Nakamura H, Into T, Matsushita K, Nakashima M (2009) Regeneration of dental pulp after pulpotomy by transplantation of CD31(−)/CD146(−) side population cells from a canine tooth. Regen Med 4(3):377–385. doi:10.2217/rme.09.5
99. Iohara K, Imabayashi K, Ishizaka R, Watanabe A, Nabekura J, Ito M, Matsushita K, Nakamura H, Nakashima M (2011) Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1. Tissue Eng Part A 17(15–16):1911–1920. doi:10.1089/ten.TEA.2010.0615
100. Colnot C (2011) Cell sources for bone tissue engineering: insights from basic science. Tissue Eng Part B Rev 17(6):449–457. doi:10.1089/ten.TEB.2011.0243
101. Khojasteh A, Motamedian SR (2016) Mesenchymal stem cell therapy for treatment of craniofacial bone defects: 10 years of experience. Regen Reconstr Restor 1(1):1–7. doi:107508/rrr.2015.01.001
102. Khojasteh A, Eslaminejad MB, Nazarian H (2008) Mesenchymal stem cells enhance bone regeneration in rat calvarial critical size defects more than platelete-rich plasma. Oral Surg Oral Med Oral Pathol Oral Radiol 106(3):356–362
103. Khojasteh A, Behnia H, Hosseini FS, Dehghan MM, Abbasnia P, Abbas FM (2013) The effect of PCL-TCP scaffold loaded with mesenchymal stem cells on vertical bone augmentation in dog mandible: a preliminary report. J Biomed Mater Res B: Appl Biomater 101(5):848–854
104. Khojasteh A (2014) Regenerative medicine in dentistry: new horizons. J Paramed Sci 5(3)
105. Handa K, Saito M, Yamauchi M, Kiyono T, Sato S, Teranaka T, Sampath Narayanan A (2002) Cementum matrix formation in vivo by cultured dental follicle cells. Bone 31(5):606–611
106. Yagyuu T, Ikeda E, Ohgushi H, Tadokoro M, Hirose M, Maeda M, Inagake K, Kirita T (2010) Hard tissue-forming potential of stem/progenitor cells in human dental follicle and dental papilla. Arch Oral Biol 55(1):68–76. doi:10.1016/j.archoralbio.2009.10.011
107. Honda MJ, Imaizumi M, Suzuki H, Ohshima S, Tsuchiya S, Satomura K (2011) Stem cells isolated from human dental follicles have osteogenic potential. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 111(6):700–708. doi:10.1016/j.tripleo.2010.08.004
108. Tsuchiya S, Ohshima S, Yamakoshi Y, Simmer JP, Honda MJ (2010) Osteogenic differentiation capacity of porcine dental follicle progenitor cells. Connect Tissue Res 51(3):197–207. doi:10.3109/03008200903267542
109. Rezai Rad M, Bova JF, Orooji M, Pepping J, Qureshi A, Piero FD, Hayes D, Yao S (2015) Evaluation of bone regeneration potential of dental follicle stem cells (DFSCs) for treatment of craniofacial defects. Cytotherapy 17(11):1572–1581. doi:10.1016/j.jcyt.2015.07.013
110. Carinci F, Papaccio G, Laino G, Palmieri A, Brunelli G, D'Aquino R, Graziano A, Lanza V, Scapoli L, Martinelli M, Pezzetti F (2008) Comparison between genetic portraits of osteoblasts derived from primary cultures and osteoblasts obtained from human pulpar stem cells. J Craniofac Surg 19(3):616–625. doi:10.1097/SCS.0b013e31816aabc8
111. Graziano A, d'Aquino R, Laino G, Proto A, Giuliano MT, Pirozzi G, De Rosa A, Di Napoli D, Papaccio G (2008) Human CD34+ stem cells produce bone nodules in vivo. Cell Prolif 41(1):1–11. doi:10.1111/j.1365-2184.2007.00497.x
112. Riccio M, Maraldi T, Pisciotta A, La Sala GB, Ferrari A, Bruzzesi G, Motta A, Migliaresi C, De Pol A (2012) Fibroin scaffold repairs critical-size bone defects in vivo supported by human amniotic fluid and dental pulp stem cells. Tissue Eng Part A 18(9–10):1006–1013. doi:10.1089/ten.TEA.2011.0542
113. Annibali S, Bellavia D, Ottolenghi L, Cicconetti A, Cristalli MP, Quaranta R, Pilloni A (2014) Micro-CT and PET analysis of bone regeneration induced by biodegradable scaffolds as carriers for dental pulp stem cells in a rat model of calvarial "critical size" defect: preliminary data. J Biomed Mater Res B Appl Biomater 102(4):815–825. doi:10.1002/jbm.b.33064

114. d'Aquino R, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A, Desiderio V, Laino G, Papaccio G (2009) Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. Eur Cell Mater 18:75–83
115. Giuliani A, Manescu A, Langer M, Rustichelli F, Desiderio V, Paino F, De Rosa A, Laino L, d'Aquino R, Tirino V, Papaccio G (2013) Three years after transplants in human mandibles, histological and in-line holotomography revealed that stem cells regenerated a compact rather than a spongy bone: biological and clinical implications. Stem Cells Transl Med 2(4):316–324. doi:10.5966/sctm.2012-0136
116. Yamada Y, Ito K, Nakamura S, Ueda M, Nagasaka T (2011) Promising cell-based therapy for bone regeneration using stem cells from deciduous teeth, dental pulp, and bone marrow. Cell Transplant 20(7):1003–1013. doi:10.3727/096368910x539128
117. Lekic PC, Rajshankar D, Chen H, Tenenbaum H, McCulloch CA (2001) Transplantation of labeled periodontal ligament cells promotes regeneration of alveolar bone. Anat Rec 262(2):193–202
118. Moshaverinia A, Xu X, Chen C, Akiyama K, Snead ML, Shi S (2013) Dental mesenchymal stem cells encapsulated in an alginate hydrogel co-delivery microencapsulation system for cartilage regeneration. Acta Biomater 9(12):9343–9350. doi:10.1016/j.actbio.2013.07.023
119. Kim SH, Kim KH, Seo BM, Koo KT, Kim TI, Seol YJ, Ku Y, Rhyu IC, Chung CP, Lee YM (2009) Alveolar bone regeneration by transplantation of periodontal ligament stem cells and bone marrow stem cells in a canine peri-implant defect model: a pilot study. J Periodontol 80(11):1815–1823. doi:10.1902/jop.2009.090249
120. Xu J, Wang W, Kapila Y, Lotz J, Kapila S (2009) Multiple differentiation capacity of STRO-1+/CD146+ PDL mesenchymal progenitor cells. Stem Cells Dev 18(3):487–496. doi:10.1089/scd.2008.0113
121. Gay IC, Chen S, MacDougall M (2007) Isolation and characterization of multipotent human periodontal ligament stem cells. Orthod Craniofac Res 10(3):149–160. doi:10.1111/j.1601-6343.2007.00399.x
122. Houshmand B, Behnia H, Khoshzaban A, Morad G, Behrouzi G, Dashti SG, Khojasteh A (2012) Osteoblastic differentiation of human stem cells derived from bone marrow and periodontal ligament under the effect of enamel matrix derivative and transforming growth factor-beta. Int J Oral Maxillofac Implants 28(6):e440–450
123. de Mendonca CA, Bueno DF, Martins MT, Kerkis I, Kerkis A, Fanganiello RD, Cerruti H, Alonso N, Passos-Bueno MR (2008) Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. J Craniofac Surg 19(1):204–210. doi:10.1097/scs.0b013e31815c8a54
124. Chadipiralla K, Yochim JM, Bahuleyan B, Huang CY, Garcia-Godoy F, Murray PE, Stelnicki EJ (2010) Osteogenic differentiation of stem cells derived from human periodontal ligaments and pulp of human exfoliated deciduous teeth. Cell Tissue Res 340(2):323–333. doi:10.1007/s00441-010-0953-0
125. Zhang H, Wang J, Deng F, Huang E, Yan Z, Wang Z, Deng Y, Zhang Q, Zhang Z, Ye J, Qiao M, Li R, Wang J, Wei Q, Zhou G, Luu HH, Haydon RC, He TC, Deng F (2015) Canonical Wnt signaling acts synergistically on BMP9-induced osteo/odontoblastic differentiation of stem cells of dental apical papilla (SCAPs). Biomaterials 39:145–154. doi:10.1016/j.biomaterials.2014.11.007
126. Martens W, Bronckaers A, Politis C, Jacobs R, Lambrichts I (2013) Dental stem cells and their promising role in neural regeneration: an update. Clin Oral Investig 17(9):1969–1983. doi:10.1007/s00784-013-1030-3
127. Martens W, Wolfs E, Struys T, Politis C, Bronckaers A, Lambrichts I (2012) Expression pattern of basal markers in human dental pulp stem cells and tissue. Cells Tissues Organs 196(6):490–500. doi:10.1159/000338654
128. Abe S, Yamaguchi S, Amagasa T (2007) Multilineage cells from apical pulp of human tooth with immature apex. Oral Science International 4(1):45–58. doi:10.1016/S1348-8643(07)80011-5
129. Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S (2008) Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells 26(7):1787–1795. doi:10.1634/stemcells.2007-0979

130. Karbanova J, Soukup T, Suchanek J, Pytlik R, Corbeil D, Mokry J (2011) Characterization of dental pulp stem cells from impacted third molars cultured in low serum-containing medium. Cells Tissues Organs 193(6):344–365. doi:10.1159/000321160
131. Kiraly M, Porcsalmy B, Pataki A, Kadar K, Jelitai M, Molnar B, Hermann P, Gera I, Grimm WD, Ganss B, Zsembery A, Varga G (2009) Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons. Neurochem Int 55(5):323–332. doi:10.1016/j.neuint.2009.03.017
132. Kiraly M, Kadar K, Horvathy DB, Nardai P, Racz GZ, Lacza Z, Varga G, Gerber G (2011) Integration of neuronally predifferentiated human dental pulp stem cells into rat brain in vivo. Neurochem Int 59(3):371–381. doi:10.1016/j.neuint.2011.01.006
133. Arthur A, Shi S, Zannettino AC, Fujii N, Gronthos S, Koblar SA (2009) Implanted adult human dental pulp stem cells induce endogenous axon guidance. Stem Cells 27(9):2229–2237. doi:10.1002/stem.138
134. Nosrat IV, Widenfalk J, Olson L, Nosrat CA (2001) Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons in vitro, and rescue motoneurons after spinal cord injury. Dev Biol 238(1):120–132. doi:10.1006/dbio.2001.0400
135. Nosrat IV, Smith CA, Mullally P, Olson L, Nosrat CA (2004) Dental pulp cells provide neurotrophic support for dopaminergic neurons and differentiate into neurons in vitro; implications for tissue engineering and repair in the nervous system. Eur J Neurosci 19(9):2388–2398. doi:10.1111/j.0953-816X.2004.03314.x
136. Huang AH, Snyder BR, Cheng PH, Chan AW (2008) Putative dental pulp-derived stem/stromal cells promote proliferation and differentiation of endogenous neural cells in the hippocampus of mice. Stem Cells 26(10):2654–2663. doi:10.1634/stemcells.2008-0285
137. Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Ogiuchi H, Okano T, Ando T (2011) PLGA artificial nerve conduits with dental pulp cells promote facial nerve regeneration. J Tissue Eng Regen Med 5(10):823–830. doi:10.1002/term.387
138. Nakashima M, Iohara K (2011) Regeneration of dental pulp by stem cells. Adv Dent Res 23(3):313–319. doi:10.1177/0022034511405323
139. Moioli EK, Clark PA, Chen M, Dennis JE, Erickson HP, Gerson SL, Mao JJ (2008) Synergistic actions of hematopoietic and mesenchymal stem/progenitor cells in vascularizing bioengineered tissues. PLoS One 3(12):1–11. doi:10.1371/journal.pone.0003922
140. Dissanayaka WL, Zhan X, Zhang C, Hargreaves KM, Jin L, Tong EH (2012) Coculture of dental pulp stem cells with endothelial cells enhances osteo-/odontogenic and angiogenic potential in vitro. J Endod 38(4):454–463. doi:10.1016/j.joen.2011.12.024
141. Barachini S, Danti S, Pacini S, D'Alessandro D, Carnicelli V, Trombi L, Moscato S, Mannari C, Cei S, Petrini M (2014) Plasticity of human dental pulp stromal cells with bioengineering platforms: a versatile tool for regenerative medicine. Micron 67:155–168. doi:10.1016/j.micron.2014.07.003
142. Iohara K, Zheng L, Wake H, Ito M, Nabekura J, Wakita H, Nakamura H, Into T, Matsushita K, Nakashima M (2008) A novel stem cell source for vasculogenesis in ischemia: subfraction of side population cells from dental pulp. Stem Cells 26(9):2408–2418. doi:10.1634/stemcells.2008-0393
143. d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, Papaccio G (2007) Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ 14(6):1162–1171
144. Bronckaers A, Hilkens P, Fanton Y, Struys T, Gervois P, Politis C, Martens W, Lambrichts I (2013) Angiogenic properties of human dental pulp stem cells. PLoS One 8(8):1–11
145. Yamaguchi TP, Dumont DJ, Conlon RA, Breitman ML, Rossant J (1993) flk-1, an flt-related receptor tyrosine kinase is an early marker for endothelial cell precursors. Development 118(2):489–498
146. Cao Y (2009) Positive and negative modulation of angiogenesis by VEGFR1 ligands. Sci Signal 2(59):re1. doi:10.1126/scisignal.259re1
147. Zhang Z, Neiva KG, Lingen MW, Ellis LM, Nor JE (2010) VEGF-dependent tumor angiogenesis requires inverse and reciprocal regulation of VEGFR1 and VEGFR2. Cell Death Differ 17(3):499–512. doi:10.1038/cdd.2009.152

148. Sakai VT, Zhang Z, Dong Z, Neiva KG, Machado MA, Shi S, Santos CF, Nor JE (2010) SHED differentiate into functional odontoblasts and endothelium. J Dent Res 89(8):791–796. doi:10.1177/0022034510368647
149. Bento LW, Zhang Z, Imai A, Nor F, Dong Z, Shi S, Araujo FB, Nor JE (2013) Endothelial differentiation of SHED requires MEK1/ERK signaling. J Dent Res 92(1):51–57. doi:10.1177/0022034512466263
150. Li X, Hou J, Wu B, Chen T, Luo A (2014) Effects of platelet-rich plasma and cell coculture on angiogenesis in human dental pulp stem cells and endothelial progenitor cells. J endodont 40(11):1810–1814
151. Gandia C, Arminan A, García-Verdugo JM, Lledo E, Ruiz A, Minana MD, Sanchez-Torrijos J, Paya R, Mirabet V, Carbonell-Uberos F (2008) Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem cells 26(3):638–645
152. Mauro A (1961) Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 9:493–495
153. Zammit P, Beauchamp J (2001) The skeletal muscle satellite cell: stem cell or son of stem cell? Differentiation 68(4–5):193–204
154. Arminan A, Gandia C, Bartual M, Garcia-Verdugo JM, Lledo E, Mirabet V, Llop M, Barea J, Montero JA, Sepulveda P (2009) Cardiac differentiation is driven by NKX2.5 and GATA4 nuclear translocation in tissue-specific mesenchymal stem cells. Stem Cells Dev 18(6):907–918. doi:10.1089/scd.2008.0292
155. Yang R, Chen M, Lee CH, Yoon R, Lal S, Mao JJ (2010) Clones of ectopic stem cells in the regeneration of muscle defects in vivo. PLoS One 5(10):1–8. doi:10.1371/journal.pone.0013547
156. Nakatsuka R, Nozaki T, Uemura Y, Matsuoka Y, Sasaki Y, Shinohara M, Ohura K, Sonoda Y (2010) 5-Aza-2'-deoxycytidine treatment induces skeletal myogenic differentiation of mouse dental pulp stem cells. Arch Oral Biol 55(5):350–357. doi:10.1016/j.archoralbio.2010.03.003
157. Antonitsis P, Ioannidou-Papagiannaki E, Kaidoglou A, Charokopos N, Kalogeridis A, Kouzi-Koliakou K, Kyriakopoulou I, Klonizakis I, Papakonstantinou C (2008) Cardiomyogenic potential of human adult bone marrow mesenchymal stem cells in vitro. Thorac Cardiovasc Surg 56(2):77–82. doi:10.1055/s-2007-989328
158. Ye NS, Chen J, Luo GA, Zhang RL, Zhao YF, Wang YM (2006) Proteomic profiling of rat bone marrow mesenchymal stem cells induced by 5-azacytidine. Stem Cells Dev 15(5):665–676. doi:10.1089/scd.2006.15.665
159. Song M, Kim H, Choi Y, Kim K, Chung C (2012) Skeletal myogenic differentiation of human periodontal ligament stromal cells isolated from orthodontically extracted premolars. Korean J Orthod 42(5):249–254. doi:10.4041/kjod.2012.42.5.249
160. Alsberg E, Hill EE, Mooney DJ (2001) Craniofacial tissue engineering. Crit Rev Oral Biol Med 12(1):64–75
161. Mao JJ (2005) Stem-cell-driven regeneration of synovial joints. Biol Cell 97(5):289–301. doi:10.1042/bc20040100
162. Alhadlaq A, Elisseeff JH, Hong L, Williams CG, Caplan AI, Sharma B, Kopher RA, Tomkoria S, Lennon DP, Lopez A, Mao JJ (2004) Adult stem cell driven genesis of human-shaped articular condyle. Ann Biomed Eng 32(7):911–923
163. Alhadlaq A, Mao JJ (2005) Tissue-engineered osteochondral constructs in the shape of an articular condyle. J Bone Joint Surg Am 87(5):936–944. doi:10.2106/jbjs.d.02104
164. Allen KD, Athanasiou KA (2006) Growth factor effects on passaged TMJ disk cells in monolayer and pellet cultures. Orthod Craniofac Res 9(3):143–152. doi:10.1111/j.1601-6343.2006.00370.x
165. Detamore MS, Athanasiou KA (2005) Use of a rotating bioreactor toward tissue engineering the temporomandibular joint disc. Tissue Eng 11(7–8):1188–1197. doi:10.1089/ten.2005.11.1188
166. Johns DE, Athanasiou KA (2007) Improving culture conditions for temporomandibular joint disc tissue engineering. Cells Tissues Organs 185(4):246–257. doi:10.1159/000102173
167. Anderson DE, Athanasiou KA (2009) A comparison of primary and passaged chondrocytes for use in engineering the temporomandibular joint. Arch Oral Biol 54(2):138–145. doi:10.1016/j.archoralbio.2008.09.018

168. Chai Y, Jiang X, Ito Y, Bringas P Jr, Han J, Rowitch DH, Soriano P, McMahon AP, Sucov HM (2000) Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. Development 127(8):1671–1679
169. Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira LV, Caplan AI, Cerruti HF (2006) Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 184(3–4):105–116. doi:10.1159/000099617
170. Yu J, He H, Tang C, Zhang G, Li Y, Wang R, Shi J, Jin Y (2010) Differentiation potential of STRO-1+ dental pulp stem cells changes during cell passaging. BMC Cell Biol 11(1):1–7
171. Morito A, Kida Y, Suzuki K, Inoue K, Kuroda N, Gomi K, Arai T, Sato T (2009) Effects of basic fibroblast growth factor on the development of the stem cell properties of human dental pulp cells. Arch Histol Cytol 72(1):51–64
172. Koyama N, Okubo Y, Nakao K, Bessho K (2009) Evaluation of pluripotency in human dental pulp cells. J Oral Maxillofac Surg 67(3):501–506. doi:10.1016/j.joms.2008.09.011
173. Gimble J, Guilak F (2003) Adipose-derived adult stem cells: isolation, characterization, and differentiation potential. Cytotherapy 5(5):362–369. doi:10.1080/14653240310003026
174. Alhadlaq A, Tang M, Mao JJ (2005) Engineered adipose tissue from human mesenchymal stem cells maintains predefined shape and dimension: implications in soft tissue augmentation and reconstruction. Tissue Eng 11(3–4):556–566. doi:10.1089/ten.2005.11.556
175. Mauney JR, Nguyen T, Gillen K, Kirker-Head C, Gimble JM, Kaplan DL (2007) Engineering adipose-like tissue in vitro and in vivo utilizing human bone marrow and adipose-derived mesenchymal stem cells with silk fibroin 3D scaffolds. Biomaterials 28(35):5280–5290. doi:10.1016/j.biomaterials.2007.08.017
176. Johns DE, Wong ME, Athanasiou KA (2008) Clinically relevant cell sources for TMJ disc engineering. J Dent Res 87(6):548–552

Chapter 9
Dental Stem Cells: Possibility for Generation of a Bio-tooth

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9.1 Introduction

Regeneration of tissues and organs requires highly specific orchestrations on cell/extracellular matrix (ECM)/scaffold interactions depending on the tissue characteristics. Bioengineering of tooth and surrounding periodontal tissues is challenging due to their structural complexity. Tooth development requires epithelial-mesenchymal interactions, and signaling pathways of these interactions are still unclear. To overcome these difficulties, various cells such as periodontal ligament (PDL) fibroblasts, osteoblasts, cementoblasts, odontoblasts and ameloblasts have been tried to induce new PDL, bone and cementum for new periodontal attachment apparatus and dentin, and enamel for new crown development. In the last decade, mesenchymal stem cells (MSCs) are also studied in periodontal tissue regeneration approaches. Currently, cell-based therapies using MSCs are very popular to regenerate dental tissues [1, 2]. In this regard, the accessibility and quality of the stem cells are very critical for cell-based dental tissue engineering.

9.2 Stem Cells in Dentistry

In the last decade, different sources including dental follicles, apical papilla, exfoliated deciduous teeth pulp, permanent teeth (premolar, molar) pulp and PDL have been investigated for MSCs isolation [3–7]. Recent studies demonstrated new and

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more accessible sources for stem cell-like populations including gingiva, palatal connective tissue and oral mucosa [8–10]. Comparison of MSCs originated from oral or dental tissues with bone marrow MSCs (BMMSCs) demonstrated that these cells possess similar characteristics for their differentiation capacities [5–12].

9.2.1 Stem Cells Studies in Reconstruction of Cranio-Facial Tissues

Bone regeneration therapies are frequently required because of trauma, infection, congenital conditions and cancer. Dental implant therapies are needed to rehabilitate patients for functional, phonation and esthetic reasons. Dental implant-supported therapies may have some difficulties if the bone tissue is insufficient at the treatment area. Cell-based therapies combined with appropriate scaffolds may help to overcome the limitations of currently used biomaterials including xenografts, autografts, allografts, and alloplastic materials in these challenging situations [13, 14]. Stem cell therapy can be beneficial on treatments of craniofacial bone defects, *i.e.* sinus lift, extraction socket preservation, and bone augmentation procedures to prepare bone for implant insertion [15]. Although there are several animal studies in goat [16], canine [17, 18] and sheep [19], supporting MSC-based therapies for the purpose of bone regeneration, only case and/or case series were published in the literature as human studies [20–23].

Recently, randomized clinical trials for stem cell-based therapies have been mostly started in dentistry. To reconstruct localized craniofacial bone defects, Kaigler et al. [24] planned a randomized and controlled clinical trial with mixed stem and progenitor cell population enriched in CD14 and CD90 positive cells isolated from bone marrow (tissue repair cells, TRC) for socket preservation after tooth extraction. Guided bone regeneration (GBR) as control group or TRC transplantation as test group were applied to the participants. No adverse affect was reported after 1-year following the therapy. The clinical, histological and radiographic evaluations of the study demonstrated that TRC therapy increased alveolar bone regeneration compared to GBR therapy. Test group needed less secondary bone grafting during implant insertion. Bony dehiscence exposure on the implants was noted 5-fold longer in the control group compared to the test group [24]. In a very recent study, same group from University of Michigan investigated transplantation of autologous cells enriched for $CD90^+$ stem cells and $CD14^+$ monocytes in the reconstruction of bone deficiencies of the maxillary sinus in a randomized and controlled clinical trial. Patients with 50–80 % maxillary sinus deficiency were randomly allocated to two groups: (i) stem cells combined with β-tricalcium phosphate scaffold group and (ii) control group (scaffold alone). While radiographic analysis showed no difference in the total bone volume gained between test and control groups, 4 months after treatment, bone density in test group was found to be higher. Bone core biopsies of the test group showed better bone quality than

control group [25]. In addition, the authors reported no adverse effects after the 1-year follow-up, suggesting that cell-based therapy is safe for maxillary sinus reconstruction and may be an alternative for other maxillofacial bone defects.

9.2.2 Stem Cell Studies in Periodontal and Peri-Implantal Regeneration

BMMSCs were used for periodontal regeneration to promote new cementum, PDL and alveolar bone [26, 27]. BMMSCs seeded biodegradable scaffolds were used for the extraction socket preservation and additional benefit for the preservation of alveolar bone walls was observed in the cell seeded group when compared to control groups [28]. Stem cells isolated from pulp (DPSCs) and PDL (PDLSCs) have been used in several animal and human studies for regenerative periodontal and peri-implantal treatment [29–33]. In canine peri-implant defect models, PDLSCs and BMMSCs were compared for their alveolar bone regeneration capacities [30], and it was found that BMMSC group provided highest new bone formation rate. Transplantation of progenitor cells were thought as an effective and safe alternative in the treatment of human periodontitis; therefore, autologous PDLSCs were applied to the periodontal defects [33]. Upon the well-documented satisfactory results in animal studies [34], further randomized clinical trials with these stem cells are warranted to determine additional benefits of dental/oral stem cell-based therapies [35].

9.2.3 Stem Cells Studies in Pulp Regeneration

Aim of the regenerative endodontics is to convert the non-vital tooth into vital substitute to pathological pulp with functional healthy pulp tissue [36]. For this purpose, DPSCs or other MSCs from different sources have been investigated for revitalization/revascularization procedures in dentistry. Recent studies reported the presence of MSCs in human inflamed pulps [37] and inflamed periapical tissues [38]. Therefore, even infected pulp tissue can be used to obtain autologous MSCs in pulp regeneration treatment. Ravindran et al. investigated differentiation ability of human PDLSCs and BMMSCs into odontogenic lineage [39]. Histological and immunohistochemical analysis revealed that a vascularized pulp-like tissue could be formed by BMMSCs, PDLSCs and DPSCs. They concluded that the biomimetic scaffolds may promote odontogenic differentiation of BMMSCs, PDLSCs and DPSCs. To regenerate pulp, stem cells and biomimetic extracellular matrix combination provides new perspective toward possible therapeutic application in endodontics. Recently, a combination of $CD31^-$ and $CD105^+$ DPSC-seeded scaffolds was used for dental pulp regeneration in a canine pulpectomy model [40]. The potential of DPSCs in regenerating pulp-like tissue was proved in immature canine

teeth [41]. From a clinical perspective, although these studies give promise, further studies are strictly needed to establish new methods and proper parameters to provide functional pulp regeneration; *i.e.* appropriate cells, scaffolds, growth factors and clinical application procedures.

9.2.4 Stem Cells in Tooth Development, Bio-tooth or Bio-root

As regeneration of a single tissue compartment of tooth and periodontium, namely bone, PDL or pulp, is even complicated matter, creating a functional whole tooth and appropriate interaction of every single tissue of this organ become a real challenge. The knowledge about tooth development was obtained from the laboratory mice. The regulation of the signals on the tooth initiation and morphogenesis is not still enough clear. In human body, biologically replacement of congenitally missing or lost teeth still remains as a dream.

Two possible ways have been proposed to obtain biological tooth:

(i) Using cells with tooth forming ability, and transplantation to the jaw bone [42, 43].
(ii) Using cells to create the every single compartment of tooth including PDL, pulp and cementum, and seeding these cells to the bio-printed tooth scaffold or decellularized natural tooth [44, 45].

A number of animal studies were performed for whole-tooth bioengineering [46, 47]. Most realistic thought seems to use cells with tooth-forming capacity, transplantation of tooth germ to the jaw, and allowing the formation of a physiological root [1]. Obtaining a biologically mimicked and fully-functional tooth is the main objective for missing teeth due to trauma or periodontal and pulpal disease [44]. Researchers actively follows recent developments in stem cell-mediated tissue regeneration in dentistry [48–50]. In order to regenerate functional pulp and PDL, researchers have explored the characteristics of MSCs isolated from dental tissues [51–53]. In this sense, differentiation ability differences of various cells have been investigated; *i.e.* DPSCs have found prone to dentinogenesis [52] and PDLSCs to cementogenesis [54]. Cells should be used according to their potentials (proliferation, differentiation and immuno-regulatory), and the targeted tissue/organ in cell-based regenerative therapies [55, 56].

9.3 Oral/Dental Tissue-Derived MSCs

Oral/dental MSCs have become more popular due to their similarities with other MSCs based on their characteristics, relative ease of obtaining and propagating. Examination of differentiation and proliferation capacities of these oral/dental tissue-derived MSCs has been previously carried out in detailed in vivo and in vitro studies. As the first report on this topic, Gronthos et al. have revealed that stem cells derived

from the wisdom teeth's dental pulp has formed dentin/pulp-like structures in vivo and in vitro [57]. The same study group has subsequently accomplished to produce ectomesenchymal stem cells from exfoliated deciduous teeth (SHEDs) [58]. Later on, cells with MSC characteristic were successfully isolated from pulp tissue of supernumerary [59], natal tooth [60] and human third molar germs of young adults [61].

9.3.1 Oral Tissue-Derived MSCs

9.3.1.1 Gingiva-Derived MSCs

Gingival tissue is a part of the unique soft tissue that surrounds teeth; covering the alveolar ridges, palatal and retromolar regions [62, 63]. In addition, because gingival tissue is a distinctive component of the oral mucosal immunity, it plays a significant role in periodontal protection and wound healing. Therefore, gingival tissue participates in the mucosal barrier to stand against bacterial infection, sudden thermal and chemical changes. Another important feature of the gingival tissue is its unique scar-free healing process after the damage occurring in oral tissue [62, 64, 65]. Thus, gingival tissue derived-cells are admitted as potential MSC source because of unique characteristics such as regeneration ability, wound closure, clonogenicity, immunomodulatory properties and multipotent differentiation capacity like other MSCs [65, 66]. A new area of research on stem cell types obtained from periodontal connective tissues where gingival tissue was firstly used for the isolation of progenitor/stromal cell population by Zhang et al. has emerged. MSCs derived from gingival tissue (GMSCs), which are clonogenic colonies, can exhibit stem cell properties and express typical MSC surface markers. They have the capacity of differentiation toward multiple mesodermal lineages in vitro, and have stable phenotype and telomerase activity in long-term cultures [66]. Recent studies have shown that GMSCs are not prone to tumor formation whether they are obtained from healthy or inflamed\hyperplastic gingival tissue, indicating a tremendous potential for therapeutic applications [67]. GMSCs are considered as an accessible cell population because gingival tissues can be obtained from general dental procedures and treated as biomedical waste [68]. Indeed, gingival tissues can be obtained during tooth extraction, dental implantation or periodontal surgery [69]. Thus, GMSCs can be easily isolated from the patient with minimum disturbance. Human gingival tissue is a potential MSC source for the future clinical use for regeneration and repair considering its accessibility and availability.

9.3.1.2 Oral Mucosa-Derived MSCs

Oral cavity is covered by the oral mucosa (OM) as known. It has been showed that characteristics of OM-derived fibroblasts and fetal-derived fibroblasts are similar in some respects [70, 71]. The human OM has been suggested as a novel source for

therapeutic adult stem cells after Marynka's study in 2008 providing first evidence that the oral mucosa lamina propria (OMLP) gives rise to a robust multipotent stem cell population [72]. The same group also reported that the adult human OMLP can generate trillions of stem cells and 95 % of them can express MSC markers and so are referred as human oral mucosa stem cells (hOM-MSCs). hOM-MSCs have the capacity to differentiate in vitro into lineages of the three germ layers. Their implantation in vivo, after stimulation with dexamethasone, resulted in the formation of lineage mixed tumors consisting of tissues that develop from cranial neural crest cells during embryogenesis [73]. A high percentage of these cells (60–80 %) expressed fundamental neural and neural crest stem cell markers, and were positive for Oct4, Sox2, and Nanog. hOM-MSCs were differentiated into mesodermal (osteogenic, chondrogenic and adipogenic), definitive endoderm and ectodermal (neuronal) lineages in culture conditions, and they also shared all known MSC markers. Therefore, hOM-MSCs might be an alternative source to provide human MSCs. hOM-MSCs could possibly be clinically used for oral diseases and tissue regeneration in the future due to their promising differentiation capacity and easy isolation property.

9.3.1.3 Palatal Connective Tissue-Derived MSCs

The palatal-derived cells were previously isolated by Roman et al. for the first time in 2012, and these cells were named as progenitor-like cells but their characteristics were not completely studied [74]. Later, the characteristics of the cells isolated from the palate tissues were investigated by the same research group [9]. This study demonstrated that the basic characteristics to define cells as MSCs were met by the cells from palatal connective tissue. Palatal connective-derived MSCs are a type of adult stem cells which are easy to isolate, culture and manipulate under in vitro conditions [10]. These cells are characterized by high plasticity and can become important cell sources for regenerative therapy.

9.3.1.4 Palatal Adipose Tissue-Derived MSCs

Autologous MSCs isolated from palatal adipose tissue might have potential clinical use in regenerative alveolar bone/cranio-facial bone and periodontal therapy, and gingival recession treatments [10, 75]. More recently, our group has designed a study in order to make a comparative analysis between MSCs obtained from adipose tissue-derived lipoaspirate (LAT) and palatal adipose tissue (PAT) based on their immunophenotypic and immunogenetic properties, proliferation and differentiation potential [unpublished data]. The results demonstrated that the cell surface marker expression profile of the PAT- and LAT-MSCs showed similarities, and they expressed all MSC markers, except CD11b, CD34, CD45, CD106, CD117 and HLA-DR. PAT-MSCs showed differentiation potential into adipocytes, osteocytes and neuro-glial like cells under proper conditions like LAT-MSCs. The level of

Alkaline Phosphatase (ALP) activity of PAT-MSCs was found to be higher than the LAT-MSCs after the osteogenic differentiation in culture [unpublished data]. Results of this study pointed that PAT-MSCs are likely to have more osteogenic potential when compared to LAT-MSCs.

9.3.2 Dental-Derived MSCs

9.3.2.1 Dental Follicle-Derived MSCs (DFSCs)

The dental follicle (DF) has a loose connective tissue structure. It is thought that the dental follicle derived from third molar and wisdom tooth contains progenitor cells which are originated from cementoblasts, PDL cells and osteoblasts [6, 76, 77]. Like the other dental stem cells, DFSCs express similar cell surface antigens, and have the capability to form hard tissue in vitro and in vivo along with displaying extensive proliferative ability [78]. On the other hand, they can form the tissues of the periodontium including alveolar bone, PDL and cementum while they express the putative stem cell markers including Notch-1 and Nestin [77]. Recent studies show that DPSCs and DFSCs derived from the same tooth and donor have the ability to form colonies, and although they show similar immunophenotypic characteristics they had different levels of gene expressions.

When DFSCs and DPSCs are compared, DFSCs seemed to proliferate faster and contained cells larger in diameter. DFSCs also exhibited a higher potential to form adipocytes and a lower potential to form chondrocytes and osteoblasts with respect to DPSCs. Unlike DFSCs, DPSCs were able to produce the transforming growth factor (TGF)-β and suppressed the proliferation of peripheral blood mononuclear cells, which could be neutralized with anti-TGF-β antibody [78].

9.3.2.2 Apical Papilla-Derived MSCs

Recent studies have described the physical and histological properties of the dental papilla located at the apex of developing human permanent teeth, and this tissue is named as the “apical papilla”. Because this tissue is loosely attached to the apex of the developing root, it can easily be detached from it [79]. Discovery of human apical papilla MSCs have been accomplished by Sonoyama et al. in 2006, and they called these cells as “stem cells from the apical papilla (SCAPs)”. In this study, it has been demonstrated that SCAPs are a promising cell source for regeneration of bio-roots for future clinical applications by utilizing them to engineer bio-roots using swine as an animal model [44]. Afterwards, the same group have shown that apical papilla comprises less cellular and vascular components in comparison with the pulp tissue. SCAPs have displayed two to three times greater proliferation rate in comparison with DPSCs. Both SCAPs and DPSCs showed weak adipogenic differentiation potentials although they were as potent as BMMSCs in terms of osteo/

dentinogenic differentiation potential. Furthermore, it has been found that the immunophenotypic properties of SCAPs and DPSCs show similarities in osteogenic and dentinogenic gene profiles of growth factor receptors. A broad variety of neurogenic markers such as nestin and neurofilament are also expressed by SCAPs [80]. Besides playing a fundamental role in pulp healing and regeneration, SCAPs also contribute to the formation of developing odontoblasts which are responsible for dentinogenesis and radicular pulp formation [80, 81]. The importance of SCAPs for the apexogenesis of developing roots and constant root maturation in teenagers with endodontic diseases have been reported in recent clinical studies [82, 83]. In addition, SCAPs are also candidates to be used for dental tissue regeneration due to their remarkable regeneration capability. In vivo recombination of SCAPs and biological scaffolds resulted in generation of dentin-pulp-like tissues in the empty root canal space and bioengineered roots that can support a porcelain crown [6, 84]. It has been hypothesized that the insulin growth factor 1 (IGF-1) has a very important role in the differentiation and proliferation of SCAPs. SCAPs were isolated from juvenile third human molar apex and treated with exogenous IGF-1 for this rationale. Afterwards, in vitro and in vivo studies were conducted for the evaluation of the effects of IGF-1 on SCAPs. The increase of osteogenesis and osteogenic differentiation potential and decrease of dentinogenesis and odontogenic differentiation potential of SCAPs by IGF-1 treatment was also reported in the study of Wang et al., indicating that SCAPs treated with IGF-1 may be used as a potential candidate for bone tissue engineering [85].

9.3.2.3 PDLSCs

PDL is a gap interlaying the cementum and alveolar bone functioning as a replacement of the follicle region, which encloses the developing tooth during the cap and bud stages. Follicle (Sharpey's fibers) or cementoblast (in cellular intrinsic fiber cementum) originated fibers may be used to insert into the cementum layer. As PDL matures during the tooth eruption, it prepares to support the functional tooth for the occlusal forces [86, 87]. Major collagen bundles (principal fibers) occupy whole mature PDL by embedding in both cementum and alveolar bone. The maximization of the forces to be placed on the tooth during mastication is caused by the arrangement of fibers in specific orientations [4, 87]. Previous studies indicate that cementoblast-like cells, adipocytes and connective tissue with rich collagen structure can be produced from cell populations found in PDL which can differentiate into mesenchymal cell lineages [88, 89]. The study of Ponnaiyan et al. proved that embryonic stem cell markers Oct4 and Nanog (weak for PDLSCs) and the mesodermal marker vimentin are expressed both in DPSCs and PDLSCs. Strong expression of MSC markers (CD73 and CD90) in DPSCs and PDSCs were also shown by immunophenotyping experiments. These results indicated that MSC markers were expressed in both stem cells at different levels, suggesting that DPSCs are more primitive stem cell type with respect to PDLSCs [90]. Functional and cellular characteristics of MSCs derived from pulp and PDL from identical donors have been

compared in a recent study [91]. The results of this study proved that DPSCs and PDLSCs differed from each other in differentiation potentials and as well as expression levels of mesenchymal (CD105) and pluripotent/multipotent stem cell–associated cell surface antigens (SSEA4, CD117, CD123, andCD29). DPSCs, and PDLSCs also had different response patterns when exposed to pro-inflammatory cytokines [91].

In one of our group's recent study, different cell behaviors were seen in MSCs isolated from pulp and PDL tissues [5]. In this study, PDLSCs expressed higher levels of HLA-G, which is a major histocompatibility complex (MHC) class I molecule that functions as an immune modulatory molecule, when compared to DPSCs based on the immunohistochemical data. HLA-G inhibits cytolytic function of natural killer (NK) and cytotoxic T cells, the alloproliferative response of CD4+ T cells, the proliferation of NK and T cells, and the maturation and function of dendritic cells which have the ability to protect tissues from the immune system attacks [92]. PDLSCs had much higher levels of IL-6 and IL-10 expressions than DPSCs. While these cytokines play an important role in immune regulation, it was also demonstrated that both IL-10 and HLA-G are essential for the full immunosuppression mediated by MSCs [93]. IL-6, a pro-inflammatory cytokine, can also mediate immunosuppressive functions that might involve in the induction of IL-10 which is an anti-inflammatory cytokine.

Immunomodulatory characteristics of PDLSCs were examined by Wada et al. as a candidate sources for new allogeneic stem cell-based therapies. It is confirmed by this research group that PDLSCs, DPSCs and BMMSCs can inhibit proliferation of peripheral blood mononuclear cells (PBMNC) via stimulation of mitogen or an allogeneic-mixed lymphocyte reaction (MLR). The results of their study stated that soluble factors produced by activated PBMNCs mediated the immunosuppressive effect of PDLSCs, BMMSCs and DPSCs [94]. Similar to Wada et al., our study showed the expression of IL-6, IL-10 and HLA-G with respect to their immunoregulatory relationship. In addition, our study also demonstrated that although PDLSCs had higher IL-6 and IL-10 mRNA expression levels, DPSCs seemed to have more stemness characteristics, and higher BMP-2 and BMP-6 mRNA expression levels, indicating that PDLSCs are more likely to be preferred in clinical trials compared to DPSCs due to their superior immunomodulatory properties [5].

Lei et al. has reported that MSC characteristics of DPSCs and PDLSCs can be sustained after in vivo implantation but when compared with PDLSCs, DPSCs seems to be more stable under in vivo conditions [95]. This study also suggested that further studies need to be done to understand the mechanisms lying beneath the determination of the reduction of lineage-specific differentiation of PDLSCs. Comparison of DPSCs and PDLSCs in our study has shown that DPSCs had higher proliferation and telomerase activity [5]. The reduction of lineage-specific differentiation of PDLSCs may explain the reason of the low proliferation and telomerase activity of PDLSCs. PDLSCs, BMMSCs and DPSCs seem to share similarities in their differentiation potentials, and cell surface marker characteristics [86]. Cementum/PDL-like structures were formed when PDLSCs were transplanted into immune compromised mice. Bone generation was observed when human

PDLSCs were expanded ex vivo and seeded into 3D scaffolds (fibrin sponge, bovine-derived substitutes) [96]. These PDLSCs also seemed to preserve their stem cell properties and tissue regeneration potentials. In conclusion, overall data proposes that the PDLSC population might be used for creating a biological root to be used like a metal implant by capping with an artificial dental crown.

9.3.2.4 DPSCs (Natal, Deciduous and Adult)

Stem cells residing in the dental pulp showing similar characteristics with BMMSCs and generating the mineralized matrix of dentin due to their ability to differentiate into odontoblasts were first reported by Gronthos and co-workers [57]. Based on the surface marker expression of both cell types, they proposed that they can both adhere to plastic, form colonies, and display similar phenotypes with each other. Two different types of DPSCs were identified, which are similar to stem and stem-like cells in subsequent studies. While hematopoietic markers (CD34/CD45/CD14) are not expressed by the first type, MSC specific markers (STRO1/CD29/CD44/CD13) are strongly expressed [58, 97, 98]. The second type of DPSCs are constituted by C-kit^{+}/CD34^{+}/CD45^{-} cells which have osteogenic differentiation potential both in vivo and in vitro [99, 100]. Then after, cells displaying stem cell characteristics were isolated from pulp tissue of deciduous and wisdom tooth [97, 99, 100], supernumerary natal tooth [4] and human third molar germs of young adults [5]. Recent studies indicate that DPSCs have the ability to differentiate into a broad range of cell lineages, including odontoblasts that can produce dentin, osteoblasts, adipocytes, skeletal and smooth muscle cells, elastic cartilage cells, endothelial and neural cells both in vivo and in vitro conditions [97–105]. Even though adult stem cells share very similar behaviors both in vivo and in vitro, they carry some specific characteristics of the tissue that they were derived from. The impact of these differences on biological and clinical processes, their origin and the generation mechanism that lies beneath are still unclear. Comparing the differences or similarities between stem cell types is one way to understand these mechanisms. Such comparisons should be focused on aspects of biological marker discovery, characterization of their proliferation capacity and differentiation potential along with other characteristics. In this sense, our research group isolated putative stem cells derived from human impacted third molar dental pulp (hDP), broadly characterized and compared with human BMMSCs [106]. We found out that in contrast to hBMMSCs, cytokeratin (CK) -18 and -19, which may be involved in the dentine repair and odontoblast differentiation, are strongly expressed in hDPSCs [106]. By showing the expression of numerous specific proteins of neural stem cells (NSCs) and neurons, the essential neuro-glia characteristics of hDPSCs were demonstrated. While these cells can differentiate into chondrogenic, osteogenic and adipogenic lineages, they also share some specific characteristics like expressing some NSC- and epithelial-related markers. hDPSCs have the ability to differentiate into both vascular endothelial and neural cells under distinct conditions in vitro. hDPSCs are located in the perivascular niche of dental pulp, and because hDPSCs are originated

from migrating cranial neural crest cells, their neurogenicity is more potent with respect to hBMMSCs. Neural crest cells differentiate into a wide range of cell types including cells of dental papilla, dental follicle and neurons of the peripheral nervous system during embryonic development. Considering this, it has been shown that transplanted DPSCs can stay alive for a long time [107] and may induce neuroplasticity [108] in the central nervous system of experimental animal models. Considerable recovery from neurological dysfunction has been reported in studies mentioning DPSCs injection into the right dorsolateral striatum of animals subjected to middle cerebral arteryocclusion (MCAO) [109].

After rats with induced cortical lesions were injected into their cerebrospinal fluid with DPSCs which are pre-differentiated into neurons, these cells integrated into the host brain and exhibited some neuronal properties, indicating that they may be used as valuable sources for neuro- and glio-genesis in vivo [110]. The neuroregenerative effects of DPSCs in rodent spinal cord injury (SCI) models have also been reported in a recent study. High levels of trophic-factor expression in the tissue, better tissue organization and the existence of many axons or oligodendrocytes and neurons with synapses in DPSCs transplanted mouse models of compressive SCI suggested that DPSCs may be possible candidates for therapeutic intervention for the treatments of SCI and central nervous system disorder in humans [111].

Partial locomotor function recovery has been reported in completely transected rat spinal cord after hDPSC transplantation [112]. However, relatively less recovery of locomotor functions was detected after transplantation of human BMMSCs or skin-derived fibroblasts. It has been stated by the same research group that hDPSCs present three major neuroregenerative activities; (i) SCI-induced apoptosis of neurons, oligodendrocytes and astrocytes are inhibited by DPSCs that improves the preservation of myelin sheaths and neuronal filaments, (ii) they directly inhibit various axon growth inhibitors including chondroitin sulfate proteoglycan and myelin-associated glycoprotein via paracrine mechanisms, and (iii) they replace the lost cells by differentiating into mature oligodendrocytes under severe conditions of SCI. In line with these findings, results of our study state that due to their cell-autonomous and paracrine neuroregenerative activities, tooth-derived stem cells may offer therapeutic benefits for treating SCI [112]. Another finding of our study is that preclinical animal disease models, including myocardial infarction, colitis and systemic lupus erythematosus (SLE) may be treated via using the significant therapeutic benefits provided by the array of trophic factors produced by engrafted DPSCs [113, 114]. In correlation, DPSCs are found to be highly proliferative, self-renewing and multipotent cell population that can actively secrete a broad range of trophic, immuno-modulatory and anti-inflammatory factors. It has also been suggested by preliminary studies that other than exhibiting self-renewal and multi-differentiation potential, dental tissue-derived MSCs also have immunomodulatory functions and potent tissue regenerative properties [115–117]. We have also shown the regulation of T-cell functions via expression and secretion of soluble factors/cytokines such as HLA-G, HGF-β1, IL-6, IL-10, TGF-β1, ICAM-1 and VCAM-1 by hDPSCs in both direct and indirect co-culture systems [117].

SHEDs can proliferate faster than DPSCs and BMMSCs (SHEDs > DPSCs > BM-MSCs). If they are cultured in neurogenic differentiation medium, SHEDs can form sphere-like clusters due to their high proliferation rate, which either adhere to the culture dish or float freely in the culture medium aggregating in clusters. Dissociation of these sphere-like clusters can be done via passaging through needles and grown on dishes coated with 0.1 % gelatin as individual fibroblastic cells afterwards, demonstrating that the process can bring about a remarkable proliferative capacity analogous to that of NSCs [58]. SHEDs have also been isolated and termed as "immature DPSCs (iDPSCs)" by another research group [118]. As well as correlating with the results of studies described above, they also found out that iDPSCs express embryonic stem cell markers, including Oct4, Nanog, tumor recognition antigens (TRA-1-60 and TRA-1-81) and stage specific embryonic antigens (SSEA-3, SSEA-4).

Successful isolation and characterization of MSCs derived from human natal dental pulp (hNDP) were first declared by our research group [119] and these hNDP-MSCs were directionally differentiated to osteogenic, chondrogenic, adipogenic, myogenic and neurogenic lineages. Unlike CD3, CD8, CD11b, CD14, CD15, CD19, CD33, CD34, CD45, CD117, and HLA-DR, hNDP-MSCs expressed CD13, CD44, CD90, CD146 and CD166. hNDP-SCs seemed more developed and metabolically active cells based on their ultrastructural characteristics. Under basal conditions and without any stimulation towards differentiation, hNDP-SCs were able to express particular adipogenic (leptin, adipophilin and PPARγ), neurogenic (γ-enolase, MAP2a,b, c-fos, nestin, NF-H, NF-L, GFAP and betaIII tubulin), myogenic (desmin, myogenin, myosin-IIa, and α-SMA), osteogenic (osteonectin, osteocalcin, osteopontin, Runx-2, and type I collagen) and chondrogenic (type II collagen, SOX9) markers along with embryonic stem cell markers including Oct4, Rex-1, FoxD-3, Sox2, and Nanog. Adipogenic, osteogenic, chondrogenic, myogenic and neurogenic differentiation potentials of hNDP-SCs have also been demonstrated [119].

In one of our recent studies, phenotypic and proteomic characteristics of hDP-SCs derived from a natal, an exfoliated deciduous and an impacted third molar tooth were comparatively analyzed [120]. All three stem cells displayed similar features on morphology, proliferation rates, expression of various cell surface markers, and differentiation potentials into adipocytes, osteocytes and chondrocytes. Furthermore, using 2DE approach coupled with MALDI-TOF/TOF, we have generated a common 2DE profile for all three stem cells. We found that 62.3 ± 7 % of the protein spots were conserved among the three MSC lines. Sixty-one of these conserved spots were identified by MALDI-TOF/TOF analysis. Classification of the identified proteins based on biological function revealed that proteins that are involved in protein folding machinery along with many structurally important proteins are predominantly expressed by all three stem cell lines. Some of these proteins may hold importance in understanding specific characteristics of hDPSCs [120].

To sum up, ongoing researches on DSCs is growing at an exceptional rate. Teeth-derived stem cells can be obtained in a convenient and minimally invasive way, and are easily accessible. Based on the discussion above, these new stem cell sources

could be exploited for cellular therapies and ultimately for the development of regenerative treatment methods. Although these cells guarantee a donor match (autologous transplant) for life, they can also be used partly for close relatives.

9.4 Induced Pluripotent Stem (iPS) Cells in Dentistry

The discovery of iPS cells by Dr. Shinya Yamanaka is a milestone in stem cell research, and created a new approach in regenerative medicine [121–123]. iPS cells are obtained by reprogramming of somatic cells with gene transfer of transcription factors (Oct4, Sox2, Klf4, and c-Myc) which are highly expressed in embryonic stem cells. In the dental field, researchers are actively working with iPS cells for tooth regeneration [124]. Tooth development requires epithelial-mesenchymal interactions during the early stages of morphogenesis, and these cells come from different embryonic layer. To form tooth/root, iPS cells should be differentiated into both epithelial and mesenchymal lineages. If iPS cells can be induced separately to epithelial cells which express ameloblasts-specific proteins *(i.e.*, cytokeratin, ameloblastin, amelogenin, enamelin) and mesenchymal cells which display odontogenic potential, and if the interactions of these two lineages could be provided later, functional tooth regeneration seems possible. Although iPS cells are promising for tooth/root regeneration and tooth like-structures obtained in mouse models, tooth regeneration process using iPS cells in humans, however, cannot be that much easy. While in mouse, the success rate of tooth regeneration using mesenchyme and epithelium derived from iPS cells can be 100%, success rate of obtaining the tooth-like structures can be only 30% in human studies. The difference of success rates between two groups can be explained with lack of uniformity in the epithelium derived from human iPS cells, and lack of the capacity to secrete extracellular matrix required for tooth regeneration [125, 126]. Furthermore, these variations can be due to differences in species and signaling at the stages of tooth formation in human *vs.* mouse. Therefore, some challenges still remain in creating root/tooth formation from human iPS cells as follows;

(i) Immunogenicity of iPS cells
(ii) No established feasible reprogramming method
(iii) Lack of reproducible method due to significant differences between species
(iv) Lack of information to accelerate human tooth development in vitro or in vivo
(v) Lack of information in regulatory mechanism for iPS cells
(vi) Insufficiency in regulation of the shape and size of the tooth

iPS cells can be differentiated into both epithelial and mesenchymal cells, and expanded and maintained for tooth bioengineering [127]. Liu et al. claimed that iPS cells had more potential in tooth regeneration when compared to other stem cells due to having better proliferation and differentiation capacities [128]. In addition to form epithelium and/or mesenchyme layer of tooth germ with iPS cells, these cells can also be used to obtain functional adult MSCs [129, 130]. For the first time, Hynes and co-workers investigated the pre-clinical utility of iPS

cell-derived MSC-like cells for the treatment of periodontal fenestration defect model in rats [131]. Their results demonstrated that iPSC-MSC-like cells increased regeneration efficiency of periodontal tissues. Yang et al. used iPS cells-derived MSCs in the treatment of experimentally induced periodontitis model in rat as well, and they observed significantly decreased inflammatory infiltrates in periodontal tissues after systemic and local application of iPSC-MSCs treatment [132]. These two studies mentioned above concluded that iPSC-MSCs might provide a promising approach for the treatment of periodontal defects, and can be used as a source for not only for periodontal tissue engineering but also orthopedic applications and dental tissue engineering [133]. Ozeki et al. reported a method to differentiate mouse iPS cells into odontoblast-like cells expressing mature odontoblast markers, dentin sialophosphoprotein, and dentin matrix protein 1, and displaying physiologic and functional characteristics of odontoblasts in vitro. The generation of odontoblast cells from iPS cells may provide new clinical application area for the treatment of dental pulp regeneration [134] Since MSC-like cells derived from different iPS cell lines might demonstrate variability in their differentiation potential, detailed characterization studies regarding iPS cell-derived MSC-like cells is critical. Furthermore, safety, efficacy and economical concerns should be taken in to consideration as well [128, 133].

9.5 Current Approach in the Treatment of Missing Teeth

There are various alternative methods for the management of oral conditions due to periodontal disease, profound caries, congenital missing teeth, failure in endodontic treatment, tumor, and trauma, which may result in partial or full edentulism [135]. While the only choices for patients were conventional prosthesis including fixed prosthesis and full/partial dentures until dental implants were discovered, dental implant supported fixed and removable prosthesis have currently been offered to patients as a promising option (Figs. 9.1, 9.2, 9.3, 9.4, and 9.5). As patient's expectations and life standards increase, more options are being presented to the patients. However, economic condition can limit the alternative interventions, and is important for decision-making for the management of tooth loss.

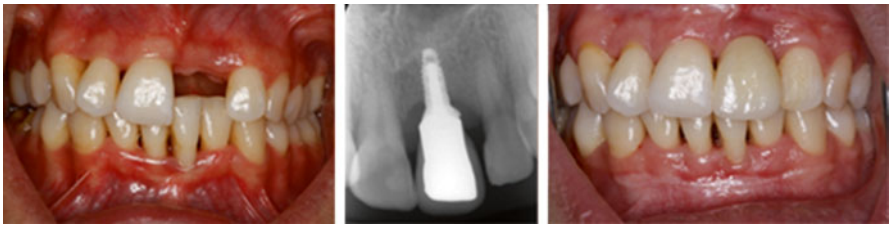

Fig. 9.1 Functional and esthetic rehabilitation of the patient with titanium implant in the case who has single missing tooth due to periodontitis

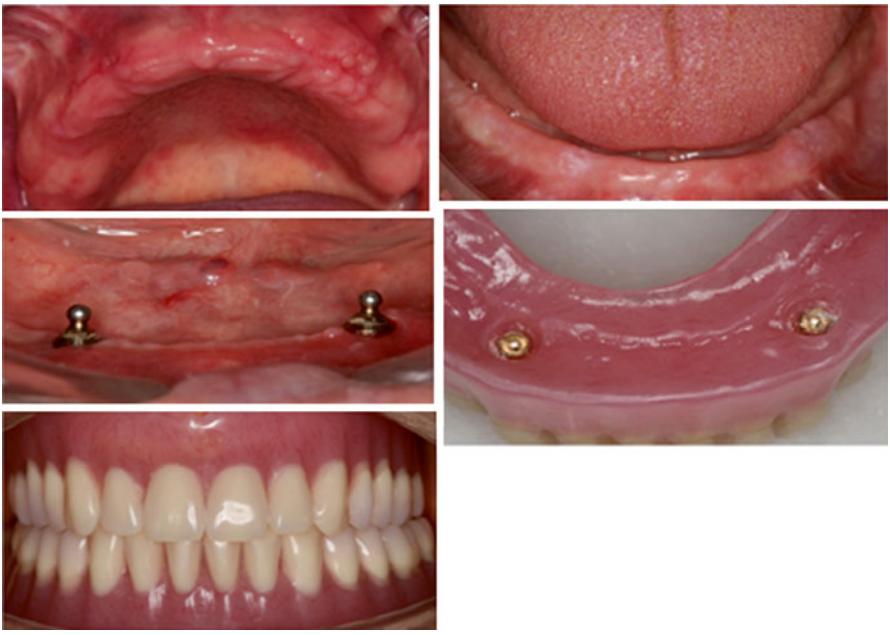

Fig. 9.2 Two-implant supported removable mandibular prosthesis using ball attachment in the edentulous patient

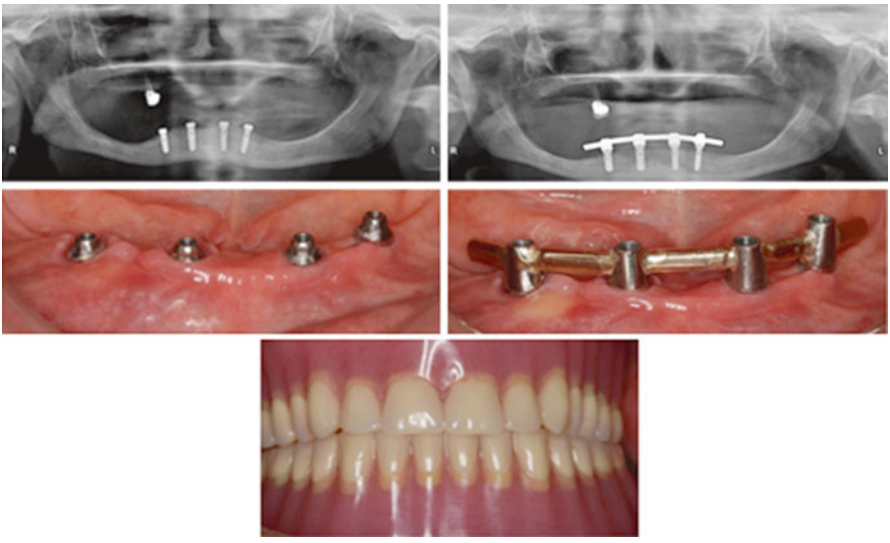

Fig. 9.3 Four-implant supported removable mandibular prosthesis with bar attachment in edentulous patient

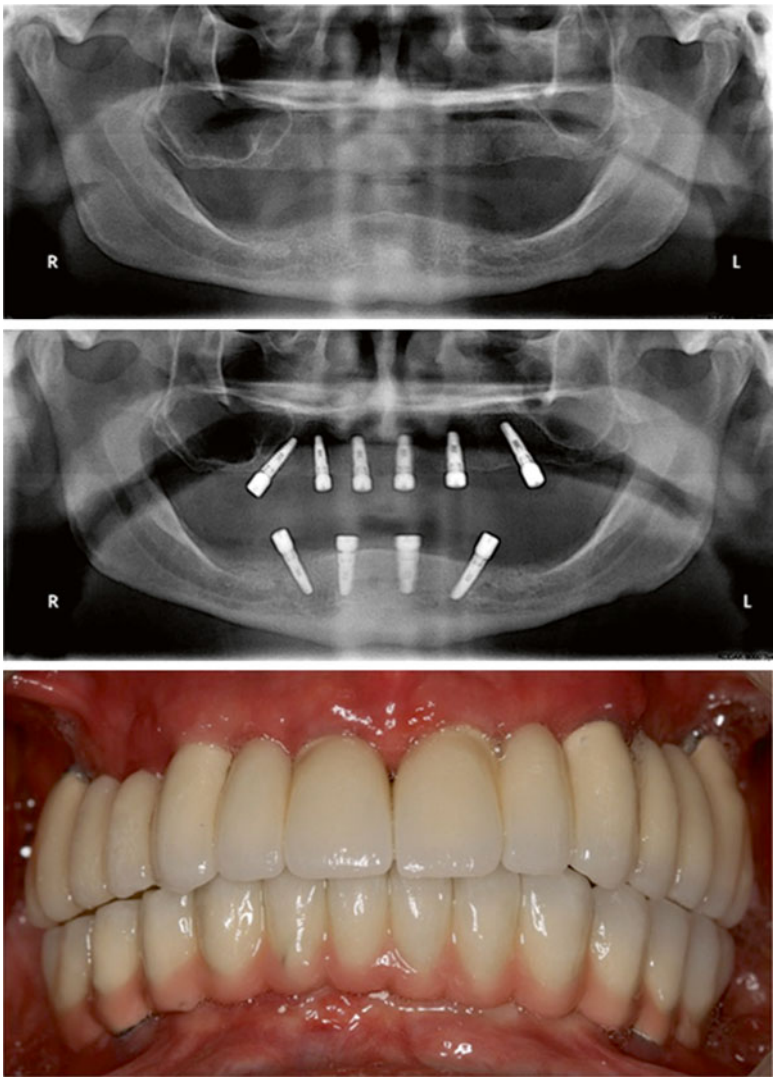

Fig. 9.4 Four-implant supported mandibular and six-implant supported maxillary fixed hybrid dental prosthesis in the edentulous patient

In dentistry, evidence-based approaches indicate that root canal treatment is the most cost-effective treatment options for the treatment of teeth with irreversible pulpitis and coronal lesions. If initial root canal treatment fails, orthograde retreatment can be the most cost-effective way. However, if root canal retreatment is not successful, extraction and/or implant-supported crown would be more cost-effective compared to traditional prosthesis including fixed or removable partial dentures [135].

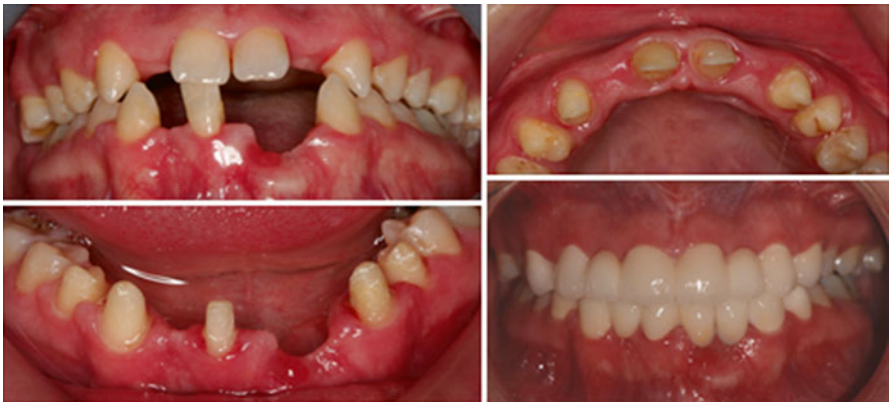

Fig. 9.5 Fixed prosthesis of the patient with conventional prosthodontic restoration

In molars with furcation involvement, non-surgical periodontal therapy is more effective for Class I types, and the therapy costs less than implant-supported single crowns. However, molars with class II-III need periodontal surgeries, and further bone graft materials and membranes for guided tissue regeneration, meaning that teeth can be saved successfully but it may not always be cost effective. Quality and survival rates of the treatment plan are very important for the replacement of missing single tooth. Implant-supported single crown provides better results in comparison with fixed partial prostheses. However, implant-supported prosthesis especially in partially or totally edentulous cases may cost more but provides superior survival rates when compared to partial or full dentures [135]. Gjengedal et al. compared the dietary intake of edentulous subjects who had conventional mandibular complete dentures or implant-supported overdenture, and recorded food avoidance of the patients [136]. The results of their study demonstrated that while there was no significant difference regarding food choices and nutrient intake between two groups, better chewing ability and greater willingness to eat more of certain food were reported in implant supported overdenture group. The chewing ability and capacity are very important for patients, and complete dentures may present oral disability. Chewing efficiency is critical to maintain quality of life and adequate nutrition. Using dentures or fixed prosthesis supported with dental implants improves life standards and nutritional status [137]. Numerous alternatives have been presented for implant supported dentures including fixed, removable or hybrid type according to bone amount (width/height), bone quality of jaw, oral hygiene, habits, systemic conditions and expectations of the patients. Esthetic, phonetic, functional and financial parameters can also be determinative for decision of the patients and dentist (prosthodontist and periodontist/oral surgeon).

For a successful treatment, osseointegration of the implants with bone should take place. Osseointegration is defined as the direct structural and functional connection between living bone and the surface of implant without intervening soft tissue. In dentistry, the implementation of osseointegration started in the mid-1960s

as results of the study performed by Branemark who was anatomist professor [138]. Osseointegration is a dynamic process in which implant characteristics (macro-topography, micro-topography and surface properties) play critical role for cell behavior [139, 140]. However, the survival rate of the dental implants are very high (95–99 %), osseointegration of bone to titanium dental implant provides very rigid connection when compared to natural tooth. Natural tooth has periodontium and periodontal ligament tissue surrounding root surface which is unique tissue in the body allows tooth for the mobility for compensation of occlusal forces or trauma, and provides the maintenance of the periodontium. PDL composes different cell types including fibroblasts, MSCs, nerve cells and extracellular matrix and firm collagen fibers [140]. These cells have the capacity to differentiate into cementoblasts and/or osteoblasts, and have roles in repair/regeneration and immunoregulation of cell within the periodontium [140].

Dental implant-supported rehabilitations have some limitations due to lack of periodontal ligament around implants that maintains periodontium and proprioception during chewing. Dental implant supported prosthesis cannot mimic biologically active system like in tooth surrounding periodontal ligament and alveolar bone. There is direct integration and rigid connection with bone and titanium implants. Rehabilitation of mouth with denture (with/without implant) may present some complications like denture induced stomatitis and traumatic ulcers. Furthermore, dental implant-supported therapies may have some early and delayed complications (failure of implants, broken implants, peri-implantitis, decementation of prosthesis) [141]. To overcome these limitations, stem cell-based tooth regeneration has been considered as a fantastic option, combining tissue engineering techniques and stem cells [142].

9.6 Future Prospective of Stem Cells in Dentistry

9.6.1 Utility of Stem Cells to Create Whole Tooth Organ.

During the last decade, stem cell-based tooth regeneration studies presented attractive approaches for lost teeth. For this purpose, embryonic, adult stem cells and recently iPS cells have been investigated as potential cell sources for tooth regeneration. Since using embryonic stem cells leads to ethical concerns, iPS and adult stem cells seem more promising approaches for regenerative dentistry.

9.6.2 What Is Bio-tooth? Is It Possible?

Regeneration of a living tooth is the final aim of dentistry for the replacement of a lost tooth. There are two different approaches to create bio-tooth; only cell or cell-scaffold based approaches.

9.6.2.1 Cell-Based Tooth Regeneration

The cell-based regeneration process can be simply defined as obtaining of tooth germ using the epithelial and mesenchymal cells (derived from embryo or iPS cells). These cells can be derived from either dental or non-dental sources.

9.6.2.2 Using Epithelial and Mesenchymal Cells Derived from Dental Source

Oshima et al. described a protocol for three-dimensional bioengineered tooth germ reconstitution using tooth germ-derived epithelial and mesenchymal cells [43, 46]. They also described methods for analysis utilized for in vitro and in vivo studies of tooth development. Oshima's group ectopically produced a bioengineered tooth containing periodontal ligament and alveolar bone, and they engrafted this bioengineered tooth into a jaw bone through bone integration. This bioengineered tooth could perform normal physiological tooth functions, including masticatory and perceptive potential, in mouse.

9.6.2.3 Using One of Epithelial or Mesenchymal Cells from Non-dental Sources

Mesenchymal cells derived from bone marrow stroma as a non-dental source were used for tooth formation firstly by Ohazama et al. in 2004 [143]. Their study demonstrated that adult BMMSCs with the embryonic inductive tooth epithelium cells could induce tooth formation in an adult body. Later, Angelova Volponi et al. showed that adult human epithelial cells (non-dental source) combined with mouse embryonic mesenchymal cells could also produce tooth-like structure in renal capsule of the mouse [144]. Micro-CT analysis of the transferred tissues revealed obvious tooth-like structures. Histological sections confirmed the presence of obvious teeth structure with dentin, enamel spaces, and well-vascularized pulp containing odontoblast-like cells expressing dentin sialophosphoprotein and lining the dentin surface. They claimed that these epithelial cells obtained from human gingival are a realistic source to be used in human bio-tooth generation [144]. They concluded that using non-embryonic sources for epithelial or mesenchymal cells is clinically feasible and needs further research to provide sufficient cell numbers for successful tooth formation.

9.6.2.4 Scaffold and Cell-Based Tooth Regeneration (Fig. 9.6)

The main aim of the process is to obtain different compartment of tooth (periodontal ligament and pulp) from MSCs (derived from adult or iPS cells) separately, and put them together into the tooth-like bio-printed scaffold mimicking calcified tooth structure.

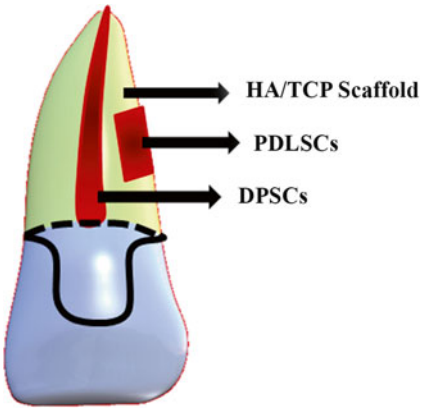

Fig. 9.6 Schematic illustration of the bio-root/tooth using dental pulp stem cell (DPSCs) and periodontal ligament stem cell (PDLSCs) seeded hydroxyapatite (HA)/ tricalcium phosphate (TCP) tooth-shaped scaffold

Sonoyama et al. aimed to establish a bio-root model to reconstruct a functional tooth in miniature pigs (minipigs) using postnatal stem cells including SCAPs and PDLSCs [44]. Their results demonstrated that this hybrid strategy using autologous DSCs may provide predictable applications. Wei et al. also performed an animal experiment to regenerate bio-root by employing similar hybrid strategy with different cell types (allogeneic DSCs) [45]. Hydroxyapatite tricalcium phosphate root shaped scaffold containing DPSCs covered by PDLSC sheet exhibited normal tooth characteristics after 6 months. In addition, dentinal tubule-like and functional periodontal ligament-like structures without any immunological response were reported. In another study, hydroxyapatite-coated dental implant was covered with embryonic dental follicle tissue, and transplanted into jaw bone of a murine tooth-loss model [145]. Using hydroxyapatite-coated dental implant and DFSCs, fibrous connection was established around the implants, a bio-hybrid organ. This bio-hybrid implant provided function, bone remodeling, and periodontal tissue regeneration including periodontal ligament and cementum. The bio-hybrid implant was claimed to be a promising approach to be used for future tooth replacement therapies. However, to create tooth-like structures, numerous concerns should be elucidated before conducting clinical studies;

- Which cell combinations are better for human approaches?
- Heterogeneity of the cells among the patient,
- Appropriate reciprocal interaction among the cells,
- The predictability of shape of the growing tooth,
- Tumorigenicity and immunogenicity of the cells (since one of cell layer is embryonic and obtained from iPS)

9.7 Biomaterials

Developing three dimensional bioengineered tooth for future replacement therapy have been investigated, and in this line, mechanically resistant to the occlusal force and biocompatible biomaterials have been tested. Biomaterials including micro and

nano-sized (or their combinations) have been used for this purpose. Biomaterials should provide appropriate micro-environment for cells to form the final structured organ. The materials used for bio-tooth applications should be resistant to chemical and physical abrasions, and provide required mechanical strength and intraoral maintenance with desired function and esthetic. For this purpose, poly(lactide-co-glycolide) (PLGA) (70/30, mol/mol) scaffolds, three types of calcium phosphate contained composites scaffolds that were composed of 50 % of PLGA and 50 % of hydroxyapatite, tricalcium phosphate (TCP) and calcium carbonate hydroxyapatite (CDHA) were evaluated [146]. The results showed that while the calcium phosphate contained compound supported tooth regeneration effectively, the PLGA/TCP scaffold would be more appropriate for the proliferation and differentiation of DPSCs. Furthermore, seeding of rat tooth bud cells on the PLGA/TCP scaffold generated dentin- and pulp-like tissues, indicating that PLGA/TCP scaffold is superior to the other three scaffolds for tooth-tissue regeneration approaches, particularly for dentin formation.

Selection of optimal scaffold for future clinical application remains a questionable, and further research is required to improve the features of the materials for tooth regeneration applications. In particular, recent developments including composites, biomaterials (nanofibrous scaffolds, hydrogel systems, laser-fabricated nanostructures) and cell-based bio-printing methods seem promising to produce proper scaffolds for dental tissue engineering.

9.7.1 Bio-implant vs. Bio-tooth?

Gault et al. evaluated PDLSC-seeded titanium implant to create bio-implant (Fig. 9.7), and named the structure as '*ligaplant*' [147]. They placed titanium dental implant to the extraction socket and reported new bone and PDL tissue development around the implants at the end of treatment. They claimed that biological mimicking of tooth with dental implant can be applicable in clinical dentistry. Their investigation demonstrated the application of ligament-anchored implants, which have advantages over osseointegrated oral implants since they don't have rigid fixation. In addition, they concluded that *ligaplant* induced the formation of new bone and new PDL in the vicinity due to their remarkable potential in periodontal tissue regeneration [147, 148]. On the other hand, as there is no cementum on the titanium surface, and collagen fibers cannot be placed around of the titanium implants like in natural tooth environment, bio-implant cannot exactly mimic natural structure around the tooth. Without cementum layer, cell-seeded titanium implant cannot provide biological expectations. Many questions remain with the *ligaplant* to be solved with long-term clinical findings [148]. However, this bio-hybrid (cells and titanium material combination) technology for tooth replacement can find a place in both periodontology/oral implantology, bio-tooth philosophy looks more applicable and more biological thought. But further pre-clinical studies in large animal models or human clinical trials using patient tissue-derived cells are needed to realize future

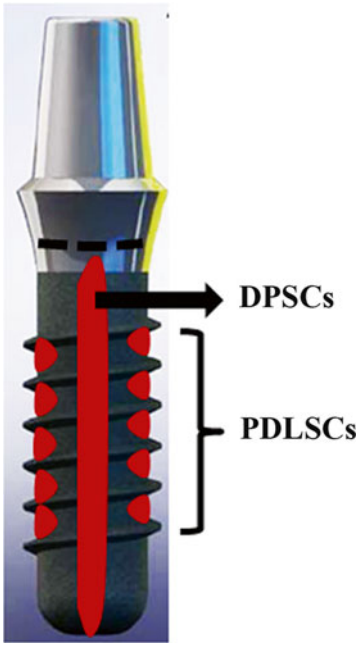

Fig. 9.7 Schematic illustration of the bio-implant using dental pulp stem cell (DPSCs) and periodontal ligament stem cell (PDLSCs) seeded titanium dental implant

human clinical applications [149]. Making a functional bio-tooth using stem cells may be much more complicated than expected. Several issues including identification and stemness of stem cells, dental morphogenesis, determination tooth type, odontogenic signals, controllable bio-tooth growth and eruption, and host-graft immunorejection in the jaws must be solved [35, 124, 149].

9.8 Conclusion and Future Trends or Directions

The final aim of the regenerative dentistry is to create functional whole tooth organ, mimicking dental hard and soft tissues. Various stem cells have been used in tooth bioengineering studies to evaluate their potential. Technologies using MSCs and iPS cells might be the new era of personalized dentistry but due to heterogeneity among the patients, studies should be focused an individually-targeted approach. Functional cell-based tooth replacement therapy requires collaborative studies conducted by bio-engineers, biologists, chemists and dentists. Mechanically and topographically appropriate biomaterials should be investigated for functional tooth organ studies. For successful tooth regeneration, more information is needed on genetic and cellular mechanisms regulating growth of the tooth crown and root, guiding tooth development, to understand the specification of important cell lineages including ameloblasts, odontoblasts, and cementoblasts.

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References

1. Jussila M, Juuri E, Thesleff I (2013) Tooth morphogenesis and renewal. In: Huang GTJ, Thesleff I (eds) Stem cells in craniofacial development and regeneration. Wiley, New York, pp 109–134
2. Foster BL, Nociti FH, Somerman MJ (2013) Tooth root development. In: Huang GTJ, Thesleff I (eds) Stem cells in craniofacial development and regeneration. Wiley, New York, pp 153–178
3. Akiyama K, Chen C, Gronthos S, Shi S (2012) Lineage differentiation of mesenchymal stem cells from dental pulp, apical papilla, and periodontal ligament. Methods Mol Biol 887:111–121
4. Hakki SS, Bozkurt SB, Hakki EE, Turac G, Yilmaz I, Karaoz E (2014) BMP-2,-6 and BMP-7 differently regulate osteogenic differentiation of human periodontal ligament stem cells (hPDLSCs). J Biomed Mater Res B 02(1):119–130
5. Hakki SS, Kayis SA, Hakki EE, Bozkurt SB, Duruksu G, Unal ZS, Turaç G, Karaoz E (2015) Comparison of mesenchymal stem cells isolated from pulp and periodontal ligament. J Periodontol 86(2):283–291
6. Huang GT, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 88(9):792–806
7. Gosau M, Götz W, Felthaus O, Ettl T, Jäger A, Morsczeck C (2013) Comparison of the differentiation potential of neural crest derived progenitor cells from apical papilla (dNC-PCs) and stem cells from exfoliated deciduous teeth (SHED) into mineralising cells. Arc Oral Biol 58(6):699–706
8. Yang H, Gao LN, An Y et al (2013) Comparison of mesenchymal stem cells derived from gingival tissue and periodontal ligament in different incubation conditions. Biomaterials 34(29):7033–7047
9. Roman A, Soancă A, Florea A, Páll E (2013) In vitro characterization of multipotent mesenchymal stromal cells isolated from palatal subepithelial tissue grafts. Microsc Microanal 19(2):370–380
10. Grimm WD, Dannan A, Giesenhagen B, Schau I, Varga G, Vukovic MA, Sirak SV (2014) Translational research: palatal-derived ecto-mesenchymal stem cells from human palate: a new hope for alveolar bone and cranio-facial bone reconstruction. Int J Stem Cells 7(1):23–29
11. Hynes K, Menicanin D, Gronthos S, Bartold PM (2012) Clinical utility of stem cells for periodontal regeneration. Periodontol 59(1):203–227
12. Xiao L, Nasu M (2014) From regenerative dentistry to regenerative medicine: progress, challenges, and potential applications of oral stem cells. Stem Cells Cloning 7:89–99
13. Padial-Molina M, O'Valle F, Lanis A, Mesa F, Ehrenfest DM, Wang HL, Galindo-Moreno P (2015) Clinical application of mesenchymal stem cells and novel supportive therapies for oral bone regeneration. Biomed Res Int 2015:341327
14. Farré-Guasch E, Wolff J, Helder MN, Schulten EA, Forouzanfar T, Klein-Nulend J (2015) Application of additive manufacturing in oral and maxillofacial surgery. J Oral Maxillofac Surg. doi:10.1016/j.joms.2015.04.019
15. Pagni G, Kaigler D, Rasperini G, Avila-Ortiz G, Bartel R, Giannobile WV (2012) Bone repair cells for craniofacial regeneration. Adv Drug Deliv Rev 64(12):1310–1319

16. Zhao W, Lu JY, Hao YM, Cao CH, Zou DR (2014) Maxillary sinus floor elevation with a tissue-engineered bone composite of deciduous tooth stem cells and calcium phosphate cement in goats. J Tissue Eng Regen Med. doi:10.1002/term.1867
17. Yu BH, Zhou Q, Wang ZL (2014) Comparison of tissue-engineered bone from different stem cell sources for maxillary sinus floor augmentation: a study in a canine model. J Oral Maxillofac Surg 72(6):1084–1092
18. Zhang W, Zhang X, Wang S, Xu L, Zhang M, Wang G, Jin Y, Zhang X, Jiang X (2013) Comparison of the use of adipose tissue-derived and bone marrow-derived stem cells for rapid bone regeneration. J Dent Res 92(12):1136–1141
19. Oshima T, Duttenhoefer F, Xavier S, Nelson K, Sauerbier S (2014) Can mesenchymal stem cells and novel gabapentin-lactam enhance maxillary bone formation? J Oral Maxillofac 72(3):485–495
20. Yamada Y, Nakamura S, Ueda M, Ito K (2013) Osteotome technique with injectable tissue-engineered bone and simultaneous implant placement by cell therapy. Clin Oral Implants Res 24(4):468–474
21. Yamada Y, Nakamura S, Klein OD, Ito K (2014) Current trends in stem cell therapy for improvement of bone quality. Histol Histopathol 29(6):691–697
22. Yamada Y, Hara K, Nakamura S, Ueda M, Ito K, Nagasaka T (2013) Minimally invasive approach with tissue engineering for severe alveolar bone atrophy case. Int J Oral Maxillofac Surg 42(2):260–263
23. Rickert D, Vissink A, Slot WJ, Sauerbier S, Meijer HJ, Raghoebar GM (2014) Maxillary sinus floor elevation surgery with BioOss® mixed with a bone marrow concentrate or autogenous bone: test of principle on implant survival and clinical performance. Int J Oral Maxillofac Surg 43(2):243–247
24. Kaigler D, Pagni G, Park CH, Braun TM, Holman LA, Yi E, Tarle SA, Bartel RL, Giannobile WV (2013) Stem cell therapy for craniofacial bone regeneration: a randomized, controlled feasibility trial. Cell Transplant 22(5):767–777
25. Kaigler D, Avila-Ortiz G, Travan S, Taut AD, Padial-Molina M, Rudek I, Wang F, Lanis A, Giannobile WV (2015) Bone engineering of maxillary sinus bone deficiencies using enriched CD90+ stem cell therapy: a randomized clinical trial. J Bone Miner Res. doi:10.1002/jbmr.2464
26. Hasegawa N, Kawaguchi H, Hirachi A, Takeda K, Mizuno N, Nishimura M, Koike C, Tsuji K, Iba H, Kato Y, Kurihara H (2006) Behavior of transplanted bone marrow-derived mesenchymal stem cells in periodontal defects. J Periodontol 77:1003–1007
27. Kawaguchi H, Hirachi A, Hasegawa N, Iwata T, Hamaguchi H, Shiba H, Takata T, Kato Y, Kurihara H (2004) Enhancement of periodontal tissue regeneration by transplantation of bone marrow mesenchymal stem cells. J Periodontol 75:1281–1287
28. Marei MK, Nouh SR, Saad MM, Ismail NS (2005) Preservation and regeneration of alveolar bone by tissue-engineered implants. Tissue Eng 11:751–767
29. Ito K, Yamada Y, Nakamura S, Ueda M (2011) Osteogenic potential of effective bone engineering using dental pulp stem cells, bone marrow stem cells, and periosteal cells for osseointegration of dental implants. Int J Oral Maxillofac Implants 26(5):947–954
30. Kim SH, Kim KH, Seo BM et al (2009) Alveolar bone regeneration by transplantation of periodontal ligament stem cells and bone marrow stem cells in a canine peri-implant defect model: a pilot study. J Periodontol 80(11):1815–1823
31. Liu Y, Zheng Y, Ding G et al (2008) Periodontal ligament stem cell-mediated treatment for periodontitis in miniature swine. Stem Cells 26(4):1065–1073
32. Khorsand A, Eslaminejad MB, Arabsolghar M et al (2013) Autologous dental pulp stem cells in regeneration of defect created in canine periodontal tissue. J Oral Implantol 39(4):433–443. doi:10.1563/AAID-JOI-D-12-00027
33. Feng F, Akiyama K, Liu Y et al (2010) Utility of PDL progenitors for in vivo tissue regeneration: a report of 3 cases. Oral Dis 16(1):20–28
34. Fawzy El-Sayed KM, Mekhemar MK, Beck-Broichsitter BE, Bähr T, Hegab M, Receveur J, Heneweer C, Becker ST, Wiltfang J, Dörfer CE (2015) Periodontal regeneration employing gingival margin-derived stem/progenitor cells in conjunction with IL-1ra-hydrogel synthetic extracellular matrix. J Clin Periodontol 42(5):448–457

35. Liu J, Yu F, Sun Y, Jiang B, Zhang W, Yang J, Xu GT, Liang A, Liu S (2015) Concise reviews: characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells 33(3):627–638
36. Bansal R, Jain A, Mittal S (2015) Current overview on challenges in regenerative endodontics. J Conserv Dent 18(1):1–6
37. Alongi DJ, Yamaza T, Song Y et al (2010) Stem/progenitor cells from inflamed human dental pulp retain tissue regeneration potential. Regen Med 5:617–631
38. Liao J, Al Shahrani M, Al-Habib M, Tanaka T, Huang GT (2011) Cells isolated from inflamed periapical tissue express mesenchymal stem cell markers and are highly osteogenic. J Endod 37:1217–1224
39. Ravindran S, Huang CC, George A (2014) Extracellular matrix of dental pulp stem cells: applications in pulp tissue engineering using somatic MSCs. Front Physiol 4:395
40. Ishizaka R, Iohara K, Murakami M, Fukuta O, Nakashima M (2012) Regeneration of dental pulp following pulpectomy by fractionated stem/progenitor cells from bone marrow and adipose tissue. Biomaterials 33:2109–2118
41. Wang Y, Zhao Y, Jia W, Yang J, Ge L (2013) Preliminary study on dental pulp stem cell-mediated pulp regeneration in canine immature permanent teeth. J Endod 39:195–201
42. Nakao K, Morita R, Saji Y, Ishida K, Tomita Y, Ogawa M, Saitoh M, Tomooka Y, Tsuji T (2007) The development of a bioengineered organ germ method. Nat Methods 4(3):227–230
43. Oshima M, Mizuno M, Imamura A, Ogawa M, Yasukawa M, Yamazaki H, Morita R, Ikeda E, Nakao K, Takano-Yamamoto T, Kasugai S, Saito M, Tsuji T (2011) Functional tooth regeneration using a bioengineered tooth unit as a mature organ replacement regenerative therapy. PLoS One 6(7):e21531
44. Sonoyama W, Liu Y, Fang D et al (2006) Mesenchymal stem cell-mediated functional tooth regeneration in swine. Plos One 20:1
45. Wei F, Song T, Ding G, Xu J, Liu Y, Liu D, Fan Z, Zhang C, Shi S, Wang S (2013) Functional tooth restoration by allogeneic mesenchymal stem cell-based bio-root regeneration in swine. Stem Cells Dev 22(12):1752–1762
46. Oshima M, Ogawa M, Yasukawa M, Tsuji T (2012) Generation of a bioengineered tooth by using a three-dimensional cell manipulation method (organ germ method). Methods Mol Biol 887:149–165
47. Takahashi C, Yoshida H, Komine A, Nakao K, Tsuji T, Tomooka Y (2010) Newly established cell lines from mouse oral epithelium regenerate teeth when combined with dental mesenchyme. In Vitro Cell Dev Biol Anim 46:457–468
48. Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K (2012) Stem cells in dentistry-Part II: clinical applications. J Prosthodont Res 56(4):229–248
49. Lin NH, Gronthos S, Marrtold P (2009) Stem cells and future periodontal regeneration. Periodontol 51:239–251
50. Steindorff MM, Lehl H, Winkel A, Stiesch M (2014) Innovative approaches to regenerate teeth by tissue engineering. Arch Oral Biol 59(2):158–166
51. Menicanin D, Mrozik KM, Wada N et al (2014) Periodontal ligament derived stem cells exhibit the capacity for long-term survival, self-renewal and regeneration of multiple tissue types in vivo. Stem Cells Dev 23:1001–1011
52. Rosa V, Zhang Z, Grande RH, Nör JE (2013) Dental pulp tissue engineering in full-length human root canals. J Dent Res 92(11):970–975
53. Syed-Picard FN, Ray HL Jr, Kumta PN, Sfeir C (2014) Scaffoldless tissue-engineered dental pulp cell constructs for endodontic therapy. J Dent Res 93:250–255
54. Torii D, Konishi K, Watanabe N, Goto S, Tsutsui T (2014) Cementogenic potential of multipotential mesenchymal stem cells purified from the human periodontal ligament. Odontology. doi:10.1007/s10266-013-0145-y
55. Otsu K, Kumakami-Sakano M, Fujiwara N, Kikuchi K, Keller L, Lesot H, Harada H (2014) Stem cell sources for tooth regeneration: current status and future prospects. Front Physiol 5:36. doi:10.3389/fphys.2014.00036

56. Yan M, Yu Y, Zhang G, Tang C, Yu J (2011) A journey from dental pulp stem cells to a biotooth. Stem Cell Rev 7(1):161–171
57. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97:13625–13630
58. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100:5807–5812
59. Huang AH, Chen YK, Lin LM, Shieh TY, Chan AW (2008) Isolation and characterization of dental pulp stem cells from a supernumerary tooth. J Oral Pathol Med 37:571–574
60. Karaoz E, Okcu A, Gacar G, Saglam O, Yuruker S, Kenar H (2010) A comprehensive characterization study of human bone marrow MSCs with an emphasis on molecular and ultrastructural properties. J Cell Physiol 226:1367–1382
61. Yalvac ME, Ramazanoglu M, Tekguc M, Bayrak OF, Shafigullina AK, Salafutdinov II, Blatt NL, Kiyasov AP, Sahin F, Palotás A, Rizvanov AA (2010) Human tooth germ stem cells preserve neuro-protective effects after long-term cryo-preservation. Curr Neurovasc Res 7:49–58
62. Palmer R, Lubbock M (1995) The soft connective tissues of the gingiva and periodontal ligament: are they unique? Oral Dis 1:230–237
63. Bath-Balogh M, Fehrenbach MJ (2011) Illustrated dental embryology, histology, and anatomy. Elsevier, St Louis, p 123
64. Schor SL, Ellis I, Irwin CR (1996) Sub- populations of fetal-like gingival fibroblasts: characterization and potential significance for wound healing and the progression of periodontal disease. Oral Dis 2(2):155e66
65. Häkkinen L, Uitto VJ, Larjava H (2000) Cell biology of gingival wound healing. Periodontol 24:127e52
66. Zhang Q, Shi S, Liu Y et al (2009) Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 183(12):7787e98
67. Tang L, Li N, Xie H, Jin Y (2011) Characterization of mesenchymal stem cells from human normal and hyperplastic gingiva. J Cell Physiol 226(3):832e42
68. Ge S, Mrozik KM, Menicanin D, Gronthos S, Bartold PM (2012) Isolation and characterization of mesenchymal stem cell-like cells from healthy and inflamed gingival tissue: potential use for clinical therapy. Regen Med 7(6):819e32
69. Egusa H, Okita K, Kayashima H et al (2010) Gingival fibroblasts as a promising source of induced pluripotent stem cells. PLoS One 5(9):e12743
70. Schor SL, Ellis I, Irwin CR et al (1996) Subpopulations of fetal-like gingival fibroblasts: characterisation and potential significance for wound healing and the progression of periodontal disease. Oral Dis 2:155–166
71. McCulloch CA, Knowles G (1991) Discrimination of two fibroblast progenitor populations in early explant cultures of hamster gingiva. Cell Tissue Res 264:87–94
72. Marynka K (2008) Human oral mucosa is highly enriched with pluripotent stem cells. Sixth ISSCR Annual Meeting, Philadelphia, PA
73. Marynka K, Treves S, Yafee M, Rachima H, Gafni Y, Cohen MA, Pitaru S (2010) The lamina propria of adult human oral mucosa harbors a novel stem cell population. Stem Cells. doi:10.1002/stem.425
74. Roman A, Soanca A, Barbu-Tudoran L, Irimie AI, Pall E (2012) Comparative evaluation of the influence of two resin-based restorative materials on the behavior of progenitor like cells. J Optoelectron Adv Mater 14:491–496
75. Köseoğlu S, Duran İ, Sağlam M, Bozkurt SB, Kırtıloğlu OS, Hakki SS (2013) Efficacy of collagen membrane seeded with autologous gingival fibroblasts in gingival recession treatment: a randomized, controlled pilot study. J Periodontol 84(10):1416–1424
76. Hakki SS, Berry JE, Somerman MJ (2001) The effect of enamel matrix protein derivative on follicle cells in vitro. J Periodontol 72(5):679–687
77. Morsczeck C, Götz W, Schierholz J et al (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix 24:155–165
78. Honda MJ, Imaizumi M, Tsuchiya S, Morsczeck C (2010) Dental follicle stem cells and tissue engineering. J Oral Sci 52:541–552

79. Estrela C, Alencar AH, Kitten GT, Vencio EF, Gava E (2011) Mesenchymal stem cells in the dental tissues: perspectives for tissue regeneration. Braz Dent J 22(2):91–98
80. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT-J (2008) Characterization of apical papilla and its residing stem cells from human immature permanent teeth – a pilot study. J Endod 34(2):166–171. doi:10.1016/j.joen.2007.11.021
81. Huang AH-C, Snyder BR, Cheng P-H, Chan AWS (2008) Putative dental pulp-derived stem/stromal cells promote proliferation and differentiation of endogenous neural cells in the hippocampus of mice. Stem Cells 26(10):2654–2663
82. Banchs F, Trope M (2004) Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? J Endod 30:196–200
83. Chueh LH, Huang GT (2006) Immature teeth with periradicular periodontitis or abscess undergoing apexogenesis: a paradigm shift. J Endod 32:1205–1213
84. Huang GT, Yamaza T, Shea LD, Djouad F, Kuhn NZ, Tuan RS, Shi S (2010) Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng Part A 16(2):605–615
85. Sainan W, Jinquan M, Zhipeng F, Yan Y, Ming Y, Gang L, Chunbo T, Zilu W, Yangyu Z, Jinhua Y, Guangdong Z (2012) Insulin-like growth factor 1 can promote the osteogenic differentiation and osteogenesis of stem cells from apical papilla. Stem Cell Res 8:346–356
86. Seo BM, Miura M, Gronthos S et al (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364(9429):149e55
87. Hakki SS, Hakki EE, Nohutcu RM (2009) Regulation of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases by basic fibroblast growth factor and dexamethasone in periodontal ligament cells. J Periodontal Res 44(6):794–802
88. McCulloch CA, Bordin S (1991) Role of fibroblast subpopulations in periodontal physiology and pathology. J Periodontal Res 26(3 Pt 1):144–154
89. Isaka J, Ohazama A, Kobayashi M et al (2001) Participation of periodontal ligament cells with regeneration of alveolar bone. J Periodontol 72:314–323
90. Ponnaiyan D, Bhat KM, Bhat GS (2012) Comparison of immuno-phenotypes of stem cells from human dental pulp and periodontal ligament. J Oral Implantol 25(1):127–134
91. Vasandan AB, Shankar SR, Prasad P, Sowmya JV, Bhonde RR, Jyothi PS (2014) Functional differences in mesenchymal stromal cells from human dental pulp and periodontal ligament. J Cell Mol Med 8:344–354
92. Carosella ED, Favier B, Rouas-Freiss N, Moreau P, Lemaoult J (2008) Beyond the increasing complexity of the immunomodulatory HLA-G molecule. Blood 15:4862–4870
93. Selmani Z, Naji A, Zidi I (2008) Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce CD4-CD25 high FOXP3+regulatory T cells. Stem Cells 26:212–222
94. Wada N, Menicanin D, Shi S, Bartold PM, Gronthos S (2009) Immunomodulatory properties of human periodontal ligament stem cells. J Cell Physiol 219(3):667–676
95. Lei M, Li K, Li B, Gao LN, Chen FM, Jin Y (2014) Mesenchymal stem cell characteristics of dental pulp and periodontal ligament stem cells after in vivo transplantation. Biomaterials 35:6332–6343
96. Trubiani O, Orsini G, Zini N et al (2008) Regenerative potential of human periodontal ligament derived stem cells on three-dimensional biomaterials: a morphological report. J Biomed Mater Res A 87:986–993
97. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, Den-Besten P, Robey PG, Shi S (2002) Stem cell properties of human dental pulp stem cells. J Dent Res 81:531–535
98. Jo YY, Lee HJ, Kook SY, Choung HW, Park JY, Chung JH, Choung YH, Kim ES, Yang HC, Choung PH (2007) Isolation and characterization of postnatal stem cells from human dental tissues. Tissue Eng 13:767–773
99. Laino G, d'Aquino R, Graziano A, Lanza V, Carinci F, Naro F, Pirozzi G, Papaccio G (2005) A new population of human adult dental pulp stem cells: a useful source of living autologous Wbrous bone tissue (LAB). J Bone Miner Res 20:1394–1402

100. d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, Papaccio G (2007) Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ 14:1162–1171
101. Gandia C, Arminan A, Garcia-Verdugo JM, Lledo E, Ruiz A, Minana MD, Sanchez-Torrijos J, Paya R, Mirabet V, Carbonell-Uberos F, Llop M, Montero JA, Sepulveda P (2008) Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells 26:638–645
102. Iohara K, Nakashima M, Ito M, Ishikawa M, Nakasima A, Akamine A (2004) Dentin regeneration by dental pulp stem cell therapy with recombinant human bone morphogenetic protein 2. J Dent Res 83:590–595
103. Nosrat IV, Widenfalk J, Olson L, Nosrat CA (2001) Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons in vitro, and rescue motoneurons after spinal cord injury. Dev Biol 238:120–132
104. Otaki S, Ueshima S, Shiraishi K, Sugiyama K, Hamada S, Yorimoto M, Matsuo O (2007) Mesenchymal progenitor cells in adult human dental pulp and their ability to form bone when transplanted into immune compromised mice. Cell Biol Int 31:1191–1197
105. Yu J, Wang Y, Deng Z, Tang L, Li Y, Shi J, Jin Y (2007) Odontogenic capability: bone marrow stromal stem cells versus dental pulp stem cells. Biol Cell 99:465–474
106. Karaoz E, Demircan PÇ, Saglam Ö, Aksoy A, Kaymaz F, Duruksu G (2011) Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow derived mesenchymal stem cells. Histochem Cell Biol 136(4):455–473
107. Arthur A, Rychkov G, Shi S et al (2008) Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells 26:1787–1795
108. Arthur A, Shi S, Zannettino AC et al (2009) Implanted adult human dental pulp stem cells induce endogenous axon guidance. Stem Cells 27:2229–2237
109. Yang K-L, Chen M-F, Liao C-H et al (2009) A simple and efficient method for generating Nurr1-positive neuronal stem cells from hu- man wisdom teeth (tNSC) and the potential of tNSC for stroke therapy. Cytotherapy 11:606–617
110. Király M, Kádár K, Horváthy DB et al (2011) Integration of neuronally pre-differentiated human dental pulp stem cells into rat brain in vivo. Neurochem Int 59:371–381
111. de Almeida FM, Marques SA, Ramalho Bdos S et al (2011) Human dental pulp cells: a new source of cell therapy in a mouse model of compressive spinal cord injury. J Neurotrauma 28:1939–1949
112. Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, Ueda M (2012) Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Invest 122(1):80–90
113. Zhao Y, Wang L, Jin Y, Shi S (2012) Fas ligand regulates the immunomodulatory properties of dental pulp stem cells. J Dent Res 91:948–954
114. Yamamoto A, Sakai K, Matsubara K, Kano F, Ueda M (2014) Multifaceted neuro-regenerative activities of human dental pulp stem cells for functional recovery after spinal cord injury. Neurosci Res 78:16–20
115. Pierdomenico L, Bonsi L, Calvitti M et al (2005) Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. Transplantation 80:836–842
116. Tomic S, Djokic J, Vasilijic S et al (2010) Immunomodulatory properties of mesenchymal stem cells derived from dental pulp and dental follicle are susceptible to activation by toll-like receptor agonists. Stem Cells Dev 20:695–708
117. Demircan PÇ, Sarıboyacı AE, Ünal ZS, Gacar G, Subaşı C, Karaoz E (2011) Immunoregulatory effects of human dental pulp-derived stem cells on T cells: comparison of transwell co-culture and mixed lymphocyte reaction. Cytotherapy 13(10):1205–2005
118. Kerkis I, Kerkis A, Dozortsev D et al (2006) Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 184:105–116
119. Karaoz E, Doğan BN, Aksoy A, Gacar G, Akyüz S, Ayhan S, Genç ZS, Yürüker S, Duruksu G, Demircan PC, Sarıboyacı AE (2010) Isolation and in vitro characterisation of dental pulp stem cells from natal teeth. Histochem Cell Biol 133(1):95–112

120. Akpinar G, Kasap M, Aksoy A, Duruksu G, Gacar G, Karaoz E (2014) Phenotypic and proteomic characteristics of human dental pulp derived mesenchymal stem cells from a natal, an exfoliated deciduous, and an impacted third molar tooth. Stem Cells Int. doi:10.1155/2014/457059
121. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676
122. Takahashi K, Tanabe K, Ohnuki M et al (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5):861–872
123. Yamanaka S (2012) Induced pluripotent stem cells: past, present, and future. Cell Stem Cell 10(6):678–684
124. Wan M, Du W, Zhou X, Xu X, Zheng L (2015) Stem cell-based tooth engineering and their potential in dental medicine. Curr Stem Cell Res Ther 10(5):443–449
125. Cai J, Cho SW, Kim JY, Lee MJ, Cha YG, Jung HS (2007) Patterning the size and number of tooth and its cusps. Dev Biol 304(2):499–507
126. Cai J, Zhang Y, Liu P, Chen S, Wu X, Sun Y, Li A, Huang K, Luo R, Wang L, Liu Y, Zhou T, Wei S, Pan G, Pei D (2013) Generation of tooth-like structures from integration-free human urine induced pluripotent stem cells. Cell Regen 2(1):6
127. Menendez L, Kulik MJ, Page AT et al (2013) Directed differentiation of human pluripotent cells to neural crest stem cells. Nature Protocols 8(1):203–212
128. Liu P, Zhang Y, Chen S, Cai J, Pei D (2014) Application of iPS cells in dental bioengineering and beyond. Stem Cell Rev 10(5):663–670
129. Hynes K, Menicanin D, Mrozik K, Gronthos S, Bartold PM (2014) Generation of functional mesenchymal stem cells from different induced pluripotent stem cell lines. Stem Cells Dev 23(10):1084–1096
130. Hynes K, Menicanin D, Gronthos S, Bartold MP (2014) Differentiation of iPSC to mesenchymal stem-like cells and their characterization. Methods Mol Biol. doi:10.1007/7651_2014_142
131. Hynes K, Menicanin D, Han J, Marino V, Mrozik K, Gronthos S, Bartold PM (2013) Mesenchymal stem cells from iPS cells facilitate periodontal regeneration. J Dent Res 92(9):833–839
132. Yang H, Aprecio RM, Zhou X, Wang Q, Zhang W, Ding Y, Li Y (2014) Therapeutic effect of TSG-6 engineered iPSC-derived MSCs on experimental periodontitis in rats: a pilot study. PLoS One 9(6):1–7
133. Hynes K, Menichanin D, Bright R, Ivanovski S, D.W. Hutmacher DW, Gronthos S, Bartold PM (2015) Induced pluripotent stem cells: a new frontier for stem cells in dentistry. J Dent Res. doi:10.1177/0022034515599769
134. Ozeki N, Mogi M, Kawai R, Yamaguchi H, Hiyama T, Nakata K, Nakamura H (2013) Mouse-induced pluripotent stem cells differentiate into odontoblastlike cells with induction of altered adhesive and migratory phenotype of integrin. PLoS One 8(11):1–13
135. Beikler T, Flemmig TF (2015) EAO consensus conference: economic evaluation of implant-supported prostheses. Clin Oral Implants Res. doi:10.1111/clr.12630
136. Gjengedal H, Dahl L, Lavik A, Trovik TA, Berg E, Boe OE, Malde MK (2012) Randomized clinical trial comparing dietary intake in patients with implant-retained overdentures and conventionally relined denture. Int J Prosthodont 25(4):340–347
137. Schimmel M, Katsoulis J, Genton L, Müller F (2015) Masticatory function and nutrition in old age. Swiss Dent J 125(4):449–454
138. Branemark PI (1983) Osseointegration and its experimental background. J Prosthet Dent 50(3):399–410
139. Hakki SS, Bozkurt SB, Hakki EE, Korkusuz P, Purali N, Koç N, Timucin M, Ozturk A, Korkusuz F (2012) Osteogenic differentiation of MC3T3-E1 cells on different titanium surfaces. Biomed Mater. doi:10.1088/1748-6041/7/4/045006
140. Hakki SS, Korkusuz P, Purali N, Korkusuz F, Bozkurt BS, Hakki EE, Onder ME, Gorur I, Nohutcu RM, Timucin M, Ozturk A (2013) Periodontal ligament cell behavior on different titanium surfaces. Acta Odontol Scand 71(3-4):906–916
141. Zembic A, Kim S, Zwahlen M, Kelly JR (2014) Systematic review of the survival rate and incidence of biologic, technical, and esthetic complications of single implant abutments supporting fixed prostheses. Int J Oral Maxillofac Implants 29:99–116

142. Sanz AR, Carrión FS, Chaparro AP (2015) Mesenchymal stem cells from the oral cavity and their potential value in tissue engineering. Periodontol 67(1):251–267
143. Ohazama A, Modino SA, Miletich I, Sharpe PT (2004) Stem-cell-based tissue engineering of murine teeth. J Dent Res 83:518–522
144. Angelova Volponi A, Kawasaki M, Sharpe PT (2013) Adult human gingival epithelial cells as a source for whole-tooth bioengineering. J Dent Res 92(4):329–334
145. Oshima M, Inoue K, Nakajima K, Tachikawa T, Yamazaki H, Isobe T, Sugawara A, Ogawa M, Tanaka C, Saito M, Kasugai S, Takano-Yamamoto T, Inoue T, Tezuka K, Kuboki T, Yamaguchi A, Tsuji T (2014) Functional tooth restoration by next-generation bio-hybrid implant as a bio-hybrid artificial organ replacement therapy. Sci Rep 13(4):6044. doi:10.1038/srep06044
146. Zheng L, Yang F, Shen H, Hu X, Mochizuki C, Sato M, Wang S, Zhang Y (2011) The effect of composition of calcium phosphate composite scaffolds on the formation of tooth tissue from human dental pulp stem cells. Biomaterials 32(29):7053–7059
147. Gault P, Black A, Romette JL, Fuente F, Schroeder K, Thillou F, Brune T, Berdal A, Wurtz T (2010) Tissue-engineered ligament: implant constructs for tooth replacement. J Clin Periodontol 37(8):750–758
148. Giannobile WV (2010) Getting to the root of dental implant tissue engineering. J Clin Periodontol 37(8):747–749
149. Zhao H, Chai Y (2015) Stem cells in teeth and craniofacial bones. J Dent Res. doi:10.1177/0022034515603972

Chapter 10
Dental Stem Cells for Bone Tissue Engineering

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10.1 Introduction

Bone defects and atrophy, which can be caused by trauma and congenital malformations, might lead to several clinical problems including malformation, movement disorders, pathological fracture and mastication disorders. Bone augmentation by applying autogenous bone grafts, guided bone regeneration, and some other therapies, are commonly used to repair bone defects in clinics. Of those, autogenous bone grafts provide the best clinical outcomes because of their remarkable osteoinductive and osteogenic potentials. However, the procedure has some disadvantages including the limited amount of tissue supply, the risk of infection transfer and damage to the donor site. For bone graft materials, conventional materials have unsatisfying osteoconduction or osteoinduction properties. Thus, studies have focused on improving various graft materials in order to avoid invasiveness and promote good outcomes. By now, numerous bone grafting materials invariably falling into the categories of allografts, xenografts and alloplastic grafts are commercially available, which have varying limitations in clinical applications. Allografts and xenografts have the potential to transmit disease and are rejected by some patients, whereas alloplastic grafts lack osteoinductive and osteogenic properties. Achieving satisfactory bone quantity and quality using these materials is still difficult; therefore, advanced bone tissue engineering approaches attempt to overcome these problems [1].

Bone tissue engineering is a part of regenerative medicine, and most commonly defined as the implantation of an artificial construct comprised of stem/progenitor cells seeded on scaffolds combined with growth factors. In this artificial system, the stem/progenitor cells act as seed cells for regenerating tissue, the growth factors act

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as morphogenetic signals for tissue induction, and the scaffolds create a microenvironment to facilitate the tissue regeneration [2]. Among these three elements that constitute the basis of bone tissue engineering, stem/progenitor cells are considered as the most crucial element. Commonly employed strategies for bone tissue engineering require a previous biopsy of live tissue containing the cells of interest for the receptor site. Cell cultures are then developed in the laboratory conditions where the cells of interest are expanded and seeded onto polymer matrices so they can later be reinserted into the organism [3].

Bone marrow mesenchymal stem cells (BMMSCs), which have been widely investigated, are considered the gold standard for bone tissue engineering [4], and have been applied in preliminary clinical trials for regenerative therapy [5]. However, collection of BMMSCs leads to varying degrees of injury at the donor site, impeding the application. Therefore, investigators have been paying more and more attention to dental stem cells (DSCs).

10.2 DSCs as Candidate Seed Cells for Bone Tissue Engineering

Several kinds of mesenchymal stem cells (MSCs) have been isolated from dental tissues and identified based on the properties of adult MSCs. These populations are divided into several cell types depending on the tissue of origin: namely, dental pulp stem cells (DPSCs) [6], stem cells from human exfoliated deciduous teeth (SHEDs) [7], periodontal ligament stem cells (PDLSCs) [8], dental follicle stem cells (DFSCs) [9], and stem cells from apical papilla (SCAPs) [10]. These cells have been the subject of numerous studies because they are easily accessible, have the potential for self renewal, and are capable of differentiation into osteo/dentinogenic cells, adipocytes, cardiomyocytes, endotheliocytes, and neurocytes [7, 11–16]. DSCs also possess immunomodulatory function, which makes them suitable candidates for allogenic transplantation of a single MSC line into multiple patients [17, 18]. All dental tissue-derived MSCs have been reported to potentially differentiate into osteocytes in vitro. In vivo studies have further proved their osteogenesis capacity forming bone-like mineralized tissue and repairing bone defects. Considering the powerful potential to differentiate into osteocytes in vitro and regenerate bone tissues in vivo, DSCs have become suitable candidates as seed cells to be used in bone tissue engineering.

To determine whether a cell line can be applied in bone tissue engineering, some characterization tests, which have still not reached an international consensus, should reliably predict the therapeutic aptitude of the MSCs. In this line, it has been reported that high levels of cell growth, proliferation, and viability in vitro, as determined by cell count, 5-bromo-2-deoxyuridine (Brdu) incorporation, and cellular adenosine triphosphate (ATP) levels, respectively, accurately determine proper lines functioning well in vascularized granular tissue regeneration in vivo [19].

10.2.1 In Vitro Properties of DSCs

In vitro characteristics of MSCs such as growth, proliferation, osteogenic differentiation, viability, angiopoiesis, and immunomodulatory functions affect mineral tissue formation which is essential for bone tissue engineering [20, 21]. In this sense, proliferation abilities of several kinds of DSCs (DPSCs, SHEDs, and PDLSCs) and BMMSCs have been compared [22]. The DSCs have been reported to possess higher proliferation potential than BMMSCs which reached the plateau phase earlier than the DSCs, but no significant difference has been noted for pulp-derived SHEDs and DPSCs. Another investigation agreed with Galler's study, reporting that SHEDs had a higher proliferation potential than BMMSCs, but the SHEDs also possessed a higher proliferation ability than DPSCs [23]. Proliferation rates of DPSCs and PDLSCs has been examined, and the results of the study proved greater cell proliferation potential for DPSCs with respect to PDLSCs [24]. SCAPs also exhibited significantly greater cell proliferation and colony forming capacity than PDLSCs [25]. Other studies have discovered that the proliferation ability of DFSCs is more powerful than that of DPSCs and PDLSCs [26, 27]. Taken together, these results suggest that the hierarchy of cell proliferation potential is DFSCs, SCAPs, DPSCs, and SHEDs > PDLSCs > BMMSCs.

Previous studies have investigated the osteogenic differentiation potential of DSCs. Although some authors have reported that DPSCs have remarkable osteogenic potential [28–31], PDLSCs have exhibited greater alkaline phosphatase (ALP) activity than DPSCs and SHEDs, revealing massive collagen and mineral deposition [22]. Similarly, osteogenically differentiated PDLSCs had more calcium nodules and greater accumulation of calcium than DFSCs and DPSCs [27] but exhibited weaker ALP activity and mineralization than BMMSCs after osteogenic induction [32]. SCAPs have been reported to display significantly increased expression of ALP, bone sialoprotein (BSP), and osteocalcin (OC), which play crucial roles in mineralization process, than PDLSCs, indicating the stronger mineralization capacity of SCAPs compared to PDLSCs [25]. Thus, overall data suggests that the hierarchy of osteogenic differentiation potential is BMMSCs, SCAPs > PDLSCs > DPSCs, SHEDs.

Vascularization ability of stem cells would also be beneficial for stem cell-mediated bone regeneration approaches. The angiogenic property of DSCs has rarely been investigated. It has been indicated that DPSCs enhance angiogenesis in vivo [33]. When DPSCs differentiate into osteoblasts, they express not only OC, but also vascular endothelial growth factor receptor-2 (VEGF-R2), an angiogenic marker. Some cells also express endothelial cell markers, such as intercellular cell adhesion molecule-1 (ICAM-1, CD54), platelet endothelial cell adhesion molecule-1 (PECAM-1), and angiotensin-converting enzyme. Furthermore, DPSC transplantation to immunocompromised rats forms a structure with the capillary network similar to bone tissue [13]. In addition, SHEDs have been observed to differentiate into endothelial cells, which line the walls of blood vessels [12].

Immunomodulatory action of MSCs is another important parameter in tissue regeneration, and BMMSCs reportedly possess an immunomodulatory function.

SHEDs have been confirmed to display an immunomodulatory effect, and DPSCs, PDLSCs, DFPCs, and SCAPs suppress the immune reaction [34]. Other studies have reported that PDLSCs have low immunogenicity and display an immunomodulatory function [35, 36]. This property of DSCs could help to decrease the reaction of allogenic stem cell transplantation and enhance bone regeneration. Moreover, the number of cells yielded per tooth is a factor that impacts whether dental MSCs can be a cell source for tissue engineering. Otabe and colleagues found that DPSCs had a higher cell yield per tooth compared to PDLSCs [31]. Thus, it can be concluded that DPSCs could be more suitable source of MSCs for regenerative medicine than PDLSCs.

10.2.2 In Vivo Osteogenesis of DSCs

In vivo investigations conducted with DSCs have mainly attempted to confirm the osteogenic ability proved by in vitro examinations. In general, DPSC and SHED based bone regeneration strategies have significantly increased bone formation compared to control animals [37–39]. It has been reported that SHEDs are capable of repairing critical-size calvarial defects in immunocompromised mice, though the generated bone tissue lacked hematopoietic marrow elements [7, 38]. Thus, it has been proposed that SHEDs are not able to differentiate into osteoblasts directly, but they recruit host osteogenic cells in vivo to form new bone tissue. One of scarce clinical trials performed with DSCs have also investigated bone regeneration capacity of DPSCs combined with collagen sponge scaffold [40]. After transplantation, it has been verified that DPSCs are capable of repairing human mandibular bone defects completely. However, another study contradicted these results, reporting no significant differences in bone formation between DPSC transplanted and control groups [41]. Apart from pulp derived DSCs, Park et al. discovered that PDLSCs significantly enhanced new bone regeneration in peri-implantitis defects in an experimental animal model [42]. Other studies have demonstrated that, similar to SHEDs, DFSCs obviously supported the generation of new bone, restoring a critical-size calvarial defect but the new bone tissue formation being due to the direct differentiation of transplanted cells is doubtful. The researchers have also implied that bone generation efficiency does not differ in DFSCs, PDLSCs and BMMSCs [43]. To increase the bone regeneration potential of DSCs, combining cells with scaffolds is a promising option. To this end, it has been found that SCAPs have given rise to bone-like mineralized tissue when combined with hydroxyapatite (HA) scaffold in vivo [44, 45].

10.3 Effect of Matrices and Scaffold on DSC-Mediated Bone Tissue Engineering

An extracellular matrix (ECM) or scaffold is an important element for tissue engineering as it provides the necessary framework for nutrient, oxygen, and metabolic waste transportation for the cells in the medium. The framework must be biocompatible, have

a suitable biodegradation profile, be non-toxic to the surrounding cells and tissues, and have optimal physical and mechanical properties with a firm consistency so that the final formed tissue is easily managed during transplantation to the body [46]. When used in tissue engineering, a matrix or scaffold should also facilitate adherence, migration, proliferation, and differentiation of the cells in question [47]. The compositions of scaffolds are conductive and inductive biomaterials, which are frequently modified by bioactive molecules to facilitate cell functions such as migration, and enhance the restoration of large defects [48]. The ideal materials for bone tissue engineering should have high porousness with an interconnected porous structure, be biocompatible and tensioactive, possess superior mechanical properties that allow shaping, and mimic the ECM of the bone regenerating environment.

Stem cells implanted in vivo without scaffolds may present a problem of uncontrolled cell migration within the body, leading to heterotopic mineralized tissue formation. A scaffold may help cells to stay together and maintain their position [49]. Moreover, the microenvironment formed by the scaffold and signaling molecules are important in guiding stem cell differentiation. For example, when SCAPs and HA scaffolds were transplanted subcutaneously into immunocompromised rats, most of the harvested mineralized tissue was bone-like, and only a small portion of the dentin-like structure was formed [44]. However, SCAPs combined with teeth-induced synthetic scaffolds resulted in the deposition of dentin-like tissue instead of bone-like tissue onto the wall of the endodontic canal in immunocompromised mice [50]. Generally, the scaffolds mimicking the natural ECM of the target tissue could be beneficial, to some extent, in the application of DSCs for different tissues.

10.3.1 Categories of Materials

Numerous materials have been used as scaffolds for bone tissue engineering including natural, synthetic, inorganic and composite materials [51]. Natural materials are biocompatible and biodegradable but the structural strength of the materials is weak to meet the requirements for bone tissue reconstruction. In addition, natural materials always carry the risk of transmitting animal-associated pathogens or might cause immune reactions [52]. Elastin, laminin, fibrin, fibronectin, collagen, silk, alginate, chitosan, and glycosaminoglycans are a few examples of natural materials. The effects of four kinds of ECM compositions including collagen type I (Col-I), collagen type IV (Col-IV), laminin, and fibronectin on DFSCs have been investigated by evaluating the cell growth and osteogenic differentiation. While laminin suppressed cell proliferation, osteoblastic differentiation, and cell adhesion, Col-I and fibronectin promoted cell growth, and Col-I or Col-IV was able to enhance osteoblastic differentiation but fibronectin did not facilitate osteogenic differentiation [53]. Coyac et al. also confirmed the enhanced osteogenic differentiation of SHEDs by treatment of ECM containing Col-I [54]. Agreeing with Tsuchiya et al. [53], Viale-Bouroncle et al. [55] reported that cell proliferation and vitality of DFSCs were slightly decreased on laminin matrix. They also confirmed that laminin inhibited early

osteogenic markers but up-regulated the late osteogenic differentiation markers of DFSCs by inducing the expression of osteopontin (OPN). In contrast, Morsczeck et al. [56] observed that laminin is differentially expressed during osteogenic differentiation of human DFSCs. Another natural material, acellular amniotic membrane (AM), which presents a basement membrane side and collagenous stromal side, was also reported to supply a preferable environment for SCAPs to differentiate into osteocytes. The collagenous stromal side of this material is more effective at facilitating osteogenesis than the basement membrane side [57]. The main component of AM is Col-I and Col-III, which may be a possible explanation for why AM possesses the favorable environment for mineralization. In addition, tissue engineered alginate scaffold enhanced osteogenic potential of DPSCs while maintaining the high viability [58]. Taken together, these findings indicate that scaffold containing Col-I or alginate may be suitable natural materials for DSC-mediated bone regeneration.

Synthetic polymers, such as polydopamine (PDA), polyglycolic acid (PGA), polylactic acid (PLA), and their copolymer polylactic-co-glycolic acid (PLGA), present satisfactory chemical and mechanical properties, and manageable degradation rates. However, the structures of the polymers are different than that of the ECM, and lack the bioactive signals [22]. Low tissue affinity is another critical issue with synthetic polymers. PLGA is a biocompatible and biodegradable synthetic polymer synthesized by using lactic acid and glycolic acid. The degradation speed of PLGA can be arranged changing the percentages of the two reactants; the degradation products are also metabolites in the human body, making them nontoxic. For these reasons, PLGA is widely used as a scaffold material in tissue engineering in today's technology [59]. Another synthetic material, PDA displays bioadhesive and bioactive characteristics. PDA has been confirmed to induce cell proliferation and stimulate osteogenic differentiation of PDLSCs by increasing the expression of integrin -α5/-β1, an adhesion receptor, resulting in enhanced cell attachment and activation of the integrin-mediated phosphoinositide 3-kinase (PI3K) pathway [60].

Inorganic materials including fluorapatite (FA), HA, and β-tricalcium phosphate (TCP) provide high compressive strength, stable chemical properties, and biodegradability, but they are often frangible as a scaffold and difficult to operate. Studies have shown that the osteogenic differentiation of human MSCs can be induced by TCP or HA but the induction of ALP activity is much higher on TCP scaffold than HA scaffold [61]. Viale-Bouroncle et al. [62] confirmed TCP-induced osteogenic differentiation in another trial conducted with DFSCs. They also found that TCP induces the apoptosis of DFSCs, indicating potential cytotoxicity of TCP and soluble particles derived from TCP [63].

In order to take advantage of the benefits of the scaffold, hybrid materials for cell delivery have been created as composite materials. Col-I with HA have been combined to form the nanometer-scale porous material, Col-I-HA [64]. It possessed functional properties that facilitated cell growth and bone formation. Another nanometer-scale scaffold incorporating FA crystals within polycaprolactone (PCL) backbone has also been investigated, and although it presented a relatively slower proliferation stimulation than the PCL scaffold, it induced mineralization without any other supplements [65]. Dahl et al. [59] investigated the chitosan coated TCP

scaffold incorporated with lysozymes, and verified promotion of bone integration, osteoconduction and osteoinduction. Annibali et al. [48] compared the bone regeneration potentials of three scaffolds (*i.e.*, granular deproteinized bovine bone with additional porcine collagen, β-TCP, and resorbable granulate ceramic) and discovered no significant differences among them.

10.3.2 Material Features

The morphology and structure of scaffold materials exist as porous scaffolds, nanofibrous materials, microparticles, and hydrogels, etc., affecting the characteristics of stem cells in bone tissue engineering.

Two kinds of nanometer scale materials (Col-I-HA and FA-PCL) have been proven to allow osteogenic induction as mentioned before. Compared to conventional HA, nanophase HA might be better for bone regeneration approaches because of its superior biomimetic structure and osteoconduction potential [59]. Graziano et al. [29] discovered that microconcavities of PLGA had significant effects on the osteoblastic differentiation of DPSCs. In addition, compared to convex and smooth surfaces, cells cultured on concave textured surface presented better cell-scaffold interactions and easily induced signaling factors secretion, resulting in quick osteogenic differentiation, bone generation, and the vascularization of newly formed tissue [29].

Recently, the applications of hydrogels have been explored in detail in bone tissue engineering. A large number of materials have been presented as hydrogels with plenty of attractive characteristics including tissue-like moisture content, appropriate mechanical characteristics similar to those of human tissue, and favorable biocompatibility [51]. Galler et al. [22] found that the number of SHEDs were equivalent in polyethyleneglycolylated (PEGylated) fibrin and collagen hydrogel. Moreover, arginine-glycine-aspartic acid tripeptide (RGD)-united alginate hydrogel accelerates differentiation into the osteoblast lineage [66]. Physical characteristics of the surfaces could be important in stem cell-based bone tissue engineering, as it has been shown that while a soft surface induced osteogenic differentiation of DFSCs, stiff surface induced osteogenic differentiation of BMMSCs [55]. Similar to BMMSCs, SHEDs possessed a greater capability for proliferation and osteogenesis in rigid matrix than in soft matrix [67].

10.4 Growth Factors in DSC-Mediated Bone Tissue Engineering

Growth factors play crucial roles in bone tissue engineering as they stimulate proliferation, differentiation, and other functions of the seed cells combined with the scaffold. These growth factors are also applied as signaling molecules to regulate tissue formation. Several growth factors have been used in bone tissue regeneration,

including bone morphogenetic proteins (BMPs), basic fibroblastic growth factor (bFGF), and transforming growth factor-β (TGF-β), insulin-like growth factors (IGFs), as well as composite growth factors such as platelet-rich plasma (PRP).

10.4.1 BMPs

Belonging to the TGF-β superfamily, BMPs comprise more than 15 human proteins. BMPs are used sequentially and repeatedly throughout cytodifferentiation and matrix secretion [2]. Differentiation into osteoblasts and the generation of bone tissue by stem cells are mainly governed by BMPs, especially BMP2, BMP4, and BMP7. In addition, BMP2 and BMP7 have been applied as assisted therapy in clinical bone regeneration approaches. Among 14 BMPs (BMP2-15), BMP9 has been reported to be the most potent inducer of osteogenic differentiation [68–70]. SCAPs were successfully differentiated into bone, cartilage, and adipocytes upon BMP9 stimulation in vitro and in vivo [69]. A recent study confirmed that BMP2 stimulated the osteogenic differentiation of DFSCs [71]. Furthermore, BMP2 and BMP9 induced osteogenic differentiation through the canonical Wnt/β-catenin pathway. Activation of the canonical Wnt pathway and knockdown of β-catenin inhibited the differentiation of DFSCs, illustrating the need for accurate levels of β-catenin in the regulation of cell differentiation induced by BMP pathway [72]. Another study has discovered that BMP9 induces ALP activity in SCAPs, which has been diminished by silencing β-catenin. Thus, β-catenin has been proposed to play an important role in BMP9-induced osteo/ondontogenic signaling [73]. BMP4 could stimulate distal-less homeobox 2 (DLX2) gene, and then enhance SCAPs' osteogenic differentiation and bone tissue regeneration by up-regulating Sp7 transcription factor 7 (OSX) [74]. BMPs have also been demonstrated to enhance osteogenic differentiation through the activation of mitogen-activated protein kinase (MAPK) pathway. In particular, BMP2 exerts its role in osteogenic induction via activation of extracellular signal-regulated kinase (ERK) pathway [75]. However, the p38 pathway has been elucidated to play a positive role in BMP9-induced differentiation, whereas the ERK pathway has the opposite effect [70]. Unfortunately, as BMP2 has been used clinically, some serious complications have been reported including ectopic bone formation, cyst-like bone void emergence, and significant soft tissue swelling. Therefore, more pre-clinical researches are needed on use of BMPs in bone tissue engineering.

10.4.2 IGFs

IGFs consist of two receptors and two ligands: IGF1 and IGF2. Numerous investigations have focused on IGF1, demonstrating its key roles in the regulation of osteoblastic differentiation or cell proliferation. IGF1 is a multifunctional peptide that can enhance

osteogenic differentiation of BMMSCs. IGF1 has a positive effect on bone tissue generation and homeostasis, and it upregulates osteogenesis-associated genes and downregulates the expression of odontoblast-specific markers in SCAPs [45]. IGF1 may regulate osteogenesis via β-catenin, the molecule involved in the canonical Wnt signaling pathway [45]. IGF1 also enhances the proliferation and osteogenic differentiation potentials of PDLSCs through ERK and c-Jun N-terminal kinase (JNK) MAPK pathways [76]. In another study, IGF1 was found to promote cell proliferation and osteogenic differentiation potentials of DPSCs via mammalian target of rapamycin (mTOR) pathway [77]. Furthermore, a recent study proposed that IGF2 treatment stimulated the osteogenic differentiation of DFSCs [71]. Insulin-like growth factor binding protein family (IGFBPs), including six members (IGFBP1-6), are an indispensable member of the IGF axis [78]. Researchers have reported that IGFBP5 expression increased upon osteogenic induction, and IGFBP5 could enhance osteogenic differentiation in PDLSCs and SCAPs. Moreover, IGFBP5 also enhanced the anti-inflammatory effect of PDLSCs through nuclear factor kappa B (NFκB) signaling pathway [79].

10.4.3 bFGF

bFGF mainly regulates cell behaviors such as proliferation and differentiation. Whether bFGF plays a positive role in the regulation of osteogenic differentiation is still controversial. It has been shown to enhance the osteogenic differentiation of BMMSCs [80] and inhibit mineralization in SHEDs [81]. Others also demonstrate that inhibition of bFGF or FGFR has reduced the colony forming capacity and prompted the mineralization rate of SHEDs, indicating that the endogenous bFGF may participate in the colony formation and osteogenic differentiation ability of SHEDs [82]. bFGF has also been shown to promote pre-osteoblast proliferation but inhibit mineralization in PDLSCs [83]. In given conditions, bFGF significantly increased the ability of cell proliferation and colony formation, enhanced the osteogenic differentiation potentials and the expressions of pluripotency markers, including Nanog, Oct4, Sox2, and Rex1 in SCAPs [84]. Others have discovered that bFGF regulated the osteogenic differentiation ability of DPSCs in a treatment-dependent manner. Their results have shown that bFGF treatment within the period of the osteogenic differentiation decreased the osteogenic differentiation potentials of DPSCs; pre-treatment with bFGF for 1 week enhanced the osteogenic differentiation, whereas pre-treatment with bFGF for 2 weeks diminished the osteogenic differentiation ability [85]. The regulatory function of bFGF is probably cell type-specific. Several reports have explained how bFGF regulates osteogenesis. bFGF suppressed the mineralization by inhibiting Notch signaling activation in both PDLSCs and SHEDs, but the interaction between the Notch pathway and bFGF is uncertain [83]. In mice dental epithelial stem cells (DESCs), FGF signaling is necessary for self-renewal and inhibition of cell differentiation. Blocking of the FGF signaling increases cell apoptosis and decreases cell proliferation in DESCs through Wnt signaling pathway [86].

10.4.4 TGF-β

TGF-β is one of the representative members of the TGF-β superfamily, and one of the most common growth factors found in the bone matrix. TGF-β promotes osteogenic differentiation in the early stage of osteoblastic maturation, and inhibits mineralization and osteogenic differentiation in the late stage [87]. In SCAPs, TGF-β1 inhibited cell proliferation and mineralization, and significantly downregulated osteogenic/dentinogenic gene expressions. In addition, interaction between nuclear factor I-C (NFIC, an antagonist of TGF-β1) and TGF-β1 controlled the cell function, and determined the differentiation potentials of SCAPs [88]. On the contrary, TGF-β1 promoted the cell growth and collagen content, and stimulated ALP activity at lower concentrations (0.1–1 ng/mL), and down-regulated the ALP activity at higher concentrations (>5 ng/mL) in SCAPs. As for the molecular mechanism, it has been discovered that TGF-β1 regulated the cell proliferation, collagen turnover, and differentiation via ALK5/Smad2 and MEK/ERK signaling pathways [89]. In a similar way, it has been proposed that low dose of TGF-β1 promoted osteogenic differentiation while repeated or high dose of TGF-β1 inhibited osteogenic differentiation in PDLSCs [90]. Persistence of TGF-β1 suppressed the osteogenic differentiation via inhibition of IGF1 and subsequent blockage of the PI3K/AKT signaling pathway [91]. DFSCs treated with TGF-β1 migrated faster, indicating that potential chemoattractant functions of TGF-β1 [92]. Connective tissue growth factor (CTGF) is a matricellular protein that upregulates the production of ECM. Mechanical stretch loading has been suggested to upregulate CTGF gene expression in PDLSCs. Compared with TGF-β1 treatment alone, application of CTGF and TGF-β1 combination significantly enhanced the expression of Col-I and fibronectin in PDLSCs [93].

10.4.5 PRP

PRP is biologically composed of plasma, leukocytes, and platelets. PRP is produced from autologous blood and contains several growth factors including IGF1, TGFβ, FGF and VEGF. A recent preclinical study has demonstrated that SHEDs, DPSCs, and BMMSCs combined with PRP have the ability to form bone tissue in dogs [39]. The use of PRP has the potential to accelerate surgical wound healing due to the local release of specific growth factors involved in angiogenesis and collagen production [47], but they are not the key cytokines exerting the bio-function of PRP. In a human cytokine array, Chemokine (C-C motif) ligand 5 (RANTES/CCL5) and ICAM-1 were defined as the two key factors in PRP. The appropriate concentration of PRP, reported to be 1 %, promoted the characteristics of human DSCs including cell proliferation, differentiation and mineralization [94]. Investigation of the effects of PRP derived from human umbilical cord blood (UCB-PRP) proved that 1–2 % (volume percentage) of UCB-PRP had positive effects on the cell proliferation and osteogenic differentiation potentials of DSCs, including DPSCs, SHEDs and PDLSCs [95]. Another study showed that 1–20 % of PRP significantly promoted the

proliferation of DPSCs, and 1–10 % of PRP significantly enhanced the osteogenic differentiation potential of DPSCs [96]. Although clinical studies conducted with PRP and DSCs are scarce, one research group successfully applied DPSCs combined with PRP to treat one case of osteoradionecrosis in clinic [97].

10.5 Other Elements Involved in DSC-Mediated Bone Tissue Engineering

10.5.1 Inflammatory Factors

In the field of bone regeneration therapy, defected or injured tissues are generally inflamed with unnatural expression of inflammatory elements. Accumulating evidence suggests that pro-inflammatory cytokines suppress differentiation into osteoblasts and bone generation. Ideal tissue restoration mediated by MSCs requires overcoming the blockade of tissue regeneration caused by inflammation.

Interleukin-11 (IL11), which belongs to the IL6 cytokine family, is involved in bone metabolism and the regulation of both osteoblast and osteoclast activities. Appropriate mechanical loading increases IL11 expression and induces osteoblastic differentiation [98]. IL11 may promote the osteoblastic differentiation of PDLSCs via the inhibition of Dickkopf-1 (DKK1) and DKK2, the extracellular antagonists of Wnt signaling, subsequently activating the canonical Wnt/β-catenin signaling pathway and shifting the cell fate [98].

Tumor necrosis factor-α (TNFα) is a cytokine that triggers the acute phase inflammatory reaction and participates in systemic inflammatory reactions. TNFα is principally secreted by macrophages, inducing inflammation, cell apoptosis, cell proliferation and differentiation. TNFα has been found to inhibit the osteogenic differentiation of PDLSCs and BMMSCs through the canonical Wnt pathway, but PDLSCs have been more sensitive to this inflammatory cytokine via diverse regulatory mechanisms [99].

10.5.2 Integrin Receptors

Integrin surface receptors, as the components of the cellular sensing system, transmit extracellular signals to intracellular signals. The ligands of integrins include laminin, fibronectin and collagen. Studies have demonstrated that integrin α5 interacts with IGF2 and IGFBP2, activating various signaling pathways to stimulate and enhance osteoblast differentiation. The stiffness of the matrix is also a regulatory factor for integrin, playing an important role in cytoskeletal changes during the osteogenic process [60]. Integrin-α2/-β1 also transmits the information of laminin from ECM into the cells, subsequently regulating the late osteogenic differentiation of DFSCs [55].

10.5.3 Chemical Agents

Various phytochemicals and plant-derived extracts are capable of stimulating bone formation and healing. Ginsenoside Rg-1, the main functional component of ginseng, promotes the proliferation and osteoblastic differentiation of PDLSCs at an optimal concentration of 10 μmol/L and also promotes neovascularization [100]. In addition, the proliferation and osteogenic potentials of PDLSCs have been enhanced by the hexane, ethyl acetate (EA), or n-butanol (BuOH) fractions of *Zanthoxylum schinifolium* extract [101]. Flavonoids and hesperetin have also been shown to recover the osteogenic capacity of PDLSCs from the inhibitory effects of high glucose [102]. Butyrate, a bacterial metabolite and inflammatory agent commonly found in dental plaque and the periodontal pocket, at low concentrations facilitated the osteogenic differentiation of DFSCs in the early stages of differentiation but inhibited calcification at the later stages [103]. Moreover, a high concentration of butyrate has been reported to be toxic to DFSCs. These findings indicate that butyrate could stimulate or suppress osteogenic differentiation in a dose-dependent manner and possibly in a cell type-specific manner.

10.5.4 Hypoxia

Hypoxic conditions normally exist in healthy human tissues; healthy bone marrow has an oxygen concentration of 2–5 %, and the oxygen level in region of a hematoma after bone fracture is less than 1 % [104, 105]. Therefore, in bone tissue engineering approaches, the effect of hypoxia on stem cells is highly important for the scientific community. By now, there are contradictory reports about the effect of hypoxia on stem cells.

Hypoxia exhibits disparate impacts on the proliferation of MSCs. The effect probably depends on the cell type, oxygen concentration, and duration in hypoxia [106]. Increased proliferation rate for DPSCs has been reported after culturing in 3 % oxygen compared to normoxia [107, 108], but the proliferation rates of DPSCs or SHEDs incubated in 1 % oxygen or normoxic conditions were not significantly different [106, 107]. Vanacker et al. [106] unveiled that hypoxia could induce the spontaneous osteogenic differentiation of SCAPs, and maintain the pro-angiogenic factor VEGF-A or survivin, an inhibitor of apoptosis protein. PDLSCs cultured under hypoxic conditions exhibited enhanced osteogenic differentiation potential in vitro and in vivo [32]. Slightly increased mineralization is occasionally seen in the central regions of the scaffold, perhaps reflecting that cells prefer hypoxic conditions for cell-mediated mineralization [54]. Another opinion is that hypoxia helps to maintain the stemness of BMMSCs and DPSCs by suppressing their differentiation [109].

Until now, hypoxia has been considered to regulate cell properties via hypoxia inducible factor 1α (HIF-1α). Usually, HIF-1α translocates to the nucleus and induces changes in a series of biological behaviors. Cobalt-(II) chloride ($CoCl_2$) is a chemical

agent used in hypoxia studies to mimic a hypoxic environment, and could inhibit the activity of prolyl hydroxylase, a key enzyme in the oxygen-sensing pathway. Osathanon et al. showed that $CoCl_2$ treatment inhibited the osteogenic differentiation and maintained the stemness of PDLSCs via HIF-1α dependent pathway [109]. Thus, the opposing effects of hypoxia based on cell type needs to be further investigated.

10.5.5 Mechanical Loading

Clinically, mechanical loading is known to be one of the important factors in bone remodeling. The phenomenon of mechanical loading on cells depends on the magnitude of stress, duration of the cyclic load and loading frequency. The alteration of cell proliferation under mechanical loading is still controversial. Low mechanical force likely stimulates cell proliferation as high force suppresses proliferation [110]. In addition, appropriate mechanical stretch and stress trigger the osteoblastic differentiation of DSCs via activation of the ERK/MAPK, JNK/MAPK, and RAS pathways [98, 109, 110]. The activations of ERK1/2 and JNK signaling pathways phosphorylate and activate the downstream mediators including c-Fos and c-Jun, and the Ang II type 1 (AP1) transcription factors. The upregulation of AP1 results in the enhanced osteogenic differentiation of DSCs [110].

10.6 Prospects

Although DSCs have been extensively investigated for more than a decade, there are still several issues remaining to be figured out, especially in the concept of bone tissue engineering. Several studies have investigated the clinical use of BMMSCs derived from the iliac crest. The outcomes have shown that autogenous BMMSCs are beneficial for generating new bone tissue and improving the clinical situations [111, 112]. In addition to BMMSCs, ample evidences indicate the potential of DSCs in bone tissue engineering. However, the clinical applications of DSCs have been rarely investigated. Previously, researchers have transplanted autogenous DPSCs to repair the mandible defects, and their results showed that the bone defects were successfully restored [40]. However, solid evidence is still lacking and a large number of issues need to be solved for the application of DSCs in bone tissue engineering:

1) It is uncertain whether DSCs could reconstruct the hematopoietic marrow elements in newly formed bone tissues. If the DSC-mediated bone tissue formation lacks hematopoietic marrow elements, how can this challenging problem be overcome?

2) The ethical issue associated with stem cell applications needs to be solved. In addition, the seed cells used in previous studies were generally autogenous stem cells to prevent immunological issues. Thus, it might be better to use autogenous DSCs in bone tissue engineering. To this end, another issue for the clinical usage of DSCs is how to amplify enough seed cells and preserve them.

3) The effect of the microenvironment on DSC-mediated bone tissue regeneration is unclear including inflammation, hypoxia, senescence, and immunity, among other issues. These parameters should be investigated in order to improve and extend the use of DSCs in clinics.

4) Optimal biomaterials and efficient growth factors should be explored and developed to enhance DSC-mediated bone tissue regeneration.

5) In vivo large animal models should be conducted to evaluate DSC-mediated bone tissue regeneration and understand their limitations. On the basis of this evidence, more clinical trials are necessary for the eventual application of DSCs.

There are additional unknown problems impeding the application of DSCs in this field. Thus, much progress is needed before DSCs are applied clinically.

References

1. Ito K, Yamada Y, Nagasaka T, Baba S, Ueda M (2005) Osteogenic potential of injectable tissue-engineered bone: a comparison among autogenous bone, bone substitute (Bio-Oss®), platelet-rich plasma, and tissue-engineered bone with respect to their mechanical properties and histological findings. J Biomed Mater Res A 73(1):63–67
2. Scheller EL, Krebsbach PH, Kohn DH (2009) Tissue engineering: state of the art in oral rehabilitation. J Oral Rehabil 36(5):368–389. doi:10.1111/j.1365-2842.2009.01939.x
3. Kaigler D, Mooney D (2001) Tissue engineering's impact on dentistry. J Dent Educ 65(5):456–462
4. Daltoe FP, Mendonca PP, Mantesso A, Deboni MCZ (2014) Can SHED or DPSCs be used to repair/regenerate non-dental tissues? A systematic review of in vivo studies. Braz Oral Res 28(1):1–7
5. Rodriguez-Lozano FJ, Bueno C, Insausti CL, Meseguer L, Ramirez MC, Blanquer M, Marin N, Martinez S, Moraleda JM (2011) Mesenchymal stem cells derived from dental tissues. Int Endod J 44(9):800–806. doi:10.1111/j.1365-2591.2011.01877.x
6. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97(25):13625–13630. doi:10.1073/pnas.240309797
7. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100(10):5807–5812. doi:10.1073/pnas.0937635100
8. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364(9429):149–155. doi:10.1016/S0140-6736(04)16627-0
9. Morsczeck C, Gotz W, Schierholz J, Zeilhofer F, Kuhn U, Mohl C, Sippel C, Hoffmann KH (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 24(2):155–165. doi:10.1016/j.matbio.2004.12.004
10. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT (2008) Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 34(2):166–171. doi:10.1016/j.joen.2007.11.021
11. Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S (2008) Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem cells 26(7):1787–1795. doi:10.1634/stemcells.2007-0979
12. Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, Smith AJ, Nor JE (2008) Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 34(8):962–969. doi:10.1016/j.joen.2008.04.009

13. d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, Papaccio G (2007) Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ 14(6):1162–1171
14. Gronthos S, Brahim J, Li W, Fisher LW, Cherman N, Boyde A, DenBesten P, Robey PG, Shi S (2002) Stem cell properties of human dental pulp stem cells. J Dent Res 81(8):531–535
15. Xu J, Wang W, Kapila Y, Lotz J, Kapila S (2009) Multiple differentiation capacity of STRO-1+/CD146+ PDL mesenchymal progenitor cells. Stem Cells Dev 18(3):487–496. doi:10.1089/scd.2008.0113
16. Yalvac ME, Ramazanoglu M, Rizvanov AA, Sahin F, Bayrak OF, Salli U, Kose GT (2010) Isolation and characterization of stem cells derived from human third molar tooth germs of young adults: implications in neo-vascularization, osteo-, adipo-and neurogenesis. Pharmacogenomics J 10(2):105–113. doi:10.1038/tpj.2009.40
17. Le Blanc K, Ringdén O (2005) Immunobiology of human mesenchymal stem cells and future use in hematopoietic stem cell transplantation. Biol Blood Marrow TR 11(5):321–334
18. Tomic S, Djokic J, Vasilijic S, Vucevic D, Todorovic V, Supic G, Colic M (2010) Immunomodulatory properties of mesenchymal stem cells derived from dental pulp and dental follicle are susceptible to activation by toll-like receptor agonists. Stem Cells Dev 20(4):695–708. doi:10.1089/scd.2010.0145
19. Deskins DL, Bastakoty D, Saraswati S, Shinar A, Holt GE, Young PP (2013) Human mesenchymal stromal cells: identifying assays to predict potency for therapeutic selection. Stem Cells Transl Med 2(2):151–158. doi:10.5966/sctm.2012-0099
20. Russell KC, Lacey MR, Gilliam JK, Tucker HA, Phinney DG, O'Connor KC (2011) Clonal analysis of the proliferation potential of human bone marrow mesenchymal stem cells as a function of potency. Biotechnol Bioeng 108(11):2716–2726. doi:10.1002/bit.23193
21. Russell KC, Phinney DG, Lacey MR, Barrilleaux BL, Meyertholen KE, O'Connor KC (2010) In vitro high-capacity assay to quantify the clonal heterogeneity in trilineage potential of mesenchymal stem cells reveals a complex hierarchy of lineage commitment. Stem Cells 28(4):788–798. doi:10.1002/stem.312
22. Galler KM, Cavender AC, Koeklue U, Suggs LJ, Schmalz G, D'Souza RN (2011) Bioengineering of dental stem cells in a PEGylated fibrin gel. Regen Med 6(2):191–200. doi:10.2217/rme.11.3
23. Huang GT, Yamaza T, Shea LD, Djouad F, Kuhn NZ, Tuan RS, Shi S (2010) Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng Part A 16(2):605–615. doi:10.1089/ten.TEA.2009.0518
24. Hakki SS, Kayis SA, Hakki EE, Bozkurt SB, Duruksu G, Unal ZS, Karaoz E (2015) Comparison of MSCs isolated from pulp and periodontal ligament. J Periodontol 86(2):283–91. doi:10.1902/jop.2014.140257
25. Chen K, Xiong H, Huang Y, Liu C (2013) Comparative analysis of in vitro periodontal characteristics of stem cells from apical papilla (SCAP) and periodontal ligament stem cells (PDLSCs). Arch Oral Biol 58(8):997–1006. doi:10.1016/j.archoralbio.2013.02.010
26. Han C, Yang Z, Zhou W, Jin F, Song Y, Wang Y, Huo N, Chen L, Qian H, Hou R, Duan Y, Jin Y (2010) Periapical follicle stem cell: a promising candidate for cementum/periodontal ligament regeneration and bio-root engineering. Stem Cells Dev 19(9):1405–1415. doi:10.1089/scd.2009.0277
27. Navabazam AR, Sadeghian Nodoshan F, Sheikhha MH, Miresmaeili SM, Soleimani M, Fesahat F (2013) Characterization of mesenchymal stem cells from human dental pulp, preapical follicle and periodontal ligament. Iran J Reprod Med 11(3):235–242
28. Carinci F, Papaccio G, Laino G, Palmieri A, Brunelli G, D'Aquino R, Pezzetti F (2008) Comparison between genetic portraits of osteoblasts derived from primary cultures and osteoblasts obtained from human pulpar stem cells. J Craniofac Surg 19(3):616–625. doi:10.1097/SCS.0b013e31816aabc8
29. Graziano A, d'Aquino R, Angelis MGCD, De Francesco F, Giordano A, Laino G, Papaccio G (2008) Scaffold's surface geometry significantly affects human stem cell bone tissue engineering. J Cell Physiol 214(1):166–172

30. Laino G, d'Aquino R, Graziano A, Lanza V, Carinci F, Naro F, Pirozzi G, Papaccio G (2005) A new population of human adult dental pulp stem cells: a useful source of living autologous fibrous bone tissue (LAB). J Bone Miner Res 20(8):1394–1402. doi:10.1359/JBMR.050325
31. Otabe K, Muneta T, Kawashima N, Suda H, Tsuji K, Sekiya I (2012) Comparison of gingiva, dental pulp, and periodontal ligament cells from the standpoint of mesenchymal stem cell properties. Cell Medicine 4(1):13–21
32. Zhang QB, Zhang ZQ, Fang SL, Liu YR, Jiang G, Li KF (2014) Effects of hypoxia on proliferation and osteogenic differentiation of periodontal ligament stem cells: an in vitro and in vivo study. Genet Mol Res 13(4):10204–10214. doi:10.4238/2014.December.4.15
33. Gandia C, Arminan A, Garcia-Verdugo JM, Lledo E, Ruiz A, Minana MD, Sanchez-Torrijos J, Paya R, Mirabet V, Carbonell-Uberos F, Llop M, Montero JA, Sepulveda P (2008) Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells 26(3):638–645. doi:10.1634/stemcells.2007-0484
34. Liu J, Yu F, Sun Y, Jiang B, Zhang W, Yang J, Liu S (2014) Characteristics and potential applications of human dental tissue-derived mesenchymal stem cells. Stem Cells 33(3):627–638. doi:10.1002/stem.1909
35. Ding G, Liu Y, Wang W, Wei F, Liu D, Fan Z, An Y, Zhang C, Wang S (2010) Allogeneic periodontal ligament stem cell therapy for periodontitis in swine. Stem Cells 28(10):1829–1838. doi:10.1002/stem.512
36. Liu O, Xu J, Ding G, Liu D, Fan Z, Zhang C, Chen W, Ding Y, Tang Z, Wang S (2013) Periodontal ligament stem cells regulate B lymphocyte function via programmed cell death protein 1. Stem Cells 31(7):1371–1382. doi:10.1002/stem.1387
37. Riccio M, Maraldi T, Pisciotta A, La Sala GB, Ferrari A, Bruzzesi G, Motta A, Migliaresi C, De Pol A (2012) Fibroin scaffold repairs critical-size bone defects in vivo supported by human amniotic fluid and dental pulp stem cells. Tissue Eng Part A 18(9–10):1006–1013. doi:10.1089/ten.TEA.2011.0542
38. Seo BM, Sonoyama W, Yamaza T, Coppe C, Kikuiri T, Akiyama K, Lee JS, Shi S (2008) SHED repair critical-size calvarial defects in mice. Oral Dis 14(5):428–434
39. Yamada Y, Ito K, Nakamura S, Ueda M, Nagasaka T (2011) Promising cell-based therapy for bone regeneration using stem cells from deciduous teeth, dental pulp, and bone marrow. Cell Transplant 20(7):1003–1013. doi:10.3727/096368910X539128
40. d'Aquino R, De Rosa A, Lanza V, Tirino V, Laino L, Graziano A, Desiderio V, Laino G, Papaccio G (2009) Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. Eur Cell Mater 18:75–83
41. Liu HC, Wang DS, Su F, Wu X, Shi ZP, Lv Y, Wang JZ (2011) Reconstruction of alveolar bone defects using bone morphogenetic protein 2 mediated rabbit dental pulp stem cells seeded on nano-hydroxyapatite/collagen/poly (L-lactide). Tissue Eng Part A 17(19–20):2417–2433. doi:10.1089/ten.TEA.2010.0620
42. Park SY, Kim KH, Gwak EH, Rhee SH, Lee JC, Shin SY, Seol YJ (2015) Ex vivo bone morphogenetic protein 2 gene delivery using periodontal ligament stem cells for enhanced re-osseointegration in the regenerative treatment of peri-implantitis. J Biomed Mater Res A 103(1):38–47. doi:10.1002/jbm.a.35145
43. Honda MJ, Imaizumi M, Tsuchiya S, Morsczeck C (2010) Dental follicle stem cells and tissue engineering. J Oral Sci 52(4):541–552
44. Abe S, Yamaguchi S, Watanabe A, Hamada K, Amagasa T (2008) Hard tissue regeneration capacity of apical pulp derived cells (APDCs) from human tooth with immature apex. Biochem Biophys Res Commun 371(1):90–93. doi:10.1016/j.bbrc.2008.04.016
45. Wang S, Mu J, Fan Z, Yu Y, Yan M, Lei G, Zhang G (2012) Insulin-like growth factor 1 can promote the osteogenic differentiation and osteogenesis of stem cells from apical papilla. Stem Cell Res 8(3):346–356. doi:10.1016/j.scr.2011.12.005
46. Khan Y, Yaszemski MJ, Mikos AG, Laurencin CT (2008) Tissue engineering of bone: material and matrix considerations. J Bone Joint Surg Am 90(Suppl 1):36–42. doi:10.2106/JBJS.G.01260

47. Boeckel DG, Shinkai RSA, Grossi ML, Teixeira ER (2012) culture-based tissue engineering as an alternative to bone grafts in implant dentistry: a literature review. J Oral Implantol 38(s1):538–545. doi:10.1563/AAID-JOI-D-11-00197
48. Annibali S, Cicconetti A, Cristalli MP, Giordano G, Trisi P, Pilloni A, Ottolenghi L (2013) A comparative morphometric analysis of biodegradable scaffolds as carriers for dental pulp and periosteal stem cells in a model of bone regeneration. J Craniofac Surg 24(3):866–871. doi:10.1097/SCS.0b013e31827ca530
49. Laino G, Graziano A, d'Aquino R, Pirozzi G, Lanza V, Valiante S, De Rosa A, Naro F, Vivarelli E, Papaccio G (2006) An approachable human adult stem cell source for hard-tissue engineering. J Cell Physiol 206(3):693–701. doi:10.1002/jcp.20526
50. Huang GJ, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 88(9):792–806. doi:10.1177/0022034509340867
51. Galler KM, D'Souza RN (2011) Tissue engineering approaches for regenerative dentistry. Regen Med 6(1):111–124. doi:10.2217/rme.10.86
52. Sahdev P, Ochyl LJ, Moon JJ (2014) Biomaterials for nanoparticle vaccine delivery systems. Pharm Res 31(10):2563–2582. doi:10.1007/s11095-014-1419-y
53. Tsuchiya S, Ohshima S, Yamakoshi Y, Simmer JP, Honda MJ (2010) Osteogenic differentiation capacity of porcine dental follicle progenitor cells. Connect Tissue Res 51(3):197–207. doi:10.3109/03008200903267542
54. Coyac BR, Chicatun F, Hoac B, Nelea V, Chaussain C, Nazhat SN, McKee MD (2013) Mineralization of dense collagen hydrogel scaffolds by human pulp cells. J Dent Res 92(7):648–654. doi:10.1177/0022034513488599
55. Viale-Bouroncle S, Gosau M, Morsczeck C (2014) Laminin regulates the osteogenic differentiation of dental follicle cells via integrin-alpha2/-beta1 and the activation of the FAK/ERK signaling pathway. Cell Tissue Res 357(1):345–354. doi:10.1007/s00441-014-1869-x
56. Morsczeck C, Schmalz G, Reichert TE, Völlner F, Saugspier M, Viale-Bouroncle S, Driemel O (2009) Gene expression profiles of dental follicle cells before and after osteogenic differentiation in vitro. Clin Oral Investig 13(4):383–391. doi:10.1007/s00784-009-0260-x
57. Chen YJ, Chung MC, Jane Yao CC, Huang CH, Chang HH, Jeng JH, Young TH (2012) The effects of acellular amniotic membrane matrix on osteogenic differentiation and ERK1/2 signaling in human dental apical papilla cells. Biomaterials 33(2):455–463. doi:10.1016/j.biomaterials.2011.09.065
58. Kanafi MM, Ramesh A, Gupta PK, Bhonde RR (2014) Dental pulp stem cells immobilized in alginate microspheres for applications in bone tissue engineering. Int Endod J 47(7):687–697. doi:10.1111/iej.12205
59. Dahl M, Jørgensen NR, Hørberg M, Pinholt EM (2014) Carriers in mesenchymal stem cell osteoblast mineralization—state-of-the-art. J Cranio Maxill Surg 42(1):41–47. doi:10.1016/j.jcms.2013.01.047
60. Lee JS, Yi JK, An SY, Heo JS (2014) Increased osteogenic differentiation of periodontal ligament stem cells on polydopamine film occurs via activation of integrin and PI3K signaling pathways. Cell Physiol Biochem 34(5):1824–1834. doi:10.1159/000366381
61. Polini A, Pisignano D, Parodi M, Quarto R, Scaglione S (2011) Osteoinduction of human mesenchymal stem cells by bioactive composite scaffolds without supplemental osteogenic growth factors. PloS One 6(10):e26211. doi:10.1371/journal.pone.0026211
62. Viale-Bouroncle S, Bey B, Reichert TE, Schmalz G, Morsczeck C (2011) beta-tricalcium-phosphate stimulates the differentiation of dental follicle cells. J Mater Sci Mater Med 22(7):1719–1724. doi:10.1007/s10856-011-4345-0
63. Viale-Bouroncle S, Buergers R, Morsczeck C, Gosau M (2013) beta-Tricalcium phosphate induces apoptosis on dental follicle cells. Calcif Tissue Int 92(5):412–417. doi:10.1007/s00223-012-9694-2
64. Ning L, Malmstrom H, Ren YF (2015) Porous collagen-hydroxyapatite scaffolds with mesenchymal stem cells for bone regeneration. J Oral Implantol 41(1):45–49. doi:10.1563/AAID-JOI-D-12-00298

65. Guo T, Li Y, Cao G, Zhang Z, Chang S, Czajka-Jakubowska A, Nor JE, Clarkson BH, Liu J (2014) Fluorapatite-modified scaffold on dental pulp stem cell mineralization. J Dent Res 93(12):1290–1295. doi:10.1177/0022034514547914
66. Moshaverinia A, Chen C, Xu X, Akiyama K, Ansari S, Zadeh HH, Shi S (2013) Bone regeneration potential of stem cells derived from periodontal ligament or gingival tissue sources encapsulated in RGD-modified alginate scaffold. Tissue Eng Part A 20(3–4):611–621. doi:10.1089/ten.TEA.2013.0229
67. Viale-Bouroncle S, Gosau M, Küpper K, Möhl C, Brockhoff G, Reichert TE, Morsczeck C (2012) Rigid matrix supports osteogenic differentiation of stem cells from human exfoliated deciduous teeth (SHED). Differentiation 84(5):366–370. doi:10.1016/j.diff.2012.08.005
68. Lamplot JD, Qin J, Nan G, Wang J, Liu X, Yin L, Tomal J, Li R, Shui W, Zhang H, Kim SH, Zhang W, Zhang J, Kong Y, Denduluri S, Rogers MR, Pratt A, Haydon RC, Luu HH, Angeles J, Shi LL, He TC (2013) BMP9 signaling in stem cell differentiation and osteogenesis. Am J Stem Cells 2(1):1–21
69. Wang J, Zhang H, Zhang W, Huang E, Wang N, Wu N, Wen S, Chen X, Liao Z, Deng F, Yin L, Zhang J, Zhang Q, Yan Z, Liu W, Zhang Z, Ye J, Deng Y, Luu HH, Haydon RC, He TC, Deng F (2014) Bone morphogenetic protein-9 effectively induces osteo/odontoblastic differentiation of the reversibly immortalized stem cells of dental apical papilla. Stem Cells Dev 23(12):1405–1416. doi:10.1089/scd.2013.0580
70. Ye G, Li C, Xiang X, Chen C, Zhang R, Yang X, Weng Y (2014) Bone morphogenetic Protein-9 induces PDLSCs osteogenic differentiation through the ERK and p38 signal pathways. Int J Med Sci 11(10):1065–1072. doi:10.7150/ijms.8473.eCollection2014
71. Saugspier M, Felthaus O, Viale-Bouroncle S, Driemel O, Reichert TE, Schmalz G, Morsczeck C (2010) The differentiation and gene expression profile of human dental follicle cells. Stem Cells Dev 19(5):707–717. doi:10.1089/scd.2009.0027
72. Silverio KG, Davidson KC, James RG, Adams AM, Foster BL, Nociti FH, Somerman MJ Jr, Moon RT (2012) Wnt/beta-catenin pathway regulates bone morphogenetic protein (BMP2)-mediated differentiation of dental follicle cells. J Periodontal Res 47(3):309–319. doi:10.1111/j.1600-0765.2011.01433.x
73. Zhang H, Wang J, Deng F, Huang E, Yan Z, Wang Z, Deng Y, Zhang Q, Zhang Z, Ye J, Qiao M, Li R, Wang J, Wei Q, Zhou G, Luu HH, Haydon RC, He TC, Deng F (2015) Canonical Wnt signaling acts synergistically on BMP9-induced osteo/odontoblastic differentiation of stem cells of dental apical papilla (SCAPs). Biomaterials 39:145–154. doi:10.1016/j.biomaterials.2014.11.007
74. Qu B, Liu O, Fang X, Zhang H, Wang Y, Quan H, Zhang J, Zhou J, Zuo J, Tang J, Tang Z (2014) Distal-less homeobox 2 promotes the osteogenic differentiation potential of stem cells from apical papilla. Cell Tissue Res 357(1):133–143. doi:10.1007/s00441-014-1833-9
75. Mimori K, Komaki M, Iwasaki K, Ishikawa I (2007) Extracellular signal-regulated kinase 1/2 is involved in ascorbic acid-induced osteoblastic differentiation in periodontal ligament cells. J Periodontol 78(2):328–334
76. Yu Y, Mu J, Fan Z, Lei G, Yan M, Wang S, Tang C, Wang Z, Yu J, Zhang G (2012) Insulin-like growth factor 1 enhances the proliferation and osteogenic differentiation of human periodontal ligament stem cells via ERK and JNK MAPK pathways. Histochem Cell Biol 137(4):513–525. doi:10.1007/s00418-011-0908-x
77. Feng X, Huang D, Lu X, Feng G, Xing J, Lu J, Xu K, Xia W, Meng Y, Tao T, Li L, Gu Z (2014) Insulin-like growth factor 1 can promote proliferation and osteogenic differentiation of human dental pulp stem cells via mTOR pathway. Dev Growth Differ 56(9):615–624. doi:10.1111/dgd.12179
78. Nguyen DV, Li Calzi S, Shaw LC, Kielczewski JL, Korah HE, Grant MB (2013) An ocular view of the IGF-IGFBP system. Growth Horm IGF Res 23(3):45–52. doi:10.1016/j.ghir.2013.03.001
79. Liu D, Wang Y, Jia Z, Wang LP, Wang J, Yang D, Song J, Wang S, Fan Z (2015) Demethylation of IGFBP5 by histone demethylase KDM6B promotes mesenchymal stem cell-mediated periodontal tissue regeneration by enhancing osteogenic differentiation and anti-inflammation potentials. Stem Cells 33(8):2523–2536. doi:10.1002/stem.2018

80. Oh SA, Lee HY, Lee JH, Kim TH, Jang JH, Kim HW, Wall I (2012) Collagen three-dimensional hydrogel matrix carrying basic fibroblast growth factor for the cultivation of mesenchymal stem cells and osteogenic differentiation. Tissue Eng Part A 18(9–10):1087–1100. doi:10.1089/ten.TEA.2011.0360
81. Li B, Qu C, Chen C, Liu Y, Akiyama K, Yang R, Shi S (2012) Basic fibroblast growth factor inhibits osteogenic differentiation of stem cells from human exfoliated deciduous teeth through ERK signaling. Oral Dis 18(3):285–292. doi:10.1111/j.1601-0825.2011.01878.x
82. Nowwarote N, Pavasant P, Osathanon T (2015) Role of endogenous basic fibroblast growth factor in stem cells isolated from human exfoliated deciduous teeth. Arch Oral Biol 60(3):408–415. doi:10.1016/j.archoralbio.2014.11.017
83. Osathanon T, Nowwarote N, Manokawinchoke J, Pavasant P (2013) bFGF and JAGGED1 regulate alkaline phosphatase expression and mineralization in dental tissue-derived mesenchymal stem cells. J Cell Biochem 114(11):2551–2561. doi:10.1002/jcb.24602
84. Wu J, Huang GT, He W, Wang P, Tong Z, Jia Q, Dong L, Niu Z, Ni L (2012) Basic fibroblast growth factor enhances stemness of human stem cells from the apical papilla. J Endod 38(5):614–622. doi:10.1016/j.joen.2012.01.014
85. Qian J, Jiayuan W, Wenkai J, Peina W, Ansheng Z, Shukai S, Shafei Z, Jun L, Longxing N (2015) Basic fibroblastic growth factor affects the osteogenic differentiation of dental pulp stem cells in a treatment-dependent manner. Int Endod J 48(7):690–700. doi:10.1111/iej.12368
86. Chang JY, Wang C, Liu J, Huang Y, Jin C, Yang C, Hai B, Liu F, D'Souza RN, McKeehan WL, Wang F (2013) Fibroblast growth factor signaling is essential for self-renewal of dental epithelial stem cells. J Biol Chem 288(40):28952–28961. doi:10.1074/jbc.M113.506873
87. Houshmand B, Behnia H, Khoshzaban A, Morad G, Behrouzi G, Dashti SG, Khojasteh A (2013) Osteoblastic differentiation of human stem cells derived from bone marrow and periodontal ligament under the effect of enamel matrix derivative and transforming growth factor-beta. Int J Oral Maxillofac Implants 28(6):e440–e450. doi:10.11607/jomi.te24
88. He W, Zhang J, Niu Z, Yu Q, Wang Z, Zhang R, Su L, Fu L, Smith AJ, Cooper PR (2014) Regulatory interplay between NFIC and TGF-β1 in apical papilla-derived stem cells. J Dent Res 93(5):496–501. doi:10.1177/0022034514525200
89. Chang HH, Chang MC, Wu IH, Huang GF, Huang WL, Wang YL, Lee SY, Yeh CY, Guo MK, Chan CP, Hsien HC, Jeng JH (2015) Role of ALK5/Smad2/3 and MEK1/ERK signaling in transforming growth factor beta 1-modulated growth, collagen turnover, and differentiation of stem cells from apical papilla of human tooth. J Endod 41(8):1272–1280. doi:10.1016/j.joen.2015.03.022
90. Ochiai H, Yamamoto Y, Yokoyama A, Yamashita H, Matsuzaka K, Abe S, Azuma T (2010) Dual nature of TGF-β1 in osteoblastic differentiation of human periodontal ligament cells. J Hard Tissue Biol 19(3):187–194
91. Ochiai H, Okada S, Saito A, Hoshi K, Yamashita H, Takato T, Azuma T (2012) Inhibition of insulin-like growth factor-1 (IGF-1) expression by prolonged transforming growth factor-β1 (TGF-β1) administration suppresses osteoblast differentiation. J Biol Chem 287(27):22654–22661. doi:10.1074/jbc.M111.279091
92. Felthaus O, Gosau M, Ettl T, Prantl L, Morsczeck C (2014) Migration of human dental follicle cells in vitro. J Periodontal Res 49(2):205–212. doi:10.1111/jre.12096
93. Yuda A, Maeda H, Fujii S, Monnouchi S, Yamamoto N, Wada N, Koori K, Tomokiyo A, Hamano S, Hasegawa D, Akamine A (2015) Effect of CTGF/CCN2 on osteo/cementoblastic and fibroblastic differentiation of a human periodontal ligament stem/progenitor cell line. J Cell Physiol 230(1):150–159. doi:10.1002/jcp.24693
94. Lee UL, Jeon SH, Park JY, Choung PH (2011) Effect of platelet-rich plasma on dental stem cells derived from human impacted third molars. Regen Med 6(1):67–79. doi:10.2217/rme.10.9610.2217/rme.11.63
95. Lee JY, Nam H, Park YJ, Lee SJ, Chung CP, Han SB, Lee G (2011) The effects of platelet-rich plasma derived from human umbilical cord blood on the osteogenic differentiation of human dental stem cells. Vitro Cell Dev Biol Anim 47(2):157–164. doi:10.1007/s11626-010-9364-5
96. Zhao SN, Liu WF, Zhang ZT (2013) Effect of platelet-rich plasma on cell proliferation and osteogenic activity of pulp stem cells. Zhonghua Kou Qiang Yi Xue Za Zhi 48(3):177–182

97. Manimaran K, Sankaranarayanan S, Ravi VR, Elangovan S, Chandramohan M, Perumal SM (2014) Treatment of osteoradionecrosis of mandible with bone marrow concentrate and with dental pulp stem cells. Ann Maxillofac Surg 4(2):189–192. doi:10.4103/2231-0746.147130
98. Monnouchi S, Maeda H, Yuda A, Hamano S, Wada N, Tomokiyo A, Koori K, Sugii H, Serita S, Akamine A (2015) Mechanical induction of interleukin-11 regulates osteoblastic/cementoblastic differentiation of human periodontal ligament stem/progenitor cells. J Periodontal Res 50(2):231–239. doi:10.1111/jre.12200
99. Liu W, Konermann A, Guo T, Jager A, Zhang L, Jin Y (2014) Canonical Wnt signaling differently modulates osteogenic differentiation of mesenchymal stem cells derived from bone marrow and from periodontal ligament under inflammatory conditions. Biochim Biophys Acta 1840(3):1125–1134. doi:10.1016/j.bbagen.2013.11.003
100. Yin LH, Cheng WX, Qin ZS, Sun KM, Zhong M, Wang JK, Gao WY, Yu ZH (2014) Effects of ginsenoside Rg-1 on the proliferation and osteogenic differentiation of human periodontal ligament stem cells. Chin J Integr Med [Epub ahead of print]. doi:10.1007/s11655-014-1856-9
101. Kim SY, An SY, Lee JS, Heo JS (2015) Zanthoxylum schinifolium enhances the osteogenic potential of periodontal ligament stem cells. Vitro Cell Dev Biol Anim 51(2):165–173. doi:10.1007/s11626-014-9824-4
102. Kim SY, Lee JY, Park YD, Kang KL, Lee JC, Heo JS (2013) Hesperetin alleviates the inhibitory effects of high glucose on the osteoblastic differentiation of periodontal ligament stem cells. PloS One 8(6):e67504. doi:10.1371/journal.pone.0067504
103. Drees J, Felthaus O, Gosau M, Morsczeck C (2014) Butyrate stimulates the early process of the osteogenic differentiation but inhibits the biomineralization in dental follicle cells (DFCs). Odontology 102(2):154–159. doi:10.1007/s10266-013-0117-2
104. Brighton CT, Krebs AG (1972) Oxygen tension of healing fractures in the rabbit. J Bone Joint Surg Am 54(2):323–332
105. Heppenstall RB, Grislis G, Hunt TK (1975) Tissue gas tensions and oxygen consumption in healing bone defects. Clin Orthop Relat Res 106:357–365
106. Vanacker J, Viswanath A, De Berdt P, Everard A, Cani PD, Bouzin C, Feron O, Diogenes A, Leprince JG, des Rieux A (2014) Hypoxia modulates the differentiation potential of stem cells of the apical papilla. J Endod 40(9):1410–1418. doi:10.1016/j.joen.2014.04.008
107. Iida K, Takeda-Kawaguchi T, Tezuka Y, Kunisada T, Shibata T, Tezuka K (2010) Hypoxia enhances colony formation and proliferation but inhibits differentiation of human dental pulp cells. Arch Oral Biol 55(9):648–654. doi:10.1016/j.archoralbio.2010.06.005
108. Sakdee JB, White RR, Pagonis TC, Hauschka PV (2009) Hypoxia-amplified proliferation of human dental pulp cells. J Endod 35(6):818–823. doi:10.1016/j.joen.2009.03.001
109. Osathanon T, Vivatbutsiri P, Sukarawan W, Sriarj W, Pavasant P, Sooampon S (2015) Cobalt chloride supplementation induces stem-cell marker expression and inhibits osteoblastic differentiation in human periodontal ligament cells. Arch Oral Biol 60(1):29–36. doi:10.1016/j.archoralbio.2014.08.018
110. Mu C, Lv T, Wang Z, Ma S, Ma J, Liu J, Yu J, Mu J (2014) Mechanical stress stimulates the osteo/odontoblastic differentiation of human stem cells from apical papilla via erk 1/2 and JNK MAPK pathways. Biomed Res Int 2014:494378. doi:10.1155/2014/494378
111. Filho Cerruti H, Kerkis I, Kerkis A, Tatsui NH, da Costa NA, Bueno DF, da Silva MC (2007) Allogenous bone grafts improved by bone marrow stem cells and platelet growth factors: clinical case reports. Artif Organs 31(4):268–273. doi:10.1111/j.1525-1594.2007.00374.x
112. Rickert D, Sauerbier S, Nagursky H, Menne D, Vissink A, Raghoebar G (2011) Maxillary sinus floor elevation with bovine bone mineral combined with either autogenous bone or autogenous stem cells: a prospective randomized clinical trial. Clin Oral Implants Res 22(3):251–258. doi:10.1111/j.1600-0501.2010.01981.x

Chapter 11
Dental Stem Cells: Their Potential in Neurogenesis and Angiogenesis

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11.1 Introduction

This chapter will focus on the neurogenic and angiogenic properties of dental stem cells (DSCs) (Fig. 11.1). Angiogenesis is the development of new blood vessels out of existing blood capillaries. The first part of the chapter will cover the angiogenic characteristics of DSCs, with emphasis on the paracrine effects that these cells exert on endogenous cell populations and tissues, as well as the fruitful attempts that have been made to upregulate their angiogenic properties. In addition, possible endothelial differentiation potential of DSCs will also be briefly discussed.

Secondly, several studies have tried to gain insight into the neural regenerative potential of DSCs. An overview will be given on the status of differentiation of DSCs towards neuronal cells and Schwann cells. In addition, the neurotrophic proteins present in the DSC secretome will be described along with the potential of preconditioning of these cells for neurodegenerative diseases.

Finally, applications of DSCs in stroke will be addressed. Stroke is a severe condition defined by interrupted or severely impaired blood flow to the affected area in the brain. Consequently, neuronal cell death occurs at the affected site, resulting in severe neurological damage. Due to their neurogenic and angiogenic properties, DSCs are considered to be strong candidates for the treatment of stroke where both angiogenesis and neurogenesis are key factors to reconstitute the affected brain area.

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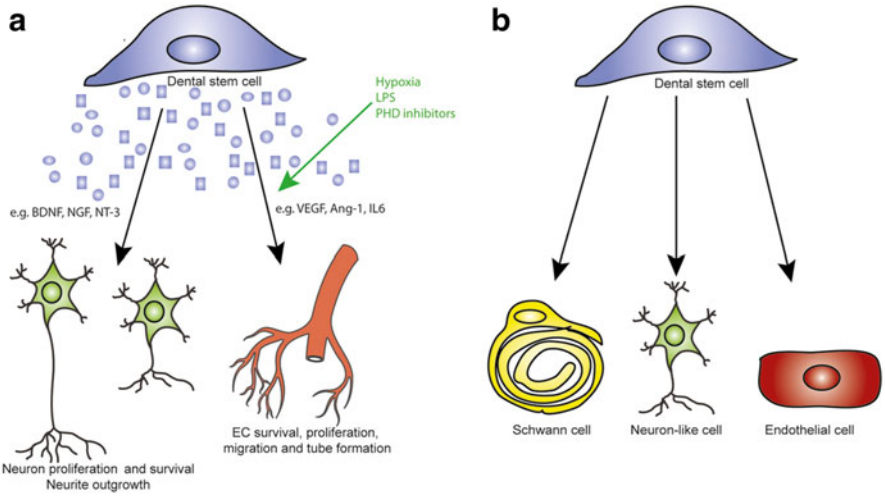

Fig. 11.1 Angiogenic and neurogenic properties of dental stem cells (DSCs). (**a**) Paracrine actions of DSCs: DSCs secrete a wide variety of angiogenic and neurogenic molecules which are able to induce responses of other tissues and cell types; For example, they produce BDNF and NGF which enhance survival and proliferation of neurons and stimulate the outgrowth of axons. In addition, angiogenic factors are generated by DSCs which activate blood vessel growth by stimulating endothelial cell migration, proliferation and tube formation. Environmental factors such as hypoxia, LPS or pharmacological agents which mimic hypoxia (such as PHD inhibitors) have been shown to increase the amount of angiogenic molecules produces by DSCs. (**b**) Differentiation potential of DSCs. Besides their classical mesenchymal differentiation potential into osteoblasts, chondroblasts and adipocytes, DSCs themselves have been shown to differentiate into cells showing subsets of typical characteristics of neurons, Schwann cells and endothelial cells hen cultured in specific conditions in vitro when cultured in specific conditions in vitro

11.2 DSCs and Angiogenesis

Angiogenesis, the sprouting of new capillaries from pre-existing blood vessels, is considered to be a well-coordinated multi-step biological process initiated in response to specific stimuli such as inflammation or hypoxia [1–3]. The subsequent increase in vascular permeability and destabilization of the pre-existing vessels cause release of sequestered growth factors and chemokines which promote endothelial proliferation and migration. Following vascular sprouting and lumen formation, the formed tubes fuse with the pre-existing capillary after which they are stabilized through attachment of pericytes and deposition of extracellular matrix [4]. These events are carefully regulated by a broad range of regulatory proteins, which form a delicate balance between stimulation and inhibition of blood vessel formation. Within the healthy human body, endothelial cells (ECs) and blood vessels usually remain in a quiescent state as the inhibitory factors have a predominant effect. However, in case of hypoxia, the balance can be tipped towards blood vessel growth due to the local production of an excess amount of pro-angiogenic growth factors [5, 6]. A disturbance

of this angiogenic balance also takes place in pathological conditions, such as myocardial infarction, diabetes mellitus, cerebral ischemic stroke and cancer [7]. Not only does angiogenesis play an important role in potential life-threatening disorders, it is also a key aspect in tissue engineering as a lack of vascular supply causes oxygen and nutrient deprivation and eventually necrosis of newly transplanted tissues. Due to the limited success of growth factor-based revascularization studies, stem cell-based therapies have been postulated as a more regenerative approach to promote angiogenesis [8, 9]. Mesenchymal stem cells (MSCs), for example, are thought to contribute to therapeutic angiogenesis through (i) the paracrine secretion of pro-angiogenic factors or (ii) direct differentiation into endothelial cells (ECs) [10–12]. The paracrine actions of DSCs and their possible transition towards ECs will be described in part 11.2.1 and 11.2.3, respectively.

11.2.1 The Paracrine Angiogenic Properties of DSCs

In this section, the angiogenic factors present in the secretome of various DSC populations will be summarized. Next part handles the effect of DSCs on endothelial cells, which align blood vessels, and are the key actors in the angiogenic process. Furthermore, as current capacities of DSCs to induce blood vessel development are suboptimal, various attempts have been made to increase their angiogenic potential by either genetic modification, hypoxia pretreatment or by incubation of the cells with various proteins or molecules, which will be discussed in Sect. 11.2.2.

11.2.1.1 DSCs Express a Wide Variety of Angiogenic Factors

Despite the elaborate characterization of DSCs and the large amount of studies suggesting their potential role in the regeneration of dental tissues, data regarding their angiogenic properties are limited. A number of studies have pointed out the expression of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF) by dental pulp stem cells (DPSCs), either under basal conditions or after injury or hypoxia [13–17]. In addition, DPSCs express urokinase-type plasminogen activator (uPA), endothelin-1 (EDN1), dipeptidyl peptidase IV (DPPIV), angiopoietin-1 (ANGPT1), colony-stimulating factor (CSF), monocyte chemoattractant protein-1 (MCP-1) and angiogenin (ANG) [18]. However, DPSCs also express anti-angiogenic factors, namely plasminogen activator inhibitor-1 (PAI-1), endostatin, thrombospondin-1 (THBS1) [18], tissue inhibitor of matrix metalloproteinase-1/4 (TIMP-1/4) and pentraxin-3 (IGFBP3) [18, 19]. Similar results were also found for stem cells from the apical papilla (SCAPs) and dental follicle stem cells (DFSCs), although the expression levels differed significantly between stem cell populations [18, 20–22]. With regard to periodontal ligament stem cells (PDLSCs) and stem cells human exfoliated deciduous teeth (SHEDs), literature merely indicates the expression of bFGF,

endostatin, insulin-like growth factor-1 (IGF1), ANGPT2 and VEGF [23–25]. As DSCs express pro-angiogenic as well as anti-angiogenic factors, it is important to determine the resulting impact on endothelial cells.

11.2.1.2 DSCs Promote Different Aspects of Angiogenesis In Vitro

As already mentioned, angiogenesis is a complex biological process which encompasses endothelial proliferation, migration and tube formation. In order to evaluate the functional impact of DSCs on endothelial cells, multiple assays can be conducted to mimic each of these aspects (Fig. 11.2a, b). Endothelial proliferation, for example, is often tested by means of colorimetric assays comprising the incubation of the cells with growth factors or conditioned medium (CM). However, limited and conflicting data are available with regard to DSCs. Iohara and co-workers, for instance, reported a

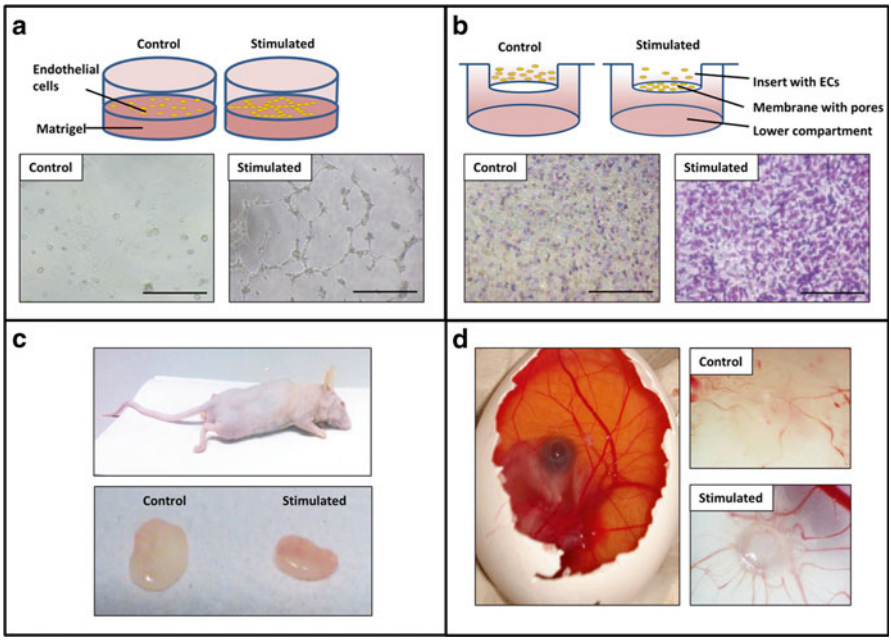

Fig. 11.2 Commonly used angiogenic assays. (**a**) In the matrigel tube formation assay, endothelial cells (ECs) are seeded onto matrigel. Under the correct angiogenic stimuli, the endothelial cells will form networks. Scalebar = 200 μm. (**b**) Transwell migration assay: ECs are allowed to migrate through a semi-permeable membrane towards a lower compartment which may contain chemotactic molecules. Scalebar = 200 μm. (**c**) Mouse matrigel assay: matrigel is mixed with angiogenic factors and injected subcutaneously in the dorsal flank of athymic nude mice. 2 or 4 weeks later mice are sacrificed and the number of blood capillaries grown within the plugs is assessed. (**d**) Chicken chorioallantoic membrane (CAM) assay: Pro-angiogenic agents are placed onto the CAM, and 48–72 hours later, blood vessels grow towards the stimulus in a typical spoke wheel pattern

significant increase of the proliferation of human umbilical cord vein endothelial cells (HUVECs) after incubation with CM of porcine $CD31^-$ $CD146^-$ DPSCs [26]. In contrast, the CM of DPSCs, SCAPs as well as DFSCs had no pronounced effect on the proliferation of human microvascular endothelial cells (HMECs) [18]. To date, there are no reports available regarding the influence of PDLSCs and SHEDs on endothelial migration. During angiogenesis, endothelial cells migrate along a gradient of chemotactic proteins. As DSCs secrete several proteins influencing endothelial migration, such as ANGPT, bFGF, CSF, EDN1 and VEGF, their chemotactic potential can be assessed in a transwell migration assay (Fig. 11.2a). In particular, DPSCs and SCAPs cause a significant increase in endothelial migration in comparison to DFSCs, which showed no substantial impact [18, 19]. With regard to endothelial tube formation (Fig. 11.2b), literature indicates an increased formation of vessel-like structures after direct co-culture of SCAPs and HUVECs [27]. Similar results were also found for DPSCs, PDLSCs and SHEDs [25, 28]. Given the close proximity of DPSCs and SCAPs to the endothelial tubes in the aforementioned studies, these results suggest a more pericyte-like role for DSCs in angiogenesis [27, 28]. However, the induction of endothelial tubulogenesis does not necessarily require cell-cell contact, as it can also be mediated by paracrine factors. Dissanayaka et al., for example, demonstrated the formation of tubular networks following indirect co-culture of human DPSCs and HUVECs encapsulated into a self-assembling peptide hydrogel [29]. A significant augmentation of endothelial tubulogenesis was also observed after the incubation of HMECs with CM of DPSCs [18]. Besides, along with more elaborate research regarding the in vitro angiogenic properties of PDLSCs and SHEDs, various in vivo tests for all types of DSCs are required as the cellular microenvironment can be determining for their influence on angiogenesis.

11.2.1.3 DSCs Induce Angiogenesis in Different In Vivo Models

In order to test angiogenesis in vivo, several proof-of-principle models are available. Janebodin et al., for instance, demonstrated the VEGF-dependent induction of angiogenesis by mouse DPSCs in a mouse matrigel plug assay [28] (Fig. 11.2c). Subcutaneous co-transplantation of PDLSCs and endothelial cells also led to significant vascularization. As there was no increase in human-derived blood vessels, PDLSCs did not differentiate into endothelial cells but acted as pericyte-like cells or secreted paracrine factors [25]. DPSCs and SCAPs were also able to induce angiogenesis in a chorioallantoic membrane assay [18, 19] (Fig. 11.2d). Next to this proof-of-principle models, DPSCs were also shown to promote angiogenesis in clinically relevant disease models. For instance, Gandia et al. reported a significant improvement of left ventricular function 4 weeks after the intracardial injection of GFP-labeled human DPSCs in rats suffering from myocardial infarction. Cardiac function improvement was not only correlated with a reduction of the infarct size and a thickening of the anterior ventricular wall, but also a significant increase in capillary density. As there were no signs of green fluorescent endothelial cells, smooth muscle cells or cardiomyocytes within the heart tissue, the observed effects were probably due to the secretion of paracrine factors, such as

VEGF or IGF [30]. A notably higher capillary density was also found in a mouse model of hindlimb ischemia after the transplantation of a side population of porcine DPSCs, namely $CD31^-$ $CD146^-$ DPSCs. Since these cells were found to be in close proximity of the newly formed vessels, the increased neovascularization was probably caused by paracrine mechanisms rather than functional incorporation into the vessels [26]. The same side population was also able to enhance functional recovery after focal cerebral ischemia in rats. Next to the secretion of neurotrophic factors, the authors also detected increased levels of VEGF, which potentially stimulated neurogenesis and vasculogenesis in the ischemic rat brain [31, 32].

11.2.2 Priming/Upregulation of Paracrine Angiogenic Actions of DSCs

Multipotent stem cells possess many characteristics that make them suitable for clinical applications. However, a major concern in the field of regenerative medicine is the survival of these stem cells after transplantation. In order to overcome this hurdle, attempts have been made to modulate the stem cells to improve cell survival and engraftment after the transplantation [33]. A recent paradigm shift has emerged, suggesting that the beneficial effects of stem cell transplantation may be due to their paracrine effects rather than their differentiation potential. Therefore, a variety of different approaches has been examined, mainly focusing on increasing stem cell survival and thereby increasing the amount of trophic factors secreted [34].

11.2.2.1 Genetic Modification

One possible approach to enhance graft survival is genetically modifying the cells to express pro-survival genes. Bone marrow derived MSCs (BMMSCs) have been modified to overexpress anti-apoptotic genes such as Bcl-2 or Akt [35–37]. Overexpression of Bcl-2 protected MSCs against apoptosis, increased VEGF secretion under hypoxic conditions in vitro, and resulted in an increased capillary density in vivo [36]. Furthermore, Gnecchi and colleagues demonstrated that CM of Akt-modified BMMSCs protected cardiomyocytes against hypoxia-induced apoptosis in vitro. Injecting CM obtained from hypoxic Akt-MSCs limited infarct size in vivo. Moreover, subjecting Akt-MSCs to hypoxia significantly increased the expression of several genes such as VEGF, bFGF, HGF and IGF1, further supporting the paracrine hypothesis [35]. Another possibility is to induce overexpression of key proteins specific to the illness of the patient (*e.g.* dopamine for patients suffering with Parkinson's disease or insulin for diabetics). To date, this line of investigation has not yet been pursued with regard to DSCs. Unfortunately, gene therapy is a relatively young field of research, of which the clinical relevance still has to be proven. Today, scientists are merely learning how exactly to modify the genes of cells, and many questions and (bio)safety concerns remain before clinical trials with genetically modified stem cells can be deemed possible [38].

11.2.2.2 Hypoxic Preconditioning

Preconditioning stem cells by exposing them to a defined stimulus may be helpful in enhancing the secretion of trophic factors. In contrast to genetic modification, which usually affects a single target, preconditioning usually results in a more global cellular response [34]. As mentioned above, hypoxia is a potent stimulus for the secretion of certain trophic factors. Not surprisingly, hypoxic preconditioning has gained a lot of attention as a standard method to improve the paracrine actions of a variety of stem cell sources [33]. By this kind of pretreatment, researchers aim to improve the resistance of cultured cells against hypoxic conditions by taking advantage of the known mechanism leading to survival during in vivo ischemia and creating in vitro simulations. Hypoxic preconditioning has been shown to increase stem cell survival and paracrine activity, and even increase angiogenesis in an in vivo model of murine hind limb ischemia [39–41].

Oxygen tension in dental pulp tissues is lower compared to that of the atmosphere, since oxygen can only reach the pulpal cells via the vasculature in the narrow root. It might be postulated that dental pulp cells are, therefore, uniquely suited to survive in hypoxic conditions as they naturally reside in a low oxygen environment in vivo. Iida and colleagues demonstrated that hDPSCs cultured under hypoxic conditions displayed an increased proliferation rate as well as an increased expression of STRO-1 [42, 43]. In contrast, hypoxic preconditioning did not influence proliferation of porcine dental pulp-derived cells [44]. Furthermore, hypoxia has been shown to enhance the expression of HIF-1α and VEGF [13]. Hypoxia also increased the migration of DPSCs according to Kanafi et al. [45]. Other stem cell populations from dental origin have also been shown to response to hypoxic preconditioning. Although hypoxia does not seem to influence the proliferation rate of SCAPs, it has been reported to increase the production of VEGF as well as influencing SCAPs' differentiation potential [22]. Subjecting human PDLSCs to hypoxia resulted in an increased secretion of VEGF and interleukin (IL)-6 after 24 and 48 hours of hypoxia. Moreover, reoxygenation resulted in an even greater increase in VEGF and IL-6 production at normal (20 %) oxygen tension conditions for 6 hours after 24 and 48 hours of hypoxia [46]. Similarly, Amemiya et al. reported increased proliferation ratios of rat PDLSCs and an augmented expression of VEGF mRNA in hypoxic conditions [47]. Yet another population are the SHEDs which have been shown to exhibit an elevated rate of cell migration under hypoxia, superior to the migration of DPSCs [45].

11.2.2.3 Pharmacological Preconditioning to Mimic Hypoxia

These reports all indicate that hypoxic preconditioning could be an effective method to improve stem cell survival and their pro-angiogenic and chemoattractive effects. However, mimicking hypoxia using pharmacological pretreatment could represent a more convenient alternative [33]. One particular group of chemical agents that mimic the hypoxic response by inhibiting the activity of prolyl hydroxylase (PHD), a key enzyme of the oxygen sensing pathway, has gained a lot of interest [48]. Typical PHD

inhibitors include cobalt chloride ($CoCl_2$), dimethyloxalglycine (DMOG) or iron chelators such as hinokitiol, deferoxamine (DFO) or L-mimosine [49–51]. Treatment with four of these PHD inhibitors ($CoCl_2$, DMOG, DFO and L-mimosine) have been reported to increase VEGF secretion and HIF-1α expression in both dental pulp-derived cells and periodontal ligament fibroblasts [49, 52]. In a similar way, Yuan et al. reported an upregulation of HIF-1α and VEGF secretion in $CoCl_2$ treated SCAPs. Furthermore, co-culture of HUVECs and SCAPs under artificial hypoxic conditions ($CoCl_2$) resulted in an increased number of endothelial tubules, tubule lengths and branching points [27]. Trimmel et al. demonstrated that L-mimosine is able to increase the VEGF production via HIF-1α in a tooth slice organ culture model in which the dental pulp is surrounded by dentin [53]. The iron chelator hinokitiol has also been demonstrated to increase HIF-1α expression and VEGF production in dental pulp cells. According to Kim et al. CM of hinokitiol-treated pulp cells enhanced angiogenesis in vitro and in vivo. CM of hinokitiol-treated pulp cells improved the capillary network formation of HUVECs compared to control CM, thereby demonstrating an increased angiogenic potential of hinokitiol-treated dental pulp cells. Moreover, a mouse matrigel plug assay showed an increased hemoglobin content and PECAM-1 expression, confirming that hinokitiol stimulates the angiogenic potential of dental pulp cells in vivo [54]. Overall data indicates a promising future for the use of hypoxia mimicking agents and more in particular the PHD inhibitors.

Besides hypoxia mimicking agents, there is a plethora of other cytokines, growth factors and chemical agents that have been investigated for their potential to augment the angiogenic profile of stem cells. For example, bacterial lipopolysaccharides (LPS) have been shown to increase VEGF production in murine BMMSCs [55], murine and human DPSCs [56, 57] and, reported to stimulate DFSC migration [58]. In addition, there are reports confirming the LPS responsiveness of PDLSCs [59, 60] and SCAPs [61], however these studies do not mention the effects LPS treatment on VEGF secretion or the angiogenic profile of these cells. Other pretreatments such as IL-1α [62] and TNF-α [63] have been reported to increase VEGF secretion in PDLSCs and adiponectin stimulates PDLSC proliferation and wound healing [64].

11.2.3 Endothelial Differentiation Potential of DSCs

11.2.3.1 DSCs Are Able to Differentiate into Endothelial Cells In Vitro

There is a growing need for ECs in regenerative medicine. ECs can align artificial vessels and EC transplantations have been shown to restore blood flow in ischemic diseases. The main disadvantage of adult ECs is that these cells are difficult to isolate and maintain in culture. It is possible to differentiate ECs from endothelial progenitor cells (EPCs), harvested from the bone marrow. Nevertheless, these EPCs lose their potential after long-term cultivation and their capacity is not enough to provide sufficient amounts of cells needed for therapeutic applications. Therefore, numerous trials have been made to establish protocols to differentiate MSCs including DSCs towards ECs in vitro.

Successful differentiation of DSCs into an EC phenotype is generally demonstrated by the increase of typical EC surface markers such as VEGF-receptor 1 and 2, CD31, CD34 and von Willibrand factor (vWF). Other commonly examined EC characteristics include increased internalization of acetylated-low density lipoproteins (LDL), and the formation of a tubular network when plated on matrigel in vitro [65]. As these assays only show phenotypical rather than functional changes, it might be inaccurate to designate these differentiated DSCs as fully functional ECs, and therefore are referred to as EC-like cells.

Differentiation of MSCs towards EC-like cells is generally accomplished by adding the angiogenic factor VEGF to the cell medium [19, 66, 67]. For example, in one of the earliest attempts to derive EC-like cells out of BMMSCs, cells were incubated in medium containing 2 % fetal calf serum (FCS) and 50 ng/ml VEGF for 7 days. The resulting cell population showed an increase in VEGFR1, VEGFR2, VE-Cadherin, VCAM-1 and vWF expressions. Differentiated cells were also able to form a characteristic capillary-like network structures when plated onto matrigel in vitro [68]. Other proteins used to drive EC-like differentiation include bFGF and epidermal growth factor (EGF) [26, 69, 70]. Besides adding molecular mediators such as growth factors to the culture media, physical cues are also applied as a successful method to skew the stem cell fate of MSCs towards EC-like cells. Shear stress [67, 71] and seeding cells on elastic nanofiber hydrogels [72] or incubating cells in 3-dimensional matrices [73, 74] have been shown to induce EC differentiation of MSCs.

d'Aquino and coworkers were the first to report the differentiation of endotheliocytes out of DPSCs. In an attempt to achieve differentiation towards osteoblasts, DPSCs were incubated in vitro for 40 days in α-MEM supplemented with 20 % FCS. Unexpectedly, cells started to differentiate into two cytotypes: 70 % became osteogenic progenitor cells while the remaining 30 % turned into VEGFR1+/CD44+/CD54+ EC-like cells. The latter cell population also expressed vWF, CD31 and angiotensin-converting enzyme [75]. In 2009, Marchionni et al. used the aforementioned protocol of Oswald et al. [68] to derive EC-like cells from DPSCs. Flow cytometry demonstrated an induction of ICAM-1, CD34 and vWF expressions. In addition, unlike control DPSCs, differentiation medium treated cells were able to form tubes when seeded on matrigel in vitro [76]. Another study described that incubation of DPSCs with 20 ng/ml VEGF and 1 % ITS supplement (a mixture of insulin, transferrin, and sodium selenite) for 10–21 days resulted in upregulation of CD31, CD34, CD105 and CD106 in only 10 % of the cells. This study did not mention any acquired functional properties (such as tube formation and uptake of lipoproteins) for these cells. In addition, Iohara et al. were able to demonstrate the existence of a CD31-/CD146- side fraction of DPSCs which highly expressed CD34 and VEGFR2, formed extensive networks on matrigel and possessed high proliferation and migration capacities upon stimulation with VEGF in comparison to other DPSC subpopulation [26]. Furthermore, when these cells were cultured in endothelial growth medium-(EGM)-2 supplemented with 2 % porcine serum, 10 ng/ml VEGF and 10 ng/ml FGF2 for 14 days, they could uptake acetylated-LDL proteins and were able to release vWF after stimulation of histamine, typical functional features of ECs. The above described studies on the formation of EC-like cells out of DPSCs did not provide evidence on

gained endothelial cell properties on the ultrastructural level (such as presence of Weibel–Palade bodies and tight junctions) nor did they study the intracellular pathways involved in the differentiation process. In addition, in most studies only a subpopulation or a fraction of DPSCs is able to acquire EC-like features, probably due to the heterogeneity and different embryonic origin of these cells. A very recent study shows that PDLSCs are also able to differentiate into EC-like cells. Incubation with low-molecular weight fraction of enamel proteins or with the synthetic tyrosine-rich amelogin peptide for 5 weeks resulted in EC-like cell differentiation with high immunoreactivity against VE-cadherin and vWF. In addition, differentiated cells also showed the ability to uptake acetylated LDL [77].

Besides adult DPSCs, EC differentiation potential of SHEDs has also been explored. Recent reports of the research group of Nör demonstrated that upon exposure to EC growth medium (EGM-2MV) supplemented with 50 ng/ml VEGF, SHEDs were able to express VEGFR2, CD31, and VE-cadherin and organize themselves into capillary-like sprouts as cultured on matrigel in vitro. Addition of VEGFR1 or mitogen-activated protein kinase kinase-1 (MEK1) shRNA, or a chemical inhibitor of the extracellular signal-regulated kinase (ERK) pathway (U0146) to the induction medium completely inhibited the differentiation towards EC-like cells, unraveling that the VEGF/MEK1/ERK pathway is a key cascade in this process [78, 79].

To our knowledge, the endothelial differentiation of other DSC populations, such as DFSCs and SCAPs, has not yet been described and thus, it is unclear whether these cells are able to transform into EC-like cells in vitro.

11.2.3.2 EC Differentiation of DSCs In Vivo?

There is tremendous evidence that DSCs induce angiogenesis in various in vivo settings. Nevertheless, incorporation of the transplanted DSCs into the host vasculature and the acquisition of an EC-like phenotype are mostly not seen. Often, transplanted DSCs are found to be in the close proximity of the blood vessels, suggesting their role as directors of angiogenesis and supporting the current concept of MSCs including DSCs functioning as pericytes [26, 30]. For example, DPSCs mixed with matrigel and transplanted subcutaneously in nude mouse induced blood vessel growth towards the engrafted cells 10 days post-surgery. Immunohistochemistry analysis showed that DSCs were in juxtaposition to the host ECs, taking the same position as pericytes [28].

11.3 Neural Regeneration Potential of DSCs

Due to the neuroectodermal origin of dental tissue [80], the neurogenic differentiation properties of DSCs are widely explored by several research groups. The most thoroughly studied DSC subtype is the DPSCs but the neurogenic properties of SHEDs, SCAPs, DFSCs and PDLSCs have also been assessed. DSCs were differentiated towards cells with neuronal- and Schwann cell-like properties with variable success

rates, as will be discussed (Fig. 11.1b). In addition, it was shown that DSCs have the potential to integrate into host neuronal tissue or to ameliorate the disease outcome in animal models of central and peripheral nervous system disorders.

11.3.1 DSCs Differentiate into Functional Neurons In Vitro and Show Promising Effects in Animal Models of Central Nervous System Pathology

In the literature, various approaches can be found to derive neurons from DSCs such as (i) transplantation of DPSCs into injured rodent brain, (ii) chemical and cytokine induction by using a mixture of neuronal inducing agents (neuro-inductive medium), or (iii) generation of neurospheres. Although a consensus has not been established for the neuronal induction protocols, EGF and bFGF [81–84] are thought to play an important roles in driving DSCs towards a neuronal cell lineage whether or not combined with neurosphere formation [85–92]. Other neuronal differentiation strategies include epigenetic reprogramming [93], Sonic Hedgehog signaling combined with bFGF and FGF8 administration [94] or using commercially available differentiation media [95]. Maturation of the neurogenically induced DSCs is normally achieved by increasing intracellular cyclic AMP (cAMP) and protein kinase C signaling and/or by specific growth factor administration [84, 86, 91–93].

Successful differentiation into neurons is usually demonstrated by upregulation of various neuronal markers including NeuN, N-tubulin, neurofilament (NF), and microtubule associated protein 2 (MAP2), and the appearance of a neuronal cell morphology (polarized cells with one axon and multiple dendrites). In addition, physiological properties have to be validated such as the coexistence of voltage-gated sodium and potassium channels, the ability to generate action potentials and the presence of synapses, neurotransmitters, and neurotransmitter receptors allowing typical neuronal communication.

With regard to the neuronal differentiation in vitro, the most explored DSC subtype are the DPSCs. The pilot study of Arthur et al. demonstrated that after exposure to neuronal inductive conditions for 3 weeks, expression of neuron-related markers such as neural cell adhesion molecule (NCAM), neurofilament-M (NF-M) and NF-H increased while the levels of early neuronal markers such as nestin and beta-III tubulin decreased. Moreover, DPSCs acquired neuronal characteristics such as inward sodium currents [81]. As this study was not able to demonstrate outward potassium currents and the generation of an action potential, numerous efforts have been made to improve the neurogenic differentiation outcome of DPSCs. In addition to a shift in marker expression towards neuronal-related proteins, the neuronally matured DPSCs of these studies acquired inward sodium currents and outward potassium currents that could be reversibly blocked by tetrodotoxin and tetraethylammonium, respectively, showing the functional characteristic of differentiated cells [86, 93]. The study conducted by Gervois and co-workers was able to show that neurogenically differentiated cells were able to fire a single action potential, suggesting successful, yet incomplete neuronal

maturation as only a single action potential and not a train of action potentials was observed [86]. Other reports describing the neurogenic differentiation potential of DPSCs were limited to immunohistochemical, morphological and gene expression analysis of the differentiated DPSCs, showing augmented expressions of MAP2, beta-III tubulin and GFAP [88, 89, 95]. Similar to human DPSCs, attempts were made to differentiate SHEDs [84, 87, 92, 94], PDLSCs [82, 83, 90, 95, 96], SCAPs [85, 95] and DFSCs [87, 91] towards neuronal cells. Neurogenically differentiated SHEDs expressed Tau, beta-III tubulin, NeuN, NCAM, GFAP and tyroxin hydroxylase (TH) and MAP2. SHEDs were also differentiated into peripheral sensory neurons by Jarmalaviciute and colleagues as demonstrated by brain-specific homeobox/POU domain protein 3A (Brn3a) and peripherin expressions (2013). However, no functional assessments were performed. PDLSCs were more thoroughly investigated and found to upregulate beta-III tubulin, GFAP, synaptophysin, MAP2, MAP1b, NeuN after neuronal differentiation, and were also reported to express specific neurotransmitter markers such as choline acetyltransferase, gamma-aminobutyric acid (GABA), GABA-transporter 1, glutamate carboxylase 65/67, the oligodendrocyte precursor markers O4 and neural/glial antigen 2. Both SCAPs and DFSCs subjected to neuroinductive media were found to express nestin, NF-M, MAP2, beta-III tubulin and GFAP. A study performed by Zou et al. showed that SCAPs could be reprogrammed towards induced pluripotent stem cells that could subsequently be differentiated towards neural-like cells expressing nestin, beta-III tubulin, NF-M, neuron specific enolase, NeuN, 2′, 3′-cyclic nucleotide-3′- phosphodiesterase (CNPase), and metabotropic- (GRM1) and ionotropic (NR1-1) glutamate receptors [97]. The results of these studies are limited to alterations in gene expression profile, neuronal marker expression and morphology, and no electrophysiological studies have been performed.

In vivo studies of transplanted DSCs in animal models of central nervous system pathology are scarce and were mainly performed with DPSCs. The results of these studies indicated that DPSCs acquired a neuronal morphology, expressed neuronal markers such as NF-M and attracted trigeminal axons after transplantation into chicken embryos [81, 98]. Moreover, the study of Kiraly and colleagues demonstrated that when neurogenically pre-differentiated DPSCs were injected into the cerebrospinal fluid of neonatal rats, DPSCs migrated towards and integrated into the host brain while retaining their functional characteristics [93, 99]. Not only did DPSCs incorporate into the host brain after transplantation, they also displayed beneficial effects in various in vivo models of neurological dysfunction including ischemic stroke [100], hypoxic-ischemic encephalopathy [101] and spinal cord injury (SCI) [102, 103]. Transplantation studies of DPSCs in animal models of Parkinson's and Alzheimer's diseases have not been performed to date. However, Apel et al. observed possible positive contributions of DPSCs in in vitro models of both neurological disorders [104]. SHEDs were used successfully in in vivo models of SCI [105] and Parkinson's disease [92], ameliorating the disease outcome. PDLSCs were found to differentiate towards neuronal cells after engraftment into the host brain [96]. DFSCs, seeded on aligned electrospun poly(ε-caprolactone)/poly-DL-lactide-co-glycolide (PCL/PLGA) fibers, were used in a spinal cord injury model. Although no significant functional improvement was observed following transplantation,

it was shown that the PCL/PLGA fibers supported nerve fiber growth and the seeded DFSCs expressed the oligodendrocyte marker Olig2 [106] To date, SCAPs have not yet been directly used in in vivo models for neurological disorders. However, Zou et al. established a SCAP-derived induced pluripotent stem cell culture that can be differentiated in vitro into neural-like cells for potential in vivo applications [97]. The proposed mechanisms of disease amelioration by the transplanted cells included integration of the transplanted cells in the host brain and/or stimulating the proliferation and differentiation of endogenous neural stem cells.

11.3.2 DSCs Differentiate into Schwann Cell–Like Cells and Actively Support Regeneration After Peripheral Nerve Injury

In addition to the neurogenic differentiation potential of DSCs, the ability of DSCs to transform into Schwann-like cells has also been evaluated. Martens et al. were able to successfully differentiate DPSCs towards Schwann cells based on a multistep process using beta-mercaptoethanol, retinoic acid, forskolin, bFGF, neuregulin-1 and PDGF-AA. They showed that Schwann cells derived from DPSCs expressed the glial markers including p75, laminin, CD104 and GFAP but not nestin, and were able to myelinate and guide neurites of dorsal root ganglia in vitro [107]. Additional attempts were made to differentiate PDLSCs derived from beagle dogs [108] and SHEDs [84] towards Schwann cell-like cells. PDLSCs were differentiated using four different protocols. All procedures had a comparable outcome, and the growth factors and supplements that were used were similar to those reported by Martens et al., with the exception of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Schwann cells derived from PDLSCs expressed GFAP and S100, as well as nestin, which contradicted the findings of Martens et al. Remarkably, with the exception of NGF and BDNF, these supplements were not used to differentiate SHEDs. These cells were differentiated towards neuronal/glial cells after exposure to bFGF, EGF, BDNF, NGF, GDNF and cAMP, and were cultured in medium supporting neuronal growth instead of glial growth. Differentiated SHEDs expressed myelin basic protein, early growth response protein 1 (ERG-1), ERG-2 and apolipoprotein E. The results of studies using SHEDs and PDLSCs have been limited to alterations in gene and/or marker expression, and morphology as no functional studies such as remyelination and neurite outgrowth assessments have been performed.

In vivo studies that apply DSCs for peripheral nerve injury have mentioned only artificial tubes seeded with DPSCs in animal models of facial nerve injury. It was shown that rat DPSCs promoted remyelination, blood vessel formation and normal nerve regeneration when applied in combination with silicon or PLGA tubes, in which positive contributions were attributed to the paracrine factors secreted by the DPSCs [109, 110]. Subsequently, same group showed that a silicon tube containing DPSCs impregnated in type I collagen gel improved the electrophysiological and functional characteristics of the facial nerve, comparable with a nerve autograft [111].

11.3.3 Preconditioning Stem Cells for Neuroregeneration

The secretome of DSCs is not only rich in angiogenic factors, but also contains numerous neurotrophic proteins. These factors regulate the growth, differentiation and survival of developing neurons and the maintenance of mature neurons. In addition, they are able to enhance axon and neurite outgrowth in both the central and peripheral nervous systems after injuries. Examples of such growth factors found in DSCs are BDNF, NGF and glial cell-derived neurotrophic factor (GDNF) [112]. In accordance to the angiogenic actions of DSCs, it is possible to further improve their neurotrophic actions as will discussed in the Sects. 11.3.3.1 and 11.3.3.2 below.

11.3.3.1 Genetic Modification

Enhancing trophic activities of cells by overexpression of related genes could be a valuable approach to maximize the efficacy of cell therapies in neurological diseases [113]. Therapeutic target genes include angiogenic factors such as VEGF, HGF and PIGF or neurotrophic factors such as BDNF, NGF and GDNF. These types of studies most frequently use MSCs and neural stem cells but so far no data are available of genetic modification of DSCs for the purpose of neuroprotection or neuroregeneration [114]. To date, there is no clinical experience using genetically modified stem cell therapy for neuroregeneration, mainly because of the difficulty of proving safety and efficacy of transplanting genetically modified cells into patients [113, 114].

11.3.3.2 Preconditioning

To date, a variety of preconditioning triggers have been tested in stem cells and stem cell-derived progenitors for the treatment of ischemic brain disorders. Hypoxia preconditioning and hypoxia mimicking agents have gained a lot of attention in the field of cell-based therapies for neurological disorders [114]. This is not surprising since HIF-1α also plays a key role in neuroprotection via its downstream targets VEGF and erythropoietin (EPO), which not only stimulate angiogenesis but also neurogenesis, which are both vital processes in the recovery of injured brains [115]. Several reports have already demonstrated that stroke animals receiving hypoxia-primed cells (such as BMMSCs and embryonic stem cells) have displayed better functional recovery compared to animals receiving non-treated cells [116–118]. Furthermore, hypoxia preconditioning has been shown to upregulate HIF-1α, BDNF, GDNF and VEGF expressions [117, 119], and increase migration [119] and homing ability compared to normoxic cells [117, 118].

Despite the promising results of transplanting hypoxia preconditioned stem/progenitor cells into ischemic brains, no data are available on the use of primed stem cells from dental origins. However, since HIF-1α and its downstream targets are also key players in the recovery of ischemic brain, other preconditioning approaches as mentioned in Sect. 11.2.2 could also be applied with regard to ischemic brain conditions and require thus further investigations.

11.4 DSCs in Stroke

Stroke is a severe condition which is defined by loss of brain function due to interrupted or severely impaired blood flow to the affected area in the brain. It is a major cause of permanent disability and ranked as the second leading cause of death worldwide [120]. Ischemic stroke is the most common type, covering 70% of all stroke cases. Since the incidence of stroke is the highest in people over age 60, the social and economic burden caused by stroke keeps on rising, given the ageing of the population. During stroke, disturbed blood supply leads to oxygen and glucose deprivation which triggers a cascade of deleterious events, including excitotoxicity, accumulation of toxic metabolites and mitochondrial failure. Consequently, neuronal cell death occurs at the affected site, resulting in severe neurological damage. Clinically, this is translated into disabilities such as paralysis, sensory disturbances, aphasia, memory loss, urinary incontinence, cognitive impairment and emotional instability. Limited stroke-induced endogenous neurogenesis can be observed in patients but not to an extent to acquire adequate functional recovery [121]. Furthermore, modern medicine is unable to sufficiently improve the functional outcome after stroke. Currently, recombinant tissue plasminogen activator is the only FDA-approved pharmacological treatment. It has to be used within 4 to 5 hours after the ischemic insult, thereby limiting its use to only 2–4% of the patients [122]. These indications highlight the urgent need for an improved treatment option for stroke patients.

11.4.1 Stem Cell Intervention as a Promising Therapy in Stroke

Stem cell-based therapy is considered to be a promising approach to minimize neurological damage and enhance functional recovery after stroke. Several preclinical studies comprising various cell types show beneficial effects on the functional outcome in animal models of stroke [123]. Furthermore, a systematic review of the first clinical trials in stroke patients suggests stem cell therapy to be feasible and effective [124]. However, further research is necessary to evaluate the clinical efficacy. Although encouraging preclinical and clinical cell based studies have been reported, still many questions concerning the optimal stem cell source, mechanisms of action, fate of the stem cells and optimal treatment protocol remain to be elucidated.

11.4.2 Human DSCs Are Ideal Candidates for Stem Cell Therapy in Stroke

In order to develop an effective cell based therapy in stroke, it is essential to exploit the optimal stem cell source. The first clinical trials primarily used BMMSCs and bone marrow derived mononuclear cells (BMMNCs). However, DSCs are proposed to be

more promising stem cell source as they can be used autologously, and can be easily isolated with low donor site morbidity and are associated with little ethical concern [125, 126]. Moreover, certain subtypes such as DPSCs seem to hold great promise for cell replacement approaches, since these neural crest-derived stem cells have neurogenic differentiation capacity and have been shown to integrate into the brain circuitry after transplantation [81, 86, 99]. Furthermore, human DPSCs have a rich secretome including BDNF, NGF, neurotrophin-3 (NT-3) and GDNF, which are considered as hallmark neurotrophic and neuroregulatory factors [112]. A recent study showed that the secretion of these factors is significantly higher in human DPSCs compared with BMMSCs [127]. When the cytoprotective effects of human DPSCs and BMMSCs were compared in an in vitro ischemic model for astrocytes, both human DPSCs and CM of DPSCs were found to have superior effects [128]. Additionally, enhancement of sensorimotor deficits after transplantation of human DPSCs in a rat stroke model has been demonstrated, and found to be mainly caused by paracrine effects rather than neural cell replacement [100]. Taken together, human DPSCs are proposed to be ideal candidates for stem cell therapy in the treatment of stroke. However, further research is strictly necessary to gain better insight into the cell biology of human DPSCs and their beneficial effects on the functional outcome after stroke.

11.4.3 Potential Beneficial Mechanisms of Action and Effects of the DSC Secretome in Stroke

Following stem cell transplantation, both paracrine effects and cell replacement can be responsible for improved functional outcome after stroke. Initially, cell replacement was believed to be the predominant mechanism. However, there is increasing evidence that transplanted human DSCs use secretome-mediated mechanisms, including neuroprotection, neuroregeneration, immune-regulation to improve disease outcome, as already demonstrated in spinal cord injury and other neurodegenerative diseases [102, 104, 129, 130]. SHEDs have been reported to provide protection against both heat stroke and cerebral ischaemia [131, 132]. These studies demonstrated a positive effect on migration and differentiation of endogenous neural progenitor cells as well as inductive effects on vasculogenesis even in case of exposure to CM, clearly demonstrating the paracrine effects of these stem cells. Even in neonatal brain, SHEDs have been found to support a neuroprotective microenvironment during perinatal hypoxia/ischemia [133].

11.5 Conclusions

Taken together, DSCs are proposed to be ideal candidates for stem cell based therapy in treatment of neurological disorders or diseases associated with limited angiogenesis. Their neural crest origin and related possible epigenetic influences make them extremely suitable to be used in neuroregeneration approaches. The main mechanism

of action is suggested to be by paracrine effects of the human DSC secretome rather than true cell replacement. As this literature overview shows, neuronal and endothelial differentiation of DSCs is possible under the right conditions in vitro but the processes are still, in most cases, very insufficient and time consuming. Preconditioning of DSCs by hypoxia or certain chemical agents has been shown to boost the angiogenic effects of DSCs and the effect of this pretreatment on the neurotrophic actions of these cells warrants further examination. Nevertheless, pharmacological pretreatment might become a valuable strategy to maximize the clinical potential of DSCs.

The limited studies available on the effect of human DSCs on stroke outcome show promising results as all studies report improved functional outcome and/or superior effects of DSCs compared to other stem cell sources. Concerning clinical application, private biobanking of dental tissue-derived stem cells is already established in a variety of countries including the United States, the United Kingdom and Switzerland. In this context, the effects of cryopreservation on DSCs has already been studied in detail and no adverse effects of this long-term storage on cell biology have been reported. However, further research is necessary to gain better insight into the cell biology of human DSCs and optimize their mode of administration before these fascinating cells become a daily practice in the clinic.

References

1. Carmeliet P (2000) Mechanisms of angiogenesis and arteriogenesis. Nat Med 6(4):389–395. doi:10.1038/74651
2. Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285(21):1182–1186. doi:10.1056/NEJM197111182852108
3. Ribatti D, Crivellato E (2012) "Sprouting angiogenesis", a reappraisal. Dev Biol 372(2):157–165. doi:10.1016/j.ydbio.2012.09.018
4. Carmeliet P, Jain RK (2011) Molecular mechanisms and clinical applications of angiogenesis. Nature 473(7347):298–307. doi:10.1038/nature10144
5. Bronckaers A, Hilkens P, Martens W, Gervois P, Ratajczak J, Struys T, Lambrichts I (2014) Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis. Pharmacol Ther 143(2):181–196. doi:10.1016/j.pharmthera.2014.02.013
6. Distler JH, Hirth A, Kurowska-Stolarska M, Gay RE, Gay S, Distler O (2003) Angiogenic and angiostatic factors in the molecular control of angiogenesis. Q J Nucl Med 47(3):149–161
7. Bhadada SV, Goyal BR, Patel MM (2011) Angiogenic targets for potential disorders. Fundam Clin Pharmacol 25(1):29–47. doi:10.1111/j.1472-8206.2010.00814.x
8. Ferrara N, Kerbel RS (2005) Angiogenesis as a therapeutic target. Nature 438(7070):967–974. doi:10.1038/nature04483
9. Staudacher DL, Flugelman MY (2006) Cell and gene therapies in cardiovascular disease with special focus on the no option patient. Curr Gene Ther 6(6):609–623
10. Giordano A, Galderisi U, Marino IR (2007) From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. J Cell Physiol 211(1):27–35. doi:10.1002/jcp.20959
11. Psaltis PJ, Zannettino AC, Worthley SG, Gronthos S (2008) Concise review: mesenchymal stromal cells: potential for cardiovascular repair. Stem Cells 26(9):2201–2210. doi:10.1634/stemcells.2008-0428
12. Sieveking DP, Ng MK (2009) Cell therapies for therapeutic angiogenesis: back to the bench. Vasc Med 14(2):153–166

13. Aranha AM, Zhang Z, Neiva KG, Costa CA, Hebling J, Nor JE (2010) Hypoxia enhances the angiogenic potential of human dental pulp cells. J Endod 36(10):1633–1637. doi:10.1016/j.joen.2010.05.013
14. Matsushita K, Motani R, Sakuta T, Yamaguchi N, Koga T, Matsuo K, Nagaoka S, Abeyama K, Maruyama I, Torii M (2000) The role of vascular endothelial growth factor in human dental pulp cells: induction of chemotaxis, proliferation, and differentiation and activation of the AP-1-dependent signaling pathway. J Dent Res 79(8):1596–1603
15. Nakashima M, Iohara K, Sugiyama M (2009) Human dental pulp stem cells with highly angiogenic and neurogenic potential for possible use in pulp regeneration. Cytokine Growth Factor Rev 20(5–6):435–440
16. Tran-Hung L, Laurent P, Camps J, About I (2008) Quantification of angiogenic growth factors released by human dental cells after injury. Arch Oral Biol 53(1):9–13. doi:10.1016/j.archoralbio.2007.07.001
17. Tran-Hung L, Mathieu S, About I (2006) Role of human pulp fibroblasts in angiogenesis. J Dent Res 85(9):819–823
18. Hilkens P, Fanton Y, Martens W, Gervois P, Struys T, Politis C, Lambrichts I, Bronckaers A (2014) Pro-angiogenic impact of dental stem cells in vitro and in vivo. Stem Cell Res 12(3):778–790. doi:10.1016/j.scr.2014.03.008
19. Bronckaers A, Hilkens P, Fanton Y, Struys T, Gervois P, Politis C, Martens W, Lambrichts I (2013) Angiogenic properties of human dental pulp stem cells. PLoS One 8(8), e71104. doi:10.1371/journal.pone.0071104
20. Guo L, Li J, Qiao X, Yu M, Tang W, Wang H, Guo W, Tian W (2013) Comparison of odontogenic differentiation of human dental follicle cells and human dental papilla cells. PLoS One 8(4), e62332. doi:10.1371/journal.pone.0062332
21. Huang G, Yamaza T, Shea LD, Djouad F, Kuhn NZ, Tuan R, Shi S (2010) Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng Part A 16(2):605–615
22. Vanacker J, Viswanath A, De Berdt P, Everard A, Cani PD, Bouzin C, Feron O, Diogenes A, Leprince JG, des Rieux A (2014) Hypoxia modulates the differentiation potential of stem cells of the apical papilla. J Endod 40(9):1410–1418. doi:10.1016/j.joen.2014.04.008
23. Huang GT, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. J Dent Res 88(9):792–806
24. Osman A, Gnanasegaran N, Govindasamy V, Kathivaloo P, Wen AS, Musa S, Abu Kasim NH (2014) Basal expression of growth-factor-associated genes in periodontal ligament stem cells reveals multiple distinctive pathways. Int Endod J 47(7):639–651. doi:10.1111/iej.12200
25. Yeasmin S, Ceccarelli J, Vigen M, Carrion B, Putnam AJ, Tarle SA, Kaigler D (2014) Stem cells derived from tooth periodontal ligament enhance functional angiogenesis by endothelial cells. Tissue Eng Part A 20(7–8):1188–1196. doi:10.1089/ten.TEA.2013.0512
26. Iohara K, Zheng L, Wake H, Ito M, Nabekura J, Wakita H, Nakamura H, Into T, Matsushita K, Nakashima M (2008) A novel stem cell source for vasculogenesis in ischemia: subfraction of side population cells from dental pulp. Stem Cells 26(9):2408–2418
27. Yuan C, Wang P, Zhu L, Dissanayaka WL, Green DW, Tong EH, Jin L, Zhang C (2014) Coculture of stem cells from apical papilla and human umbilical vein endothelial cell under hypoxia increases the formation of three-dimensional vessel-like structures in vitro. Tissue Eng Part A. doi:10.1089/ten.TEA.2014.0058
28. Janebodin K, Zeng Y, Buranaphatthana W, Ieronimakis N, Reyes M (2013) VEGFR2-dependent angiogenic capacity of pericyte-like dental pulp stem cells. J Dent Res 92(6):524–531. doi:10.1177/0022034513485599
29. Dissanayaka WL, Hargreaves KM, Jin L, Samaranayake LP, Zhang C (2014) The interplay of dental pulp stem cells and endothelial cells in an injectable peptide hydrogel on angiogenesis and pulp regeneration in vivo. Tissue Eng Part A. doi:10.1089/ten.TEA.2014.0154
30. Gandia C, Arminan A, Garcia-Verdugo JM, Lledo E, Ruiz A, Minana MD, Sanchez-Torrijos J, Paya R, Mirabet V, Carbonell-Uberos F, Llop M, Montero JA, Sepulveda P (2008) Human

dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. Stem Cells 26(3):638–645
31. Ishizaka R, Hayashi Y, Iohara K, Sugiyama M, Murakami M, Yamamoto T, Fukuta O, Nakashima M (2013) Stimulation of angiogenesis, neurogenesis and regeneration by side population cells from dental pulp. Biomaterials 34(8):1888–1897. doi:10.1016/j.biomaterials.2012.10.045
32. Sugiyama M, Iohara K, Wakita H, Hattori H, Ueda M, Matsushita K, Nakashima M (2011) Dental pulp-derived CD31(−)/CD146(−) side population stem/progenitor cells enhance recovery of focal cerebral ischemia in rats. Tissue Eng Part A 17(9–10):1303–1311. doi:10.1089/ten.TEA.2010.0306
33. Muscari C, Giordano E, Bonafe F, Govoni M, Pasini A, Guarnieri C (2013) Priming adult stem cells by hypoxic pretreatments for applications in regenerative medicine. J Biomed Sci 20:63. doi:10.1186/1423-0127-20-63
34. Baraniak PR, McDevitt TC (2010) Stem cell paracrine actions and tissue regeneration. Regen Med 5(1):121–143. doi:10.2217/rme.09.74
35. Gnecchi M, He H, Noiseux N, Liang OD, Zhang L, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS, Dzau VJ (2006) Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J 20(6):661–669. doi:10.1096/fj.05-5211com
36. Li W, Ma N, Ong LL, Nesselmann C, Klopsch C, Ladilov Y, Furlani D, Piechaczek C, Moebius JM, Lutzow K, Lendlein A, Stamm C, Li RK, Steinhoff G (2007) Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. Stem Cells 25(8):2118–2127. doi:10.1634/stemcells.2006-0771
37. Noiseux N, Gnecchi M, Lopez-Ilasaca M, Zhang L, Solomon SD, Deb A, Dzau VJ, Pratt RE (2006) Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation. Mol Ther 14(6):840–850. doi:10.1016/j.ymthe.2006.05.016
38. Phillips MI, Tang YL (2008) Genetic modification of stem cells for transplantation. Adv Drug Deliv Rev 60(2):160–172. doi:10.1016/j.addr.2007.08.035
39. Kubo M, Li TS, Suzuki R, Shirasawa B, Morikage N, Ohshima M, Qin SL, Hamano K (2008) Hypoxic preconditioning increases survival and angiogenic potency of peripheral blood mononuclear cells via oxidative stress resistance. Am J Physiol Heart Circ Physiol 294(2):H590–H595. doi:10.1152/ajpheart.00856.2007
40. Potier E, Ferreira E, Andriamanalijaona R, Pujol JP, Oudina K, Logeart-Avramoglou D, Petite H (2007) Hypoxia affects mesenchymal stromal cell osteogenic differentiation and angiogenic factor expression. Bone 40(4):1078–1087. doi:10.1016/j.bone.2006.11.024
41. Rosova I, Dao M, Capoccia B, Link D, Nolta JA (2008) Hypoxic preconditioning results in increased motility and improved therapeutic potential of human mesenchymal stem cells. Stem Cells 26(8):2173–2182. doi:10.1634/stemcells.2007-1104
42. Iida K, Takeda-Kawaguchi T, Tezuka Y, Kunisada T, Shibata T, Tezuka K (2010) Hypoxia enhances colony formation and proliferation but inhibits differentiation of human dental pulp cells. Arch Oral Biol 55(9):648–654. doi:10.1016/j.archoralbio.2010.06.005
43. Sakdee JB, White RR, Pagonis TC, Hauschka PV (2009) Hypoxia-amplified proliferation of human dental pulp cells. J Endod 35(6):818–823
44. Agata H, Kagami H, Watanabe N, Ueda M (2008) Effect of ischemic culture conditions on the survival and differentiation of porcine dental pulp-derived cells. Differentiation 76(9):981–993. doi:10.1111/j.1432-0436.2008.00282.x
45. Kanafi MM, Ramesh A, Gupta PK, Bhonde RR (2013) Influence of hypoxia, high glucose, and low serum on the growth kinetics of mesenchymal stem cells from deciduous and permanent teeth. Cells Tissues Organs 198(3):198–208. doi:10.1159/000354901
46. Motohira H, Hayashi J, Tatsumi J, Tajima M, Sakagami H, Shin K (2007) Hypoxia and reoxygenation augment bone-resorbing factor production from human periodontal ligament cells. J Periodontol 78(9):1803–1809. doi:10.1902/jop.2007.060519
47. Amemiya H, Matsuzaka K, Kokubu E, Ohta S, Inoue T (2008) Cellular responses of rat periodontal ligament cells under hypoxia and re-oxygenation conditions in vitro. J Periodontal Res 43(3):322–327. doi:10.1111/j.1600-0765.2007.01032.x

48. Osathanon T, Vivatbutsiri P, Sukarawan W, Sriarj W, Pavasant P, Sooampon S (2015) Cobalt chloride supplementation induces stem-cell marker expression and inhibits osteoblastic differentiation in human periodontal ligament cells. Arch Oral Biol 60(1):29–36. doi:10.1016/j.archoralbio.2014.08.018
49. Agis H, Watzek G, Gruber R (2012) Prolyl hydroxylase inhibitors increase the production of vascular endothelial growth factor by periodontal fibroblasts. J Periodontal Res 47(2):165–173. doi:10.1111/j.1600-0765.2011.01415.x
50. Asikainen TM, White CW (2007) HIF stabilizing agents: shotgun or scalpel? Am J Physiol Lung Cell Mol Physiol 293(3):L555–L556. doi:10.1152/ajplung.00251.2007
51. Lee MJ, Kim JW, Yang EG (2010) Hinokitiol activates the hypoxia-inducible factor (HIF) pathway through inhibition of HIF hydroxylases. Biochem Biophys Res Commun 396(2):370–375. doi:10.1016/j.bbrc.2010.04.099
52. Muller HD, Cvikl B, Gruber R, Watzek G, Agis H (2012) Prolyl hydroxylase inhibitors increase the production of vascular endothelial growth factor in dental pulp-derived cells. J Endod 38(11):1498–1503. doi:10.1016/j.joen.2012.08.003
53. Trimmel K, Cvikl B, Muller HD, Nurnberger S, Gruber R, Moritz A, Agis H (2015) L-mimosine increases the production of vascular endothelial growth factor in human tooth slice organ culture model. Int Endod J 48(3):252–260. doi:10.1111/iej.12307
54. Kim MK, Park HJ, Kim YD, Ryu MH, Takata T, Bae SK, Bae MK (2014) Hinokitiol increases the angiogenic potential of dental pulp cells through ERK and p38MAPK activation and hypoxia-inducible factor-1alpha (HIF-1alpha) upregulation. Arch Oral Biol 59(2):102–110. doi:10.1016/j.archoralbio.2013.10.009
55. Botero TM, Mantellini MG, Song W, Hanks CT, Nor JE (2003) Effect of lipopolysaccharides on vascular endothelial growth factor expression in mouse pulp cells and macrophages. Eur J Oral Sci 111(3):228–234
56. Botero TM, Son JS, Vodopyanov D, Hasegawa M, Shelburne CE, Nor JE (2010) MAPK signaling is required for LPS-induced VEGF in pulp stem cells. J Dent Res 89(3):264–269. doi:10.1177/0022034509357556
57. Matsushita K, Motani R, Sakuta T, Nagaoka S, Matsuyama T, Abeyama K, Maruyama I, Takada H, Torii M (1999) Lipopolysaccharide enhances the production of vascular endothelial growth factor by human pulp cells in culture. Infect Immun 67(4):1633–1639
58. Chatzivasileiou K, Lux CA, Steinhoff G, Lang H (2013) Dental follicle progenitor cells responses to Porphyromonas gingivalis LPS. J Cell Mol Med 17(6):766–773. doi:10.1111/jcmm.12058
59. Wada N, Maeda H, Yoshimine Y, Akamine A (2004) Lipopolysaccharide stimulates expression of osteoprotegerin and receptor activator of NF-kappa B ligand in periodontal ligament fibroblasts through the induction of interleukin-1 beta and tumor necrosis factor-alpha. Bone 35(3):629–635. doi:10.1016/j.bone.2004.04.023
60. Yamaji Y, Kubota T, Sasaguri K, Sato S, Suzuki Y, Kumada H, Umemoto T (1995) Inflammatory cytokine gene expression in human periodontal ligament fibroblasts stimulated with bacterial lipopolysaccharides. Infect Immun 63(9):3576–3581
61. Zhang J, Zhang Y, Lv H, Yu Q, Zhou Z, Zhu Q, Wang Z, Cooper PR, Smith AJ, Niu Z, He W (2013) Human stem cells from the apical papilla response to bacterial lipopolysaccharide exposure and anti-inflammatory effects of nuclear factor I C. J Endod 39(11):1416–1422. doi:10.1016/j.joen.2013.07.018
62. Bando Y, Noguchi K, Kobayashi H, Yoshida N, Ishikawa I, Izumi Y (2009) Cyclooxygenase-2-derived prostaglandin E2 is involved in vascular endothelial growth factor production in interleukin-1alpha-stimulated human periodontal ligament cells. J Periodontal Res 44(3):395–401. doi:10.1111/j.1600-0765.2008.01118.x
63. Oyama T, Sakuta T, Matsushita K, Maruyama I, Nagaoka S, Torii M (2000) Effects of roxithromycin on tumor necrosis factor-alpha-induced vascular endothelial growth factor expression in human periodontal ligament cells in culture. J Periodontol 71(10):1546–1553. doi:10.1902/jop.2000.71.10.1546

64. Nokhbehsaim M, Keser S, Nogueira AV, Cirelli JA, Jepsen S, Jager A, Eick S, Deschner J (2014) Beneficial effects of adiponectin on periodontal ligament cells under normal and regenerative conditions. J Diabetes Res 2014:796565. doi:10.1155/2014/796565
65. Voyta JC, Via DP, Butterfield CE, Zetter BR (1984) Identification and isolation of endothelial cells based on their increased uptake of acetylated-low density lipoprotein. J Cell Biol 99(6):2034–2040
66. Al-Khaldi A, Eliopoulos N, Martineau D, Lejeune L, Lachapelle K, Galipeau J (2003) Postnatal bone marrow stromal cells elicit a potent VEGF-dependent neoangiogenic response in vivo. Gene Ther 10(8):621–629. doi:10.1038/sj.gt.3301934
67. Bai K, Huang Y, Jia X, Fan Y, Wang W (2010) Endothelium oriented differentiation of bone marrow mesenchymal stem cells under chemical and mechanical stimulations. J Biomech 43(6):1176–1181. doi:10.1016/j.jbiomech.2009.11.030
68. Oswald J, Boxberger S, Jorgensen B, Feldmann S, Ehninger G, Bornhauser M, Werner C (2004) Mesenchymal stem cells can be differentiated into endothelial cells in vitro. Stem Cells 22(3):377–384. doi:10.1634/stemcells.22-3-377
69. Chen MY, Lie PC, Li ZL, Wei X (2009) Endothelial differentiation of Wharton's jelly-derived mesenchymal stem cells in comparison with bone marrow-derived mesenchymal stem cells. Exp Hematol 37(5):629–640
70. Chung N, Jee BK, Chae SW, Jeon YW, Lee KH, Rha HK (2009) HOX gene analysis of endothelial cell differentiation in human bone marrow-derived mesenchymal stem cells. Mol Biol Rep 36(2):227–235. doi:10.1007/s11033-007-9171-6
71. Fischer LJ, McIlhenny S, Tulenko T, Golesorkhi N, Zhang P, Larson R, Lombardi J, Shapiro I, DiMuzio PJ (2009) Endothelial differentiation of adipose-derived stem cells: effects of endothelial cell growth supplement and shear force. J Surg Res 152(1):157–166. doi:10.1016/j.jss.2008.06.029
72. Wingate K, Bonani W, Tan Y, Bryant SJ, Tan W (2012) Compressive elasticity of three-dimensional nanofiber matrix directs mesenchymal stem cell differentiation to vascular cells with endothelial or smooth muscle cell markers. Acta Biomater 8(4):1440–1449. doi:10.1016/j.actbio.2011.12.032
73. Valarmathi MT, Davis JM, Yost MJ, Goodwin RL, Potts JD (2009) A three-dimensional model of vasculogenesis. Biomaterials 30(6):1098–1112. doi:10.1016/j.biomaterials.2008.10.044
74. Zhang G, Drinnan CT, Geuss LR, Suggs LJ (2010) Vascular differentiation of bone marrow stem cells is directed by a tunable three-dimensional matrix. Acta Biomater 6(9):3395–3403. doi:10.1016/j.actbio.2010.03.019
75. d'Aquino R, Graziano A, Sampaolesi M, Laino G, Pirozzi G, De Rosa A, Papaccio G (2007) Human postnatal dental pulp cells co-differentiate into osteoblasts and endotheliocytes: a pivotal synergy leading to adult bone tissue formation. Cell Death Differ 14(6):1162–1171
76. Marchionni C, Bonsi L, Alviano F, Lanzoni G, Di Tullio A, Costa R, Montanari M, Tazzari PL, Ricci F, Pasquinelli G, Orrico C, Grossi A, Prati C, Bagnara GP (2009) Angiogenic potential of human dental pulp stromal (stem) cells. Int J Immunopathol Pharmacol 22(3):699–706
77. Amin HD, Olsen I, Knowles J, Dard M, Donos N (2014) A tyrosine-rich amelogenin peptide promotes neovasculogenesis in vitro and ex vivo. Acta Biomater 10(5):1930–1939. doi:10.1016/j.actbio.2013.11.027
78. Bento LW, Zhang Z, Imai A, Nor F, Dong Z, Shi S, Araujo FB, Nor JE (2013) Endothelial differentiation of SHED requires MEK1/ERK signaling. J Dent Res 92(1):51–57. doi:10.1177/0022034512466263
79. Sakai VT, Zhang Z, Dong Z, Neiva KG, Machado MA, Shi S, Santos CF, Nor JE (2010) SHED differentiate into functional odontoblasts and endothelium. J Dent Res 89(8):791–796. doi:10.1177/0022034510368647
80. Miletich I, Sharpe PT (2004) Neural crest contribution to mammalian tooth formation. Birth Defects Res C Embryo Today 72(2):200–212. doi:10.1002/bdrc.20012
81. Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S (2008) Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. Stem Cells 26(7):1787–1795

82. Coura GS, Garcez RC, de Aguiar CB, Alvarez-Silva M, Magini RS, Trentin AG (2008) Human periodontal ligament: a niche of neural crest stem cells. J Periodontal Res 43(5):531–536. doi:10.1111/j.1600-0765.2007.01065.x
83. Fortino VR, Chen RS, Pelaez D, Cheung HS (2014) Neurogenesis of neural crest-derived periodontal ligament stem cells by EGF and bFGF. J Cell Physiol 229(4):479–488. doi:10.1002/jcp.24468
84. Jarmalaviciute A, Tunaitis V, Strainiene E, Aldonyte R, Ramanavicius A, Venalis A, Magnusson KE, Pivoriunas A (2013) A new experimental model for neuronal and glial differentiation using stem cells derived from human exfoliated deciduous teeth. J Mol Neurosci. doi:10.1007/s12031-013-0046-0
85. Abe S, Hamada K, Miura M, Yamaguchi S (2012) Neural crest stem cell property of apical pulp cells derived from human developing tooth. Cell Biol Int 36(10):927–936. doi:10.1042/CBI20110506
86. Gervois P, Struys T, Hilkens P, Bronckaers A, Ratajczak J, Politis C, Brone B, Lambrichts I, Martens W (2014) Neurogenic maturation of human dental pulp stem cells following neurosphere generation induces morphological and electrophysiological characteristics of functional neurons. Stem Cells Dev. doi:10.1089/scd.2014.0117
87. Morsczeck C, Vollner F, Saugspier M, Brandl C, Reichert TE, Driemel O, Schmalz G (2010) Comparison of human dental follicle cells (DFCs) and stem cells from human exfoliated deciduous teeth (SHED) after neural differentiation in vitro. Clin Oral Investig 14(4):433–440. doi:10.1007/s00784-009-0310-4
88. Osathanon T, Nowwarote N, Pavasant P (2011) Basic fibroblast growth factor inhibits mineralization but induces neuronal differentiation by human dental pulp stem cells through a FGFR and PLCgamma signaling pathway. J Cell Biochem 112(7):1807–1816. doi:10.1002/jcb.23097
89. Osathanon T, Sawangmake C, Nowwarote N, Pavasant P (2014) Neurogenic differentiation of human dental pulp stem cells using different induction protocols. Oral Dis 20(4):352–358. doi:10.1111/odi.12119
90. Techawattanawisal W, Nakahama K, Komaki M, Abe M, Takagi Y, Morita I (2007) Isolation of multipotent stem cells from adult rat periodontal ligament by neurosphere-forming culture system. Biochem Biophys Res Commun 357(4):917–923. doi:10.1016/j.bbrc.2007.04.031
91. Vollner F, Ernst W, Driemel O, Morsczeck C (2009) A two-step strategy for neuronal differentiation in vitro of human dental follicle cells. Differentiation 77(5):433–441. doi:10.1016/j.diff.2009.03.002
92. Wang J, Wang X, Sun Z, Wang X, Yang H, Shi S, Wang S (2010) Stem cells from human-exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells. Stem Cells Dev 19(9):1375–1383. doi:10.1089/scd.2009.0258
93. Kiraly M, Porcsalmy B, Pataki A, Kadar K, Jelitai M, Molnar B, Hermann P, Gera I, Grimm WD, Ganss B, Zsembery A, Varga G (2009) Simultaneous PKC and cAMP activation induces differentiation of human dental pulp stem cells into functionally active neurons. Neurochem Int 55(5):323–332. doi:10.1016/j.neuint.2009.03.017
94. Nourbakhsh N, Soleimani M, Taghipour Z, Karbalaie K, Mousavi SB, Talebi A, Nadali F, Tanhaei S, Kiyani GA, Nematollahi M, Rabiei F, Mardani M, Bahramiyan H, Torabinejad M, Nasr-Esfahani MH, Baharvand H (2011) Induced in vitro differentiation of neural-like cells from human exfoliated deciduous teeth-derived stem cells. Int J Dev Biol 55(2):189–195. doi:10.1387/ijdb.103090nn
95. Lee JH, Um S, Song IS, Kim HY, Seo BM (2014) Neurogenic differentiation of human dental stem cells in vitro. J Korean Assoc Oral Maxillofac Surg 40(4):173–180. doi:10.5125/jkaoms.2014.40.4.173
96. Bueno C, Ramirez C, Rodriguez-Lozano FJ, Tabares-Seisdedos R, Rodenas M, Moraleda JM, Jones JR, Martinez S (2013) Human adult periodontal ligament-derived cells integrate and differentiate after implantation into the adult mammalian brain. Cell Transplant 22(11):2017–2028. doi:10.3727/096368912X657305

97. Zou XY, Yang HY, Yu Z, Tan XB, Yan X, Huang GT (2012) Establishment of transgene-free induced pluripotent stem cells reprogrammed from human stem cells of apical papilla for neural differentiation. Stem Cell Res Ther 3(5):43. doi:10.1186/scrt134
98. Arthur A, Shi S, Zannettino AC, Fujii N, Gronthos S, Koblar SA (2009) Implanted adult human dental pulp stem cells induce endogenous axon guidance. Stem Cells 27(9):2229–2237
99. Kiraly M, Kadar K, Horvathy DB, Nardai P, Racz GZ, Lacza Z, Varga G, Gerber G (2011) Integration of neuronally predifferentiated human dental pulp stem cells into rat brain in vivo. Neurochem Int 59(3):371–381. doi:10.1016/j.neuint.2011.01.006
100. Leong WK, Henshall TL, Arthur A, Kremer KL, Lewis MD, Helps SC, Field J, Hamilton-Bruce MA, Warming S, Manavis J, Vink R, Gronthos S, Koblar SA (2012) Human adult dental pulp stem cells enhance poststroke functional recovery through non-neural replacement mechanisms. Stem Cells Transl Med 1(3):177–187. doi:10.5966/sctm.2011-0039
101. Fang CZ, Yang YJ, Wang QH, Yao Y, Zhang XY, He XH (2013) Intraventricular injection of human dental pulp stem cells improves hypoxic-ischemic brain damage in neonatal rats. PLoS One 8(6), e66748. doi:10.1371/journal.pone.0066748
102. Sakai K, Yamamoto A, Matsubara K, Nakamura S, Naruse M, Yamagata M, Sakamoto K, Tauchi R, Wakao N, Imagama S, Hibi H, Kadomatsu K, Ishiguro N, Ueda M (2012) Human dental pulp-derived stem cells promote locomotor recovery after complete transection of the rat spinal cord by multiple neuro-regenerative mechanisms. J Clin Invest 122(1):80–90. doi:10.1172/JCI59251
103. Yamamoto A, Sakai K, Matsubara K, Kano F, Ueda M (2013) Multifaceted neuro-regenerative activities of human dental pulp stem cells for functional recovery after spinal cord injury. Neurosci Res. doi:10.1016/j.neures.2013.10.010
104. Apel C, Forlenza OV, de Paula VJ, Talib LL, Denecke B, Eduardo CP, Gattaz WF (2009) The neuroprotective effect of dental pulp cells in models of Alzheimer's and Parkinson's disease. J Neural Transm 116(1):71–78. doi:10.1007/s00702-008-0135-3
105. Taghipour Z, Karbalaie K, Kiani A, Niapour A, Bahramian H, Nasr-Esfahani MH, Baharvand H (2012) Transplantation of undifferentiated and induced human exfoliated deciduous teeth-derived stem cells promote functional recovery of rat spinal cord contusion injury model. Stem Cells Dev 21(10):1794–1802. doi:10.1089/scd.2011.0408
106. Li X, Yang C, Li L, Xiong J, Xie L, Yang B, Yu M, Feng L, Jiang Z, Guo W, Tian W (2015) A therapeutic strategy for spinal cord defect: human dental follicle cells combined with aligned PCL/PLGA electrospun material. Biomed Res Int 2015:197183. doi:10.1155/2015/197183
107. Martens W, Sanen K, Georgiou M, Struys T, Bronckaers A, Ameloot M, Phillips J, Lambrichts I (2014) Human dental pulp stem cells can differentiate into Schwann cells and promote and guide neurite outgrowth in an aligned tissue-engineered collagen construct in vitro. FASEB J 28(4):1634–1643. doi:10.1096/fj.13-243980
108. Li X, Gong P, Liao D (2010) In vitro neural/glial differentiation potential of periodontal ligament stem cells. Arch Med Sci 6(5):678–685. doi:10.5114/aoms.2010.17080
109. Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Ogiuchi H, Okano T, Ando T (2011) PLGA artificial nerve conduits with dental pulp cells promote facial nerve regeneration. J Tissue Eng Regen Med 5(10):823–830. doi:10.1002/term.387
110. Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Okano T, Ogiuchi H (2008) Tubulation with dental pulp cells promotes facial nerve regeneration in rats. Tissue Eng Part A 14(7):1141–1147. doi:10.1089/ten.tea.2007.0157
111. Sasaki R, Matsumine H, Watanabe Y, Takeuchi Y, Yamato M, Okano T, Miyata M, Ando T (2014) Electrophysiologic and functional evaluations of regenerated facial nerve defects with a tube containing dental pulp cells in rats. Plast Reconstr Surg 134(5):970–978. doi:10.1097/PRS.0000000000000602
112. Nosrat IV, Smith CA, Mullally P, Olson L, Nosrat CA (2004) Dental pulp cells provide neurotrophic support for dopaminergic neurons and differentiate into neurons in vitro; implications for tissue engineering and repair in the nervous system. Eur J Neurosci 19(9):2388–2398. doi:10.1111/j.0953-816X.2004.03314.x
113. Chen C, Wang Y, Yang GY (2013) Stem cell-mediated gene delivering for the treatment of cerebral ischemia: progress and prospectives. Curr Drug Targets 14(1):81–89

114. Liu X, Ye R, Yan T, Yu SP, Wei L, Xu G, Fan X, Jiang Y, Stetler RA, Liu G, Chen J (2014) Cell based therapies for ischemic stroke: from basic science to bedside. Prog Neurobiol 115:92–115. doi:10.1016/j.pneurobio.2013.11.007
115. Yu SP, Wei Z, Wei L (2013) Preconditioning strategy in stem cell transplantation therapy. Transl Stroke Res 4(1):76–88. doi:10.1007/s12975-012-0251-0
116. Theus MH, Wei L, Cui L, Francis K, Hu X, Keogh C, Yu SP (2008) In vitro hypoxic preconditioning of embryonic stem cells as a strategy of promoting cell survival and functional benefits after transplantation into the ischemic rat brain. Exp Neurol 210(2):656–670. doi:10.1016/j.expneurol.2007.12.020
117. Wei L, Fraser JL, Lu ZY, Hu X, Yu SP (2012) Transplantation of hypoxia preconditioned bone marrow mesenchymal stem cells enhances angiogenesis and neurogenesis after cerebral ischemia in rats. Neurobiol Dis 46(3):635–645. doi:10.1016/j.nbd.2012.03.002
118. Wei N, Yu SP, Gu X, Taylor TM, Song D, Liu XF, Wei L (2013) Delayed intranasal delivery of hypoxic-preconditioned bone marrow mesenchymal stem cells enhanced cell homing and therapeutic benefits after ischemic stroke in mice. Cell Transplant 22(6):977–991. doi:10.3727/096368912X657251
119. Kim YS, Noh MY, Cho KA, Kim H, Kwon MS, Kim KS, Kim J, Koh SH, Kim SH (2014) Hypoxia/reoxygenation-preconditioned human bone marrow-derived mesenchymal stromal cells rescue ischemic rat cortical neurons by enhancing trophic factor release. Mol Neurobiol. doi:10.1007/s12035-014-8912-5
120. Feigin VL, Forouzanfar MH, Krishnamurthi R, Mensah GA, Connor M, Bennett DA, Moran AE, Sacco RL, Anderson L, Truelsen T, O'Donnell M, Venketasubramanian N, Barker-Collo S, Lawes CM, Wang W, Shinohara Y, Witt E, Ezzati M, Naghavi M, Murray C, Global Burden of Diseases I, Risk Factors S, the GBDSEG (2014) Global and regional burden of stroke during 1990–2010: findings from the Global Burden of Disease Study 2010. Lancet 383(9913):245–254
121. Jin K, Wang X, Xie L, Mao XO, Zhu W, Wang Y, Shen J, Mao Y, Banwait S, Greenberg DA (2006) Evidence for stroke-induced neurogenesis in the human brain. Proc Natl Acad Sci U S A 103(35):13198–13202. doi:10.1073/pnas.0603512103
122. Molina CA (2011) Reperfusion therapies for acute ischemic stroke: current pharmacological and mechanical approaches. Stroke 42(1 Suppl):S16–S19. doi:10.1161/STROKEAHA.110.598763
123. Lees JS, Sena ES, Egan KJ, Antonic A, Koblar SA, Howells DW, Macleod MR (2012) Stem cell-based therapy for experimental stroke: a systematic review and meta-analysis. Int J Stroke 7(7):582–588. doi:10.1111/j.1747-4949.2012.00797.x
124. Jeong H, Yim HW, Cho YS, Kim YI, Jeong SN, Kim HB, Oh IH (2014) Efficacy and safety of stem cell therapies for patients with stroke: a systematic review and single arm meta-analysis. Int J Stem Cells 7(2):63–69. doi:10.15283/ijsc.2014.7.2.63
125. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97(25):13625–13630
126. Hilkens P, Gervois P, Fanton Y, Vanormelingen J, Martens W, Struys T, Politis C, Lambrichts I, Bronckaers A (2013) Effect of isolation methodology on stem cell properties and multilineage differentiation potential of human dental pulp stem cells. Cell Tissue Res 353(1):65–78. doi:10.1007/s00441-013-1630-x
127. Mead B, Logan A, Berry M, Leadbeater W, Scheven BA (2014) Paracrine-mediated neuroprotection and neuritogenesis of axotomised retinal ganglion cells by human dental pulp stem cells: comparison with human bone marrow and adipose-derived mesenchymal stem cells. PLoS One 9(10), e109305. doi:10.1371/journal.pone.0109305
128. Song M, Jue SS, Cho YA, Kim EC (2015) Comparison of the effects of human dental pulp stem cells and human bone marrow-derived mesenchymal stem cells on ischemic human astrocytes in vitro. J Neurosci Res 93(6):973–983. doi:10.1002/jnr.23569
129. de Almeida FM, Marques SA, Ramalho Bdos S, Rodrigues RF, Cadilhe DV, Furtado D, Kerkis I, Pereira LV, Rehen SK, Martinez AM (2011) Human dental pulp cells: a new source of cell therapy in a mouse model of compressive spinal cord injury. J Neurotrauma 28(9):1939–1949. doi:10.1089/neu.2010.1317

130. Huang AH, Snyder BR, Cheng PH, Chan AW (2008) Putative dental pulp-derived stem/stromal cells promote proliferation and differentiation of endogenous neural cells in the hippocampus of mice. Stem Cells 26(10):2654–2663
131. Inoue T, Sugiyama M, Hattori H, Wakita H, Wakabayashi T, Ueda M (2013) Stem cells from human exfoliated deciduous tooth-derived conditioned medium enhance recovery of focal cerebral ischemia in rats. Tissue Eng Part A 19(1–2):24–29. doi:10.1089/ten.TEA.2011.0385
132. Tseng LS, Chen SH, Lin MT, Lin YC (2014) Transplantation of human dental pulp-derived stem cells protects against heat stroke in mice. Cell Transplant. doi:10.3727/096368914X678580
133. Yamagata M, Yamamoto A, Kako E, Kaneko N, Matsubara K, Sakai K, Sawamoto K, Ueda M (2013) Human dental pulp-derived stem cells protect against hypoxic-ischemic brain injury in neonatal mice. Stroke 44(2):551–554. doi:10.1161/STROKEAHA.112.676759

Chapter 12
Dental Stem Cell Differentiation Toward Endodermal Cell Lineages: Approaches to Control Hepatocytes and Beta Cell Transformation

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12.1 Introduction

The World Health Organization (WHO) reports liver cirrhosis and diabetes mellitus (DM) form the 12th and 7th highest causes of death in the USA, respectively. Furthermore, mortality cases due to liver cirrhosis and DM were 14 and 21, respectively, for every 100,000 of the population in 2012 [1]. Having said that, much emphasis is being placed on controlling such mortality rates by introducing advanced treatments and equipment facilities upon detection at the early stages [2]. However, despite such efforts, the problems relating to these conditions have not been adequately resolved. In DM for example, though life-long insulin injection/pump seems to be a do-able treatment modality, the problems related with hypoglycemia which is life-threatening still remain and the illness requires serious

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attention [3]. It should be noted that the actual cause of DM is primarily due to lack of insulin-producing cells which could have been damaged due to various reasons. This has initiated a global search for the best solution to overcome these drawbacks, and cell replacement therapy has of late become a popular topic in the field of medicine. A wide array of cell choices to replace damaged tissues or organs is available, which were proposed by numerous studies. Dental stem cells (DSCs), among the available cell sources, are emerging as potential candidates due to their inherent plasticity and ability to differentiate into cells of germ layers despite their neural crest origin. This has created significant possibilities which, with enough attention and efforts, could be employed to our advantage. In this sense, this chapter discusses the generation of endoderm and the applicability of DSCs toward two most common endpoints of endodermal lineages, namely hepatocyte-like cells and islet like-cells.

12.2 Endoderm Generation

The endoderm is one of the three germ layers derived from the inner cell mass (ICM) of blastocysts [4] and begins to develop around embryonic (E) 7. It comprises of 3 regions, and definitive endoderm (DE) progenitors found in the foregut region will give rise to liver and pancreas [5, 6].

12.2.1 Liver Development

Shortly after DE formation, three major developmental stages take place in the onset of liver ontogeny, namely hepatic endoderm specification, liver budding and final differentiation. Fate mapping studies have demonstrated that the development of this organ arises from the lateral domain of the developing ventral foregut region [7, 8]. During the closure of the foregut, the medial and lateral domains merge together to stipulate hepatic endoderm. After the hepatic endoderm specification, inductive signalling response promotes liver bud emergence and leads to differentiation. Overall, the liver is composed of many specialized cell types and of those, hepatocytes are the principal cells comprising of 70 % of the whole liver mass.

12.2.1.1 Hepatocyte Formation

Hepatocyte formation occurs via a progressive series of reciprocal interaction between various germ layers. In brief, extracellular transcriptional machinery such as fibroblast growth factors (FGFs) from the cardiac mesoderm and bone morphogenic proteins (BMPs) from the septum transversum mesenchyme (STM) work in concert to produce inductive signals to the ventral endoderm to adopt hepatic induction by

repressing Wnt signalling [9, 10]. The cellular response to the signalling cues during this period eventually prompt new gene expression signals that are essential for cell differentiation from the epithelium at the intracellular level involving liver enriched transcription factors (LETFS) such as the homeobox factor (HEX) [11], hepatocyte nuclear factor (HNF) and GATA genes [12]. Simultaneously, initially suppressed Wnt signalling is also necessary for liver bud emergence and differentiation [11, 13]. At this point, specified hepatic cells are denoted as hepatoblast or progenitors [14]. They are bipotential as they give rise to both hepatocytes and cholangiocytes [15] that expresses both adult hepatic genes (HNF4α, albumin) and biliary cell genes (cytokeratin 19) [16, 17]. Liver diverticulum appears once the hepatoblast transform from a columnar to a pseudo-stratified epithelium. These progenitors then embed into the stromal environment of the STM in which they tend to interact and receive stimulatory signals from adjacent endothelial cells [18, 19] for liver bud formation. For instance, the presence of the hepatocyte growth factor (HGF) and transforming growth factor (TGF) produced by the surrounding non-parenchymal liver cells enhance hepatoblast proliferation and liver bud growth [20, 21]. Meanwhile, the bipotent status of the hepatoblast is then regulated by cytokines such as Oncostatin M (OSM) in combination with glucocorticoid hormone and HGF to differentiate into hepatocyte [22–24] and maintain proper balance of the number of hepatocytes under the influence of an integrated signalling and transcriptional network (Fig. 12.1)

12.2.1.2 Pancreatic Development

Pancreas is unique in nature due to having both exocrine and endocrine functions represented by acinar tissues and islets of Langerhans, respectively. Two distinct phases of development, namely primary transition (E9.5-E12.5) and secondary transition (E13.5-E16.5), define the morphogenesis of the pancreas from DE in which the former is involved particularly in organ determination and corresponds to a period of active proliferation of pancreatic progenitors [25], whereas the latter corresponds to the specification of multi-potent precursor cells toward the differentiated lineage [26]. As such, pancreas begins to develop as early as E9 in which two outpouchings, namely ventral and dorsal pancreas, start to emerge from the endodermal lining of the duodenum. These outpouchings eventually fuse due to gut rotation and form the head, body and tail of the pancreas [27–30].

Beta (β) Cell Formation

Though there are distinct cell types with respective functions in the endocrine portion of the pancreas, it is interesting to note that the neogenesis of β cells occurs side by side with other cell types as well. Expression of Neurogenin 3 (Ngn3) and Hairy Enhancer of Split-1 (HES1) from the pancreatic precursors emerging from DE play vital role in determining the subsequent development into

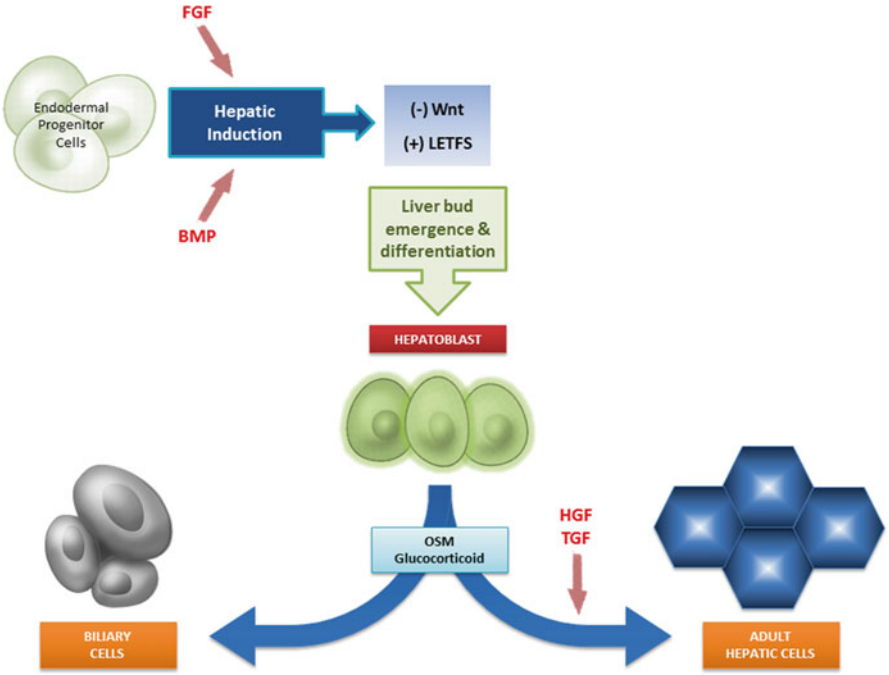

Fig. 12.1 A schematic representation of hepatocytes and biliary cell formation from endodermal progenitor cells. Hepatic induction begins with the influence of fibroblast growth factor (FGF) and bone morphogenetic protein (BMP) and eventually forms hepatoblast. Maturation of either hepatocytes or biliary cell largely depends on the signalling from hepatocyte growth factor (HGF) and transforming growth factor (TGF)

either exocrine or endocrine precursors [31, 32]. Studies showed that the ratio of Aristaless related homeobox (Arx) and Paired box 4 (Pax4) are crucial in deciding the fate of $Ngn3^{+}$ endocrine progenitors at as early as E9.5 [25, 26, 30]. For example, knockout of Pax4 results in the total absence of β cells but not α cells. In normal pancreatic development, expression of Pax4 peaks between E13.5 and E15.5, which coincides with the period of maximal differentiation of β cell precursors. Consequently after endocrine specification, Ngn3 co-localizes with Pax4, suggesting that the latter may be one of the targets of the former [33–35]. As such, when the ratio of Arx/Pax4 is low during secondary transition from E13.5 onwards, a subsequent progenitor with three signature markers, namely Paired box 6 (Pax6), Pax4 and NK2 homeobox 2 (Nkx 2.2), will begin to emerge [36].

During the later stages of β cell differentiation, the v-maf avian musculo aponeurotic fibrosarcoma oncogene homolog A and B (MAFA and MAFB) transcription factors also play prominent roles. MAFA/MAFB expressions in β cells lead to an increase in pancreatic and duodenal homeobox 1 (PDX1) expression initiating the insulin transcription. It was shown that in the absence of MAFB, fewer α and β cells were present, although total endocrine cell mass did not change [37]. MAFA

expression starts shortly after insulin expression [38] and persists in adult β cells. It was reported in MAFB-deficient mice, insulin expression reduced and delayed until MAFA became expressed, but the resulting insulin$^+$ cells had low expression of PDX1, NK6 homeobox 1 (NKX6-1), and Glucose transporter, type 2 (GLUT2; SLC2A2) [37], and therefore were unlikely to represent true β cells.

Finally, upon β cell specification, the transcription of insulin is commenced and maintained by MAFB PDX1, NEUROD1, PAX6, and MAFA whereas other factors such as Nkx 2.2, Nkx 6.1, and Homeobox 9 (Hb9) are crucial for continuation of β cell neogenesis [39, 40]. Figure 12.2 summarizes the progressive development from pancreatic precursors and their subsequent sub-types and eventual differentiation into β cells.

12.2.1.3 Liver and Pancreatic Dysfunctions and Current Treatments

Among the various organs in the human body, the liver is believed to provide the highest prospects for regenerative medicine due to its ability to regenerate following chemical or physical abuses [41]. The abuses could arise due to trauma [42], drugs [43], and microbial agents including viruses and bacteria [44, 45]. Despite huge array of medication and treatment modalities to treat liver injuries, it should be noted that until recently acute and chronic liver diseases as well as other metabolic disorders were treated by whole organ transplantation or orthotopic liver transplantation (OLT). Despite the success of this techniques, major impediments such as financial cost, long term immunosuppression issues, major surgery requirements, and many

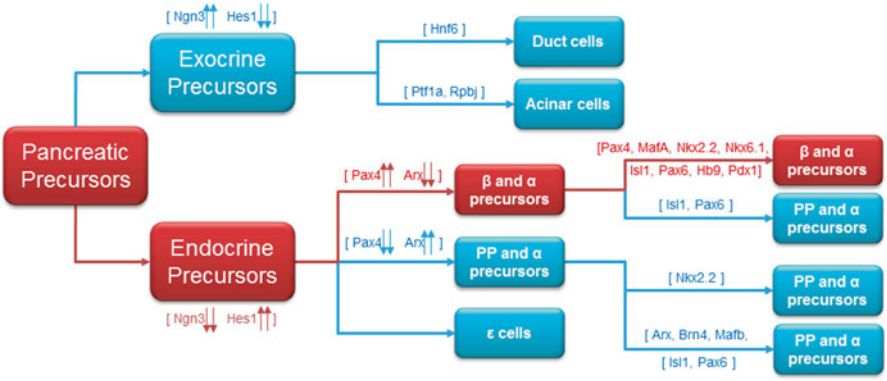

Fig. 12.2 Progressive development of pancreatic related cells from their respective precursors and their corresponding transcription factors (Ngn3: Neurogenin 3; Hes1: Hairy Enhancer of Split-1; Hnf6: Hepatocyte nuclear factor 6; Ptf1a: α subunit of pancreas-specific transcription factor 1; Rpbj: Recombination Signal Binding Protein For Immunoglobulin Kappa J; Pax4: Paired box 4; Arx: Aristaless related homeobox; MafA: v-maf avian musculoaponeuroticfibrosarcoma oncogene homolog A; Nkx 2.2: NK2 homeobox 2; Nkx 6.1: NK6 homeobox 1; Isl1: ISL LIM homeobox 1; Pax6: Paired box 6; Hb9: Homeobox 9; Pdx1: pancreatic and duodenal homeobox 1; Brn4: Brain 4; MafB: v-maf avian musculoaponeuroticfibrosarcoma oncogene homolog B). Adapted from Ben-Othman et al. [26]

other obstacles have brought the cell-based therapies to treat liver diseases to the forefront. The transplantation of hepatocytes do not require major surgical procedures and since the cells can be cryopreserved and they are theoretically available at any time they are required for transplantation as compared to organ transplantation. Besides hepatocyte transplantation, alternative sources of hepatocyte have emerged, for instance immortalized human hepatocytes [46, 47] and the widely available of stem cells-derived hepatocytes [48–50].

Dysfunctions of the pancreas are due to a number of factors, of those the most common is inflammation, leading to pancreatitis. This usually occurs when the digestive enzymes are activated before being released into the small intestines thus attacking the pancreas itself [51, 52]. Of the two types of pancreatitis disorders, acute pancreatitis occurs when the inflammation lasts for a short period of time while chronic inflammation develops when the damage is long-lasting [53, 54]. Depending on the severity of the inflammation, this condition can be treated by medication, having a low-fat diet, and surgery [55, 56]. Damage to the endocrine portion is inevitable in which defects in either insulin secretion or insulin action results in diabetes mellitus (DM). Though there are a variety of categories with known and also unknown etiology, the main characteristic of this condition lies in the ability of β cells to secrete insulin and is somewhat correlates with the lifestyle of patients as noted by the American Diabetes Association [57]. Type 1 diabetes, for example occurs when β cell destruction occurs, usually leading to absolute insulin deficiency [58–60]. Also known as insulin-dependent diabetes, type 1 DM ensues when cellular-mediated autoimmune destruction of the β cells of the pancreas takes place [61]. Furthermore, the rate of β cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults) [62]. Some patients, particularly children and adolescents, may exhibit ketoacidosis as the first manifestation of the disease [63]. Others have uncertain fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress [64]. As the name implies, this particular condition can be controlled by taking interval insulin injections [65].

Type 2 diabetes, however, accounts for almost 95 % of DM patients and ranges from predominantly insulin resistance with relative insulin deficiency to an insulin secretory defect with insulin resistance [66]. Most patients with this form of diabetes are obese, and the obesity itself causes some degree of insulin resistance [67]. Patients who are not obese according to the traditional weight criteria may have an increased percentage of body fat distributed mainly in the abdominal region [68]. In addition, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity [69]. Type 2 DM is controlled via the administration of drugs such as remogliflozinetabonate (RE), a sodium-dependent glucose transporter 2 (SGLT2) inhibitor [70], glucagon-like peptide-1 receptor agonist [71] and the use of newly developed techniques like resveratrol treatment [72]. Though insulin resistance may be improved with weight reduction and/or pharmacological treatment of hyperglycemia, it is seldomly restored to normal conditions (Fig. 12.3).

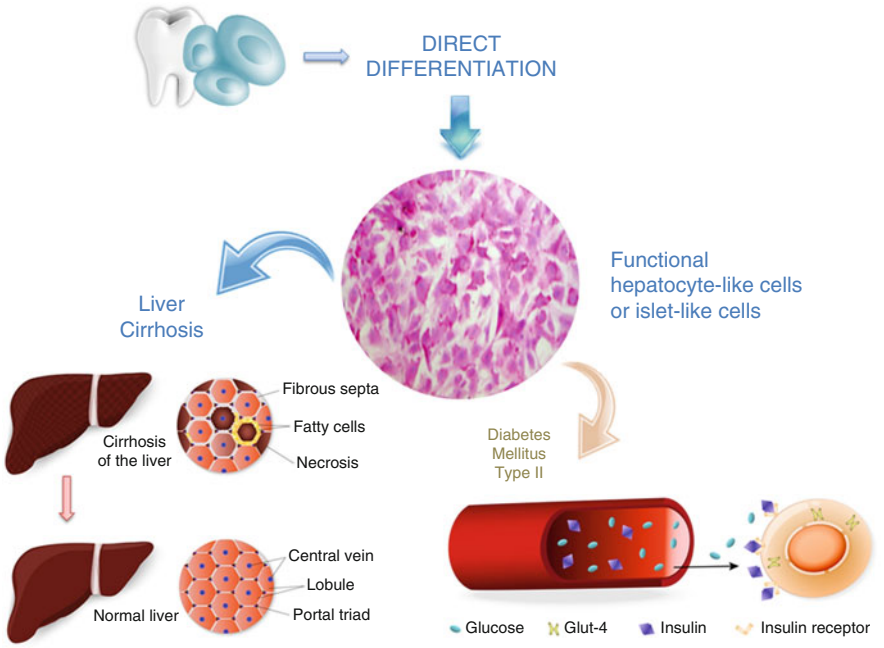

Fig. 12.3 Ideal usage of dental stem cells (DSCs) in treating two most prevalent issues in regards to endoderm, *i.e.* liver cirrhosis and diabetes mellitus Type 2. With the direct differentiation technology, cells upon large scale expansion with high purity and functional abilities are expected to be the next level of possible therapy

12.3 Stem Cells (SCs) for Liver and Pancreatic Regeneration

12.3.1 Adult SC

Adult SCs are undifferentiated cells found throughout the body that proliferate to replenish dying cells and regenerate damaged tissues. These cells are pluripotent by nature in which they have the capacity to differentiate into certain cell lineage upon exposure to growth and/or differentiating factors [73]. These cells are commonly found in bone marrow, blood vessels and skin, and recently dental pulps are also being extensively explored due to the presence of pulp tissues containing progenitor/stem cells. These cells was first discovered by Gronthos et al. [74], claiming to have similar immunophenotype in vitro with human bone marrow stromal cells (BMMSCs), and have the ability to form a dentin/pulp-like complex when transplanted on immunocompromised mice. Furthermore, their procurement which is less invasive and do not require stringent ethical pressure like in embryonic stem

cells (ESCs) as well as absence of viral transfection like we observe in induced pluripotent stem cells (iPSCs) have rendered them to be more research-friendly; thus, inspiring extensive works on them [75].

12.3.2 Dental Stem Cell (DSCs)

DSCs are basically categorized based on their location where isolation takes place. The wide sources of SCs derived from dental origin have triggered tremendous amount of work to facilitate our understanding about them and to search for possible ways to employ them to our advantage. The cell types from this origin include SCs from human exfoliated deciduous teeth (SHEDs), adult dental pulp SCs (DPSCs), SCs from the apical part of the papilla (SCAPs), SCs from the dental follicle (DFSCs) and periodontal ligament SCs (PDLSCs) [76]. The SHEDs are isolated from pulp of human deciduous teeth, and have a higher proliferation rate compared to DPSCs [77]. DPSCs have been isolated and grown from pulp tissue of permanent human dental pulp. SCAPs are isolated from human teeth found at the toot root apex [78] while DFSCs are isolated from dental follicle surrounding the developing tooth germ which have long been considered a multipotent tissue based on its ability to generate cementum, bone and periodontal ligament [79]. PDLSCs are derived from root surface of extracted teeth and can differentiate into cells or tissues very similar to periodontium [80].

12.3.2.1 DSCs and Directed Differentiation Toward Hepatocyte-Like Cells

Formulating an induction protocol to differentiate DSCs into hepatocyte-like cell involves a number of factors. First and foremost are the cytokine and growth factors acting as extracellular stimulating agents in differentiation process of DSCs into hepatocyte-like cells. Among the frequently exposed growth factors are HGF, OSM and insulin transferrin selenium (ITS), meanwhile the non-proteinaceous chemical compounds that enhance and maintain hepatocyte differentiation are dexamethasone (DEX) and nicotinamide (NA).

Ikeda et al. [81] was the first group to illustrate the potential of SCs derived from third molar tooth germs to differentiate in hepatocyte-like cells. They have reported apparent changes in morphology and also secretion of albumin confirming the presence of hepatocyte-like cells after differentiation process. The other groups worked on this subject were Ishkitiev et al. [82–84] and Okada et al. [85] who employed a two-step protocol including induction and maturation phases. In the induction phase, culture media was incorporated with 20 ng/mL HGF and 5 mM NA for the first 5 days. Subsequently for the maturation phase, 10 ng/mL OSM, 10 nmol/L DEX and 1 % ITS were added into the culture media, and cells were incubated in this particular media for about 16 days. The generated hepatocyte-like cells not only displayed hepatocyte-like morphology but also they possessed functional properties of a hepatic

cells. Apart from that, the presence of damaged liver conditioned medium can also augment the differentiation ability of MSCs into hepatocyte-like cells. In addition, our group have also worked on differentiation of DPSCs toward hepatocyte-like cells using the protocol established by Ishkitiev et al. [82–84] and Okada et al. [85], and we have also observed similar characteristics as previously described [86].

12.3.2.2 DSCs and Directed Differentiation Toward Islet-Like Cells

The ability of stem cell of dental origin to differentiate into islet-like cells was first demonstrated by Huang et al [87] in an attempt to PDLSCs. They have introduced chemicals such as monothioglycerol, sodium butyrate and NA which were established in ESC model. These factors collectively trigger Wnt pathway, the integrin-mediated signalling pathway as well as Notch signalling pathway [88, 89]. This study was the first of its kind to illustrate the expression of ESC markers in sub-population of PDLSCs displaying a broad differentiation potential.

Next, our group was the first to show the ability of DPSCs to differentiate toward islet-like cell aggregates (ICAs) using a three step protocol [90]. The presence of activin A in the differentiation protocol helps in stimulating TGF signalling pathway among other signalling pathways as discussed previously [91]. Moreover, the number of ICAs that we managed to harvest at day 10 post-differentiation were 156 ± 23, indicating sufficient production of ICAs from DPSCs for transplantation purpose.

Similar to our group, a three-step protocol was employed by Sawangmake et al. [92] on DPSCs and PDLSCs, showing the former generating more insulin-producing cells (IPCs) than the latter. Here, they investigated the effect of Notch signalling in the differentiation ability of these cells. Notch maintained the pool of PDX-1-positive early pancreatic progenitors by suppressing Ngn3 expression in order to prevent premature endocrine differentiation. Furthermore, it was also reported that Notch signalling targets (Hes1 and Hey1) were highly expressed, indicating the involvement of Notch signalling in pancreatic islet differentiation.

Next, another team from Korea have attempted to replicate the differentiation of PDLSCs into ICAs but in a Matrigel 3D culture system using our established protocol with slight modifications [93]. Here, the usage of glucagon-like peptide-1 (GLP-1) agonists are believed to promote β cell differentiation by acting on several intracellular pathways such as the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), the Hedgehog, the mitogen-activated protein kinases (MAPK), and the protein kinase A (PKA) pathways to modify the expression of β cell transcription factors, including Pdx1.

Carnevale et al. [94] have conducted a study to understand the transformation efficacy of a particular sub-population of DPSCs, which are positive for c-kit, CD34, and STRO-1 markers, into ICAs via using simple and multiple-step approaches. They introduced retinoic acid which is essential for pancreatic development through the induction of Pdx1 expression [91] and promoting the generation of $Ngn3^{+}$ endocrine progenitors. The authors claimed to have generated ICAs with high expression of Pdx-1 and Glut2 as early as day 7 upon exposure to induction media. Another group from Japan described a method of obtaining IPCs from

DPSCs and PDLSCs by first performing sorting of cells to obtain $CD117^+$ cells [95]. This marker was specifically selected for its importance in maintaining SC properties of the mesenchymal and hematopoietic SCs [96]. Moreover, its activation leads to apoptosis, cell differentiation, proliferation, chemotaxis and cell adhesion [97]. Again in 2014, Ishkitiev's group tried to improve their differentiation protocol by adding hydrogen sulphide (H_2S) at the concentration of 0.1 ng/mL [98]. It was reported that exposure to H_2S activates all signalling functions of the PI3K-AKT pathway. They have reported that the expressions of insulin, glucagon, somatostatin and pancreatic polypeptide significantly up-regulated after differentiation as compared to those not treated with H_2S. The summary of efforts taken by researchers to show the ability of DSCs toward both hepatocyte-like cells as well as pancreatic-islets like cells is presented in Table 12.1.

Table 12.1 Summary of studies employing DSCs differentiating toward hepatocyte-like cells as well as islet-like cells

Differentiation toward hepatocyte-like cells				
No.	Cell source	Growth factors introduced	Results	References
1	DSCs from 3rd molar tooth germs	**Step 1 medium (5 days):** low-glucose DMEM + 2 % FBS + 2 mM L-glutamine + 100 ng/ml acidic fibroblast growth factor (a-FGF)	The morphology changed from a bipolar-spindle and fibroblast-like to a polygonal and an epithelial-like morphology. Immature hepatocyte markers, AFP, CK19 were declining in expression toward the end of differentiation. Albumin secretion too were in concurrent with its corresponding gene expression	Ikeda et al. [81]
		Step 2 medium (5 days): low-glucose DMEM + 2 % FBS + 2 mM L-glutamine + 20 ng/mL hepatocyte growth factor (HGF)		
		Step 3 medium (11 days): low-glucose DMEM + 2 % FBS + 2 mM L-glutamine + 20 ng/ml HGF + 10 nmol/l dexamethasone + insulin-transferrin-selenium-X (ITS-X) + 10 ng/ml oncostatin M (OSM)		
2	SHED	**Step 1 (5 days):** DMEM + 20 ng/mL HGF + 2 % fetal calf serum	The morphology of the cells changed from spindle shaped to polygonal. Expression of genes and proteins albumin, AFP, HNF-4a, and IGF-1 were present. Glycogen storage in the differentiated cells were profound indicating presence of matured hepatocyte-like cells	Ishkitiev et al. [82]
		Step 2 (15 days): DMEM + 10 ng/mL OSM + 10 nmol/L dexamethasone + 1 % ITS-X		

(continued)

Table 12.1 (continued)

Differentiation toward hepatocyte-like cells				
No.	Cell source	Growth factors introduced	Results	References
3	CD117+ SHED and DPSC	**Step 1:** DMEM + 1 % ITS-X + 100 mg/mL of embryotrophic factor **Step 2 (5 days):** DMEM + 1 % ITS-X + 100 mg/mL of embryotrophic factor + 20 ng/mL HGF **Step 3 (15 days):** DMEM + 10 ng/mL OSM + 10 nmol/L dexamethasone + 1 % ITS-X	The morphology of the cells changed from spindle shaped to polygonal. All specific hepatic markers such as albumin, AFP, HNF-4a, IGF-I, and CPS-1 were positive. Glycogen storage and urea secretion were prominent in differentiated SHED and DPSC	Ishkitiev et al. [83]; Okada et al. [85]; Ishkitiev et al. [84]
4	DPSC	**Step 1 (5 days):** DMEM + 20 ng/mL HGF + 2 % FBS **Step 2 (16 days):** DMEM + 10 ng/mL Oncostatin M + 10 nmol/L dexamethasone + 1 % ITS-X	The morphology of the cells changed from spindle shaped to polygonal at Day 21. The gene, protein and its functional profile showed a steady down-regulation of early endoderm markers (GATA4, GATA6, SOX17, HNF4α, HNF3β and AFP) with the up-regulation of hepatic specific markers (TDO, TO, TAT, ALB, AAT, CK18).	Vasanthan et al. [86]

Differentiation toward islet-like cells				
No.	Cell source	Growth factors introduced	Results	References
1	PDLSC	DMEM/F12 + 1 % non-essential amino acids (NEAA) + 2 mM glutamine + 1 % ITS + 450 μM monothioglycerol + 1 mM sodium butyrate + 10 mM nicotinamide (NA) + 5 mg/ml albumin fraction V	Cell aggregates were formed within 24 h of induction and after 10 days, genes such as Isl-1, Pdx-1, Glut2 and Somatostatin were expressed. C-peptide was also secreted indicating the maturity stage of differentiated cells	Huang et al. [87]

(continued)

Table 12.1 (continued)

Differentiation toward islet-like cells				
No.	Cell source	Growth factors introduced	Results	References
2	DPSC	**SFM-A**: DMEM-KO + 1 % BSA + 1× ITS + 4 nM activin A + 1 nM sodium butyrate + 50 μM β-mercaptoethanol (BME) **SFM-B**: DMEM-KO + 1 % BSA + 1× ITS + 0.3 mM taurine **SFM-C**: DMEM-KO + 1.5 % BSA + 1× ITS + 3 mM taurine + 100 nM glucagon-like peptide + 1 (GLP-1) mM NA + 1X NEAA	DPSCs that proliferated as an adherent monolayer aggregated into spherical cells when the medium was changed from serum containing medium to day 1 SFM-A. After 5 days of incubation in SFM-C, most of the day 10 ICAs stained positive for DTZ. PCR analysis of day 10 ICAs showed a greater transcript abundance of pancreatic β-cells markers such as Pdx-1, Ngn3, Pax4, Nkx6.1, Isl-1, Insulin, and Glut2 as compared with undifferentiated DPSCs.	Govindasamy et al. [90]
3	DPSC and PDLSC	**SFM-A**: DMEM-KO + 1 % BSA + 1x ITS + 4 nM activin A + 1 nM sodium butyrate + 50 μM BME **SFM-B**: DMEM-KO + 1 % BSA + 1× ITS + 0.3 mM taurine **SFM-C**: DMEM-KO + 1.5 % BSA + 1× ITS + 3 mM taurine + 100 nM GLP-1 + 1 mM NA + 1X NEAA	As early as Day 5, cell aggregates became denser and bigger in appearance. The total colony count of hDPSCs-derived cell aggregation was significantly higher than that of hPDLSCs. Key markers such as Pdx-1, Ngn3 and Isl-1 were highly expressed and pro-insulin secretion was also significantly noted suggesting the differentiation capacity of these cells.	Sawangmake et al. [92]
4	PDLSC	**Step 1 medium**: DMEM/F12 + 17.5 mM glucose + 1 % BSA + ITS + 4 nM activin A + 1 mM sodium butyrate + 50 mM BME **Step 2 medium**: DMEM/F12 + 17.5 mM glucose + 1 % BSA + ITS + 0.3 mM taurine **Step 3 medium**: DMEM/F12 + 17.5 mM glucose + 1.5 % BSA + ITS + 3 mM taurine + GLP-1 + 1 mM NA + 1× NEAA	They have demonstrated the down-regulation of pluripotent markers, Oct4 and Nanog in ICAs and up-regulation of endodermal markers, FoxA2, Gata2 and Sox17. In addition, pancreatic cell markers, such as Glut2, Pdx1, Isl-1, and Pax6 were significantly upregulated in ICAs as compared with undifferentiated PDLSCs. The authors further stated that they have previously tested the use of 2D culture system, however islet like clusters did not appear in this as they did in 3D culture systems. These clusters, apart from expressing early endoderm markers, they too were capable of responding to glucose in a manner similar to pancreatic β cells.	Lee et al. [93]

(continued)

Table 12.1 (continued)

Differentiation toward islet-like cells				
No.	Cell source	Growth factors introduced	Results	References
5	DPSC	**Pre-induction medium (2 days)**: L-DMEM +5 % FBS + 5 μM trans-retinoic acid (RA) + 0.5 mM BME **Induction medium (2 days)**: H-DMEM + 10 mM NA, 5 μM RA, 0.5 mM BME **Maintaining-1 medium (2 days)**: H-DMEM + 5 % FBS + 10 mM NA, 10 μM zinc sulphate + 10 μM selenium **Maintaining-2 medium (2 days)**: L-DMEM + 5 % FBS, 10 mM NA, 10 μM zinc sulphate and 10 μM selenium Maintaining-1 medium and Maintaining-2 medium to be replaced every 2 days until induction process reaches 21 days	After 7 days of differentiation the cells started to form islet-like structures that became evident after 14 days of induction. Furthermore, expression of PDX-1, insulin and Glut-2 in undifferentiated and differentiated cells was evaluated by immunofluorescence assay and showed intense labelling of Pdx-1, Isl-1 and Glut2. This was further validated with Western blotting. Insulin release assay too revealed significant differences when challenged with two different glucose concentrations signifying maturation of ICAs generated.	Carnevale et al. [94]
6	DPSC and PDLSC	**5 days**: DMEM-KO + 100 U/mL penicillin + 100 μg/mL streptomycin + 0.25 μg/mL amphotericin + 20 ng/mL HGF + 10 ng/mL aFGF **7 days**: DMEM-KO + 100 U/mL penicillin + 100 μg/mL streptomycin + 0.25 μg/mL amphotericin + 20 ng/mL EGF + 100 μM BME **5 days**: DMEM-KO + 100 U/mL penicillin + 100 μg/mL streptomycin + 0.25 μg/mL amphotericin + 20 ng/mL HGF + 10 mM NA + 100 μg/mL embryotrophic factors (ETF)	Immunocytochemistry of both cell lines revealed clusters of cells expressing all tested markers, such as INS, GCG, PPY, SST, Glut2 and AMY2A after *in vitro* differentiation. Flow cytometric analysis confirmed the fluorescent data presented above and showed that between 10 and 20 % of DPSCs and up to 50 % of SHED expressed the pancreatic markers after in vitro differentiation. The expression of insulin remained low in both cell cultures. The glucose-stimulated insulin release test proved the functionality of the insulin producing cells.	Ishkitiev et al. [95])

(continued)

Table 12.1 (continued)

Differentiation toward islet-like cells				
No.	Cell source	Growth factors introduced	Results	References
7	DPSC and PDLSC	**5 days**: DMEM-KO + 20 ng/mL HGF + 10 ng/mL aFGF + 4nM activin A + 1 μM RA **7 days**: IMEM + 20 ng/mL EGF + 100 μM BME, +1 % ITS-X + 100 μg/mL ETF + 20 ng/ml BMP 11 + 0.1 ng/mL H_2S **5 days**: α-MEM + 1 % ITS-x + 20 ng/mL HGF + 10 mM NA + 100 μg/mL ETF + 20 ng/mL BMP11	Gene expression revealed that only HHEX, Pdx1 and Nkx6.1 among the factors controlling Ins, GCG, PPY and SST expression were positive in both cell lines. Flow cytometry also revealed that the presence of H_2S increased the number of cells positive for Ins compared with those in the H_2S^- group. Similarly, exposure of the cells to H_2S contributed to increased intracellular or extracellular Isl-1 concentration upon glucose challenge.	Okada et al. [98]

12.4 Mechanisms and Critical Pathways Involved in Liver and Pancreatic Regeneration

12.4.1 Liver

The generation of liver cells from MSCs involve the process of mesenchymal to epithelial transition (MET). The main aspect that changes during this process is the structural changes in which epithelial cells that appear closely connected to their neighbours with the help of adherens junctions and desmosomes transform from loosely organized MSCs.

All the above structures are regulated by the presence of many cell communicative signalling pathways in three phases; namely specification of endoderm, formation of hepatoblast and finally determination/maturation of hepatocytes. Nodal signalling/TGFβ is the top of the molecular hierarchy that plays an important role in endoderm pattern formation and development [99, 100]. Nevertheless, this signalling pathway initiates both mesodermal formation at lower dosages and endodermal formation at antagonistic dosages. At the latter point, Nodal members release inhibitory signals generated by PI3K/AKT signalling network through an insulin/IGF pathway. Nodal signalling acts together with another important signalling pathway, Wnt/β-catenin, in specifying definitive endoderm formation. Nodal signalling subsequently stimulates expression of a set of endodermal transcription factors including SOX 17, GATA and Fork Head domain proteins FOXA1-3 (HNF3α/β/γ)

that are identified as the targets of FGF and BMP signallings [12] which then regulate a cascade of genes related to the formation of hepatoblast.

During the hepatoblast formation by both hepatic liver cells and biliary epithelial cells [14], Wnt/β-catenin is known to promote cell proliferation and differentiation along with the formation of progenitor hepatocytes [101]. The activation of this signalling pathway allows the conversion of non-hepatic endodermal cells to a hepatoblast condition. Contrarily, activation of TGF-β/Nodal signalling suppresses the expression of hepatogenic transcription factors such as HNF3β, and simultaneously enhances biliary epithelial cells transcription factors such as onecut1 (OC1), OC2, and HNFβ1 [102]. However, the secretion of growth factors, in particular HGF, by mesenchymal cells or non-parenchymal liver cells induces the expression of CCAAT/enhancer binding protein alpha (C/EBPα) that improves differentiation of hepatocytes. Hepatoblast proliferation is further enhanced with HGF stimulus via Wnt/β-catenin and HGF/c-Met pathways.

Once the hepatoblast is directed to the hepatocyte lineage and separated from biliary lineage, the maturation process take place in which the cells acquire metabolic properties. This process is controlled by a dynamic network compromising six transcription factors (HNF1α, HNF1β, HNF3β, HNF4α1 and HNF6) that functionally cross-regulate each other. Hepatocyte maturation is then determined not only by the presence of these genes but also by their abundance. Another cell intrinsic cue that promotes hepatocyte maturation is OSM, a member of interleukin-6 (IL-6) subfamily, which is usually produced by hematopoietic cells. This cytokine activates the signalling pathway of the signal transducer and the activator of transcription 3 (STAT3). On the other hand, ITS has been shown to effectively augment proliferation rate of hepatocytes through PI3K pathway. Meanwhile, DEX induces the expression of the hepatocyte nuclear factor of 4 and C/EBPα via regulation of the PI3K signalling pathway, wherein these two factors belong to the hepatocyte nuclear factors and are vital transcriptions for hepatocyte maturation. DEX eventually inhibits the growth inhibitory molecules of the hepatocyte such as CXC chemokine receptor and thus allows hepatocyte development.

12.4.2 Pancreas

In general, mechanisms of islet regeneration can be categorised into reversible epithelial-to-mesenchymal (EMT) transition, self-duplication, and re-ignition of the embryonic developmental program [103]. Regardless of their mechanism, the genesis of pancreatic cells takes place via critical pathways in the presence of their respective transcription factors.

First of all, Wnt/β-catenin signalling pathway plays a vital role in β cell proliferation as described in Thu et al. [104]. It gets activated when Wnt ligands bind to specific cell surface receptors, called Frizzled (Fzd), leading to activation of the intracellular Ca^{2+}, planar cell polarity, or the β-catenin/canonical branch of the pathway.

The presence of Wnt signalling stimulates expression of multiple β cell cycle regulators, including β-catenin, cyclin D1 and D2, resulting in enhanced islet proliferation [105, 106]. It was shown that these Wnt signals promoted the expression of Pitx2, a transcriptional activator which is directly associated with cis-regulatory elements in cyclin D2 gene. Furthermore, it was reported in García-Jiménez et al. [107] that the deletion of β-catenin contributed to a silencing effect in the Wnt pathway. In addition, this pathway is also implicated in foregut endoderm specification in which repressing Wnt signalling in the anterior endoderm was required for maintaining the foregut fate, whereas high levels of Wnt signalling in the posterior endoderm enhanced intestinal development [108].

Next, the PI3K signalling pathway has been shown to negatively regulate cellular differentiation especially in regards to β cells formation and maturation. Initially, this pathway was shown to be important in maintaining self-renewal through leukemia inhibitory factor (LIF)-activated signalling [109]. Consistent with this observation, it was later reported that activation of AKT which is a major downstream component of this pathway, had similar effects in maintaining the pluripotency of cells [110]. Furthermore, it was also reported that the suppression of PI3Ks facilitated the differentiation of ESCs into mesendoderm and definitive endoderm under circumstances in which activin-Nodal signalling was highly active [111]. Moreover, suppressing the PI3K pathway was reported to promote endocrine differentiation of human fetal pancreatic cells [112] perhaps suggesting an additional negative regulatory action of PI3K at a later stage in the β cell differentiation pathway.

Apart from this, Notch signalling also plays its role in deciding between the endocrine and progenitor/exocrine fates in the developing pancreas [113]. In this signalling system, ligand activation would normally lead to intracellular cleavage of the Notch receptor. The activated intracellular domain of Notch receptors interacts with the DNA-binding protein, recombination signal binding protein for immunoglobulin kappa J region (RBP-Jk), to activate expression of the negative basic helix-loop-helix (bHLH) HES genes, which, in turn, repress expression of downstream target genes including the Ngn genes [114, 115]. The repression of Ngn3 transcription through Hes activation prevents premature endocrine differentiation. This step serves to expand the pool of pancreatic progenitors before differentiation is initiated and notch inhibition results in Ngn3 expression, and further differentiation towards the endocrine fate [116].

Last but not least, another crucial signalling pathway worth discussing is the Hedgehog signalling pathway which has a dual role in endocrine pancreatic regeneration [117]. For instance, Hebrok et al. [118] have shown that Indian hedgehog (Ihh), together with Patched-1 (Ptc-1), a receptor and negative regulator of hedgehog activity, is expressed in pancreatic tissue. Meanwhile, sonic hedgehog, another hedgehog family member, is repressed by activin, a TGF-β signalling molecule, to promote pancreas development. The TGF-β pathway is the central network in generation of mesendoderm and DE, which leads to activation of Smoothened (Smo), a G-protein coupled-like receptor [119]. Thereafter, signalling is mediated in the Hedgehog signalling complex, which modifies the activity of Gli transcription factors in order to regulate expression of target genes.

12.5 In Vivo Studies

12.5.1 Hepatocyte-Like Cells Related Studies

Studies on the efficacy of DSCs to function as hepatocyte-like cells have been tested in animal models as early as 2008 by Ikeda et al. They have employed three-step approach whereby in the first step, cells isolated from third molar were exposed to aFGF for 5 days followed by HGF treatment for additional 5 days, and finally treatment with a cocktail of HGF, OSM, DEX and ITS-X for 11 days. They have transplanted 1×10^7 cells through portal vein of nude rats (F344) in which liver injury was created with intra-peritoneal (i.p.) carbon tetrachloride injection. They have discovered engraftment of cells with expressions of albumin (ALB), cytokeratin18, and cytokeratin19 coupled with proliferative behaviour. In addition, DSC transplanted rats showed suppression of liver fibrosis and steatonecrosis, thus, proving possible use of DSCs in liver tissue engineering.

Next, Ishkitiev et al. [84] investigated the in vivo efficacy of DSCs on two types of liver diseases, namely acute liver injury and secondary biliary cirrhosis. The damage for the former model was created via 80–90 % surgical resection whereas for the latter, the injury was induced via ligation of bile duct. A total of 2×10^6 SHEDs were transplanted to spleen of nude rats (F344/NJc1) after treating stem cells with HGF for 5 days, followed by OSM and DEX treatments. Histological analysis of the rat livers revealed that the engrafted human hepatic cells were distributed all over the regenerated organs of the transplanted animals in both models. Markers such as ALB, alpha feto protein (AFP), insulin-like growth factor-1 (IGF-1), and carbamoyl phosphate synthetase-1 (CPS-1) were also highly expressed in the regenerated organs indicating functional incorporation of SHEDs to the liver. Blood examinations also revealed a similar pattern, whereby decrement of white blood cells, total bilirubin, and blood urea nitrogen were profound in animals transplanted with human cells. However, the authors further noted that since SHEDs were originally transplanted into the spleen, cells expressing human-specific markers were found in the spleens of the transplanted animals.

12.5.2 Islet-Like Cells Related Studies

Despite numerous studies describing the potential of stem cells originated from dental sources, studies on the efficacy of DSC-derived ICAs in pre-clinical models are indeed very limited. Kanafi et al. [120] conducted a study to assess the efficacy of SHEDs and DPSCs in streptozotocin-induced DM model in mice. It was demonstrated that about 90 % of diabetic mice transplanted with encapsulated stem cell-derived islets survived, and were restored to normoglycemia within 2 weeks after transplantation and maintained normoglycemia for 2 months while their body weight and glucose levels in urine reverted to normal conditions. So far, this is the

only reported study showing a positive correlation of DSCs from the pre-clinical point of view. It is hoped that more of such studies will be conducted to further validate this observation and, as a matter of fact, our group is in the midst of conducting a pre-clinical study on the efficacy of SHEDs in streptozotocin-induced DM rat models. The idea is to evaluate the ability of IPCs generated from SHEDs to reduce the blood sugar levels to their normal ranges.

12.6 Remaining Challenges and Clinical Perspectives

Though a vast amount of in vitro studies investigating the potential of DSCs differentiating toward specific endodermal-related lineage is abundant, the translatability in either pre-clinical or clinical aspects are indeed very limited. The challenges described in preceding sub-chapter above shows that the smooth sailing journey of cell replacement therapy is still far from reality. As such, a collective effort must be taken by researchers worldwide to address these issues to be able to prepare ourselves regarding treatments for liver cirrhosis and DM.

One of the major challenge we face when dealing with DSCs is the small quantity of starting material. Unlike SCs from Wharton's' jelly or adipose tissue, the amount of cells upon dental pulp processing at passage 0 would be at most 1×10^6 cells, if proper measures in ensuring sterility is taken into account. Furthermore, in general transplantation scenario, 3×10^6 cells would ideally be transplanted for each kg of body weight [121]. As such, for a 70 kg person, the optimum amount of cells to be transplanted is 2.1×10^8. Looking at the limited ability of DSCs to proliferate, it is believed that hypoxic culture condition in 3D-bioreactor can be used to up-scale the proliferation rate. We have also previously discussed the effect of different commercially available culture media for the purpose of large scale expansion, especially on the proliferative behaviour on DSCs as well as their ability to differentiate towards selected cell lineage [122]. Perhaps combining the usage of 3D-bioreactor or serum-free micro-carrier system coupled with optimized culture media would enhance the output capacity [123, 124].

The next issue is pertaining on the immunological tolerance upon cell transplantation regardless of the origin of cells, such that use of whether autologous-based or allogeneic-based is still a subject of debate [125, 126]. Preferably, autologous-based therapy would be highly recommended to avoid immunological rejection [127, 128]. For instance, in order to regulate blood glucose homeostasis, islet transplantation has been considered as a safer and easier method for treating DM. However, the amount of cells required to achieve a substantial effect would be tremendous whereby performing it in autologous-based condition would be very challenging. As such, the only feasible approach is via pooled allogeneic-based islet transplantation. If not carefully planned, the success rate of such treatment would be very low possibly due to actions of immunological rejection. Here perhaps pre-conditioning of cells prior to transplantation might be helpful. This technique would prime the cells to behave accordingly when they are exposed to a specific microenvironment.

A number of in vitro studies have shown the improvement on the cell survivability when they were pre-conditioned thus providing us an avenue to look at with the aim of enhancing the outcome of transplantation works [18, 129–133].

Another approach worth considering for islet transplantation is the idea of encapsulating the islets with physical barrier to avoid immunological rejection while allowing transition of insulin, sugar, nutrients and oxygen. The material for encapsulation can be brown algae derived alginate which is currently being used in clinical trials [134]. Another material, polysulphone can also be utilized but it needs to be modified in order to reduce insulin adsorption [135]. Despite this, few disadvantages include inability of cytokines to move across the barrier and lack of nutrient causes further impairment upon transplantation [136, 137]. This has led to an invention of polymeric layers that can be coated directly onto islets surface which minimizes the distance between the islets and capsule by 1000-fold that later increases the islet survival [138, 139].

Further, to create synergistic effect while performing these techniques, additional factors such as the route of transplantation, dosages and duration of SCs treatment are believed to play central roles as well. Though intravenous is a very popular route of SC transfusion due to ease of access [140], SC administration near the injury site due to its better healing potential remains inevitable [141, 142]. Furthermore, it was shown that multiple delivery techniques have amplified cell homing ability to target area [143, 144]. Therefore, a thorough study is needed to investigate all these considerations before we could proceed toward clinical application.

12.7 Conclusions

DSCs are indeed very promising candidates in the promotion of regenerative medicine. The fact that these cells can be differentiate into endodermal lineage in both in vitro and in vivo conditions proved that in the long run, cell replacement therapies can perhaps become a reality. Furthermore, since dental pulps could be harvested from exfoliated teeth, perhaps they can serve as an ideal source for SCs banking. This would be very helpful in instances where pooled donors for allogeneic transplantation are desired. Though there are serious issues requiring further attention, we believe that with the technological advances prevalent today, these challenges can be overcome. We can only hope that in the near future, all of these concepts will be finally settled and this field will have progressed much close to definitive solutions for endodermal related diseases.

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References

1. Mozaffarian D, Benjamin EJ, Go AS et al (2015) Executive summary: heart disease and stroke statistics—2015 Update A Report from the American Heart Association. Circulation 131(4):434–441
2. Dope SA, Nair VV, Sudha R et al (2015) Prevalence of complications of diabetes mellitus type II in patients aged more than 30 years in Kanyakumari district. Indian J Pub Health Res Dev 6(1):275–280
3. Meah F, Juneja R (2015) Insulin tactics in type 2 diabetes. Med Clin North Amer 99(1):157–186
4. Sathananthan H (2007) Derivation of embryonic stem cells for therapy: new technologies. Reprod Biomed Online 14(5):635–639
5. Kaestner KH (2015) An epigenomic road map for endoderm development. Cell Stem Cell 16(4):343–344
6. Zorn AM, Wells JM (2009) Vertebrate endoderm development and organ formation. Ann Rev Cell Dev Biol 25:221–251
7. Hanley KP, Hearn T, Berry A et al (2010) In vitro expression of NGN3 identifies RAB3B as the predominant Ras-associated GTP-binding protein 3 family member in human islets. J Endocrino 207(2):151–161
8. Tremblay KD, Zaret KS (2005) Distinct populations of endoderm cells converge to generate the embryonic liver bud and ventral foregut tissues. Dev Biol 280:87–99
9. Jung J, Zheng M, Goldfarb M et al (1999) Initiation of mammalian liver development from endoderm by fibroblast growth factors. Science 284:1998–2003
10. McLin VA, Rankin SA, Zorn AM (2007) Repression of Wnt/{beta}-catenin signaling in the anterior endoderm is essential for liver and pancreas development. Development 134:2207–2217
11. Bort R, Signore M, Tremblay K et al (2006) Hex homeobox gene controls the transition of the endoderm to a pseudostratified, cell emergent epithelium for liver bud development. Dev Biol 290:44–56
12. Cirillo LA, Lin FR, Cuesta I et al (2002) Opening of compacted chromatin by early developmental transcription factors HNF3 (FoxA) and GATA-4. Mol Cell 9(2):279–289
13. Ober EA, Verkade H, Field HA et al (2006) Mesodermal Wnt2b signalling positively regulates liver specification. Nature 442:688–691
14. Germain L, Blouin MJ, Marceau N (1988) Biliary epithelial and hepatocytic cell lineage relationships in embryonic rat liver as determined by the differential expression of cytokeratins, alpha-fetoprotein, albumin, and cell surface-exposed components. Cancer Res 48(17):4909–4918
15. Herrera MB, Bruno S, Buttiglieri S, Tetta C, Gatti S, Deregibus MC, Bussolati B, Camussi G (2006) Isolation and characterization of a stem cell population from adult human liver. Stem Cells 24(12):2840–2850
16. Shafritz DA, Oertel M, Menthena A et al (2006) Liver stem cells and prospects for liver reconstitution by transplanted cells. Hepatology 43(2 Suppl 1):S89–S98
17. Zhao R, Duncan SA (2005) Embryonic development of the liver. Hepatology 41:956–967
18. Lammert E, Cleaver O, Melton D (2003) Role of endothelial cells in early pancreas and liver development. Mech Dev 120(1):59–64
19. Matsumoto K, Yoshitomi HR et al (2001) Liver organogenesis promoted by endothelial cells prior to vascular function. Science 294:559–563
20. Cleaver O, Melton DA (2003) Endothelial signaling during development. Nat Med 9(6):661–668
21. Lemaigre F, Zaret KS (2004) Liver development update: new embryo models, cell lineage control, and morphogenesis. Curr Opin Genet Dev 14(5):582–590
22. Michalopoulos GK, Bowen WC, Mule K et al (2003) HGF-, EGF-, and dexamethasone-induced gene expression patterns during formation of tissue in hepatic organoid cultures. Gene Expr 11:55–75

23. Snykers S, Vanhaecke T, Papeleu P et al (2006) Sequential exposure to cytokines reflecting embryogenesis: the key for in vitro differentiation of adult bone marrow stem cells into functional hepatocyte-like cells. Toxicol Sci 94(2):330–341
24. Suzuki A, Iwama A, Miyashita H et al (2003) Role for growth factors and extracellular matrix in controlling differentiation of prospectively isolated hepatic stem cells. Development 130:2513–2524
25. Courtney M, Rabe T, Collombat P et al (2014) Pax4 and Arx represent crucial regulators of the development of the endocrine pancreas. New J Sci 2014, http://dx.doi.org/10.1155/2014/981569
26. Ben-Othman N, Courtney M, Vieira A et al (2013) From pancreatic islet formation to beta-cell regeneration. Diab Res Clin Prac 101(1):1–9
27. Fukuda A, Kawaguchi Y, Furuyama K et al (2006) Ectopic pancreas formation in Hes1-knockout mice reveals plasticity of endodermal progenitors of the gut, bile duct, and pancreas. J Clin Invest 116(6):1484–1493
28. Jeon J, Correa-Medina M, Ricordi C et al (2009) Endocrine cell clustering during human pancreas development. J Histochem Cytochem 57(9):811–824
29. Sander M, Sussel L, Conners J et al (2000) Homeobox gene Nkx6. 1 lies downstream of Nkx2. 2 in the major pathway of beta-cell formation in the pancreas. Development 127(24):5533–5540
30. Xu X, D'Hoker J, Stange G et al (2008) β cells can be generated from endogenous progenitors in injured adult mouse pancreas. Cell 132(2):197–207
31. Collombat P, Hecksher-Sørensen J, Krull J et al (2007) Embryonic endocrine pancreas and mature β cells acquire α and PP cell phenotypes upon Arx misexpression. J Clin Invest 117(4):961–970
32. Zorn AM, Wells JM (2007) Molecular basis of vertebrate endoderm development. Int Rev Cyto 259:49–111
33. Collombat P, Hecksher-Sørensen J, Serup P et al (2006) Specifying pancreatic endocrine cell fates. Mech Dev 123(7):501–512
34. Smith SB, Gasa R, Watada H et al (2003) Neurogenin3 and hepatic nuclear factor 1 cooperate in activating pancreatic expression of Pax4. J Biol Chem 278:38254–38259
35. Wang J, Elghazi L, Parker SE et al (2004) The concerted activities of Pax4 and Nkx2.2 are essential to initiate pancreatic beta-cell differentiation. Dev Biol 266:178–189
36. Osipovich AB, Long Q, Manduchi E et al (2014) Insm1 promotes endocrine cell differentiation by modulating the expression of a network of genes that includes Neurog3 and Ripply3. Development 141(15):2939–2949
37. Artner I, Blanchi B, Raum JC et al (2007) MafB is required for islet β cell maturation. Proc Nat Acad Sci U S A 104:3853–3858
38. Artner I, Le Lay J, Hang Y et al (2006) MafB: an activator of the glucagon gene expressed in developing islet a- and b-cells. Diabetes 55:297–304
39. Carrasco M, Rojas A, Delgado I et al (2015) Regulation of pancreatic islet formation. Islets of Langerhans. Springer, The Netherlands, pp 109–128
40. van der Meulen T, Huising MO et al (2015) Role of transcription factors in the transdifferentiation of pancreatic islet cells. J Mol Endocrino 54(2):R103–R117
41. Michalopoulos GK, DeFrances MC (1997) Liver regeneration. Science 276(5309):60–66
42. Pachter HL, Todd SR (2015) Liver trauma: parenchymal repair and resectional debridement. In: Ivatury RR (ed) Operative techniques for severe liver injury. New York, Springer, pp 57–66
43. Pillai A, Thapar M (2015) Drug-induced liver injury after soy protein supplement use. ACG Case Rep J 2(3):178–180
44. Gu Q, Guan H, Shi Q et al (2015) Curcumin attenuated acute Propionibacterium acnes-induced liver injury through inhibition of HMGB1 expression in mice. Int Immunopharmaco 24(2):159–165
45. Ramachandran A, Lebofsky M, Yan HM et al (2015) Hepatitis C virus structural proteins can exacerbate or ameliorate acetaminophen-induced liver injury in mice. Arch Toxico 89(5):773–783
46. Cai J, Ito M, Nagata H et al (2002) Treatment of liver failure in rats with end-stage cirrhosis by transplantation of immortalized hepatocytes. Hepatology 36(2):386–394

47. Kobayashi N, Noguchi H, Watanabe T et al (2000) A new approach to develop a biohybrid artificial liver using a tightly regulated human hepatocyte cell line. Hum Cell 13(4):229–235
48. Avital I, Feraresso C, Aoki T et al (2002) Bone marrow-derived liver stem cell and mature hepatocyte engraftment in livers undergoing rejection. Surgery 132(2):384–390
49. Davila JC, Cezar GG, Thiede M et al (2004) Use and application of stem cells in toxicology. Toxicol Sci 79(2):214–223
50. Ruhnke M, Ungefroren H, Zehle G et al (2003) Long –term culture and differentiation of rat embryonic stem cell –like cells into neuronal, glial, endothelial, and hepatic lineages. Stem Cells 21(4):428–436
51. Tseng DS, Hjalmar C, Inne HM et al (2014) Exocrine pancreatic insufficiency in patients with pancreatic or peri-ampullary cancer: a systematic review. Pancreatology 14(3):S43
52. Wang HM, Dong JH, Li Q et al (2014a) A stress response pathway in mice upregulates somatostatin level and transcription in pancreatic delta cells through Gs and β-arrestin. Diabetologia 57(9):1899–1910
53. Wang S, Tong Z, Li W et al (2015) Early severe acute pancreatitis: a subgroup of critical acute pancreatitis. Ann Surg 261(4):e103–e104
54. Williams JA (2014) Pancreatic polypeptide. The Pancreapedia: Exocrine Pancreas Knowledge Base. doi:10.3998/panc.2014.4
55. Campbell J, Sanders D, Lee S et al (2014) PTH-097 how common is pancreatic exocrine insufficiency in secondary care gastroenterology clinics? A dual centre study. Gut 63(Suppl 1):A253
56. Tsiotos GG, León LD, Sarr MG (1998) Long-term outcome of necrotizing pancreatitis treated by necrosectomy. Brit J Surg 85(12):1650–1653
57. American Diabetes Association (2013) Diagnosis and classification of diabetes mellitus. Diabetes Care 36(Supplement 1):S67–S74
58. Atkinson MA, Eisenbarth GS (2001) Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 358(9277):221–229
59. D'Haese JG, Ceyhan GO, Demir IE et al (2014) Pancreatic enzyme replacement therapy in patients with exocrine pancreatic insufficiency due to chronic pancreatitis: a 1-year disease management study on symptom control and quality of life. Pancreas 43(6):834–841
60. Hughes DS, Narendran P (2014) Alpha cell function in type 1 diabetes. Brit J Diab Vas Dis 14(2):45–51
61. Leese GP, Wang J, Broomhall J et al (2003) Frequency of severe hypoglycemia requiring emergency treatment in Type 1 and Type 2 diabetes A population-based study of health service resource use. Diab Care 26(4):1176–1180
62. von Eckardstein A, Widmann C (2014) HDL, beta cells and diabetes. Cardiovasc Res. doi:10.1093/cvr/cvu143
63. Elleri D, Dunger DB, Hovorka R (2011) Closed-loop insulin delivery for treatment of type 1 diabetes. BMC Med 9(1):120
64. Skyler JS, Ricordi C (2011) Stopping type 1 diabetes: attempts to prevent or cure type 1 diabetes in man. Diabetes 60(1):1–8
65. Steil GM, Rebrin K, Darwin C et al (2006) Feasibility of automating insulin delivery for the treatment of type 1 diabetes. Diabetes 55(12):3344–3350
66. Kahn SE, Cooper ME, Del Prato S (2014) Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet 383(9922):1068–1083
67. Cortés VA, Cautivo KM, Rong S et al (2014) Leptin ameliorates insulin resistance and hepatic steatosis in Agpat2–/– lipodystrophic mice independent of hepatocyte leptin receptors. J Lipid Res 55(2):276–288
68. Linden MA, Fletcher JA, Morris EM et al (2014) Combining metformin and aerobic exercise training in the treatment of type 2 diabetes and NAFLD in OLETF rats. Amer J Physio-Endocrino Met 306(3):E300–E310
69. Sullivan SD, Jablonski KA, Florez JC et al (2014) Genetic risk of progression to type 2 diabetes and response to intensive lifestyle or metformin in prediabetic women with and without a history of gestational diabetes mellitus. Diab Care 37(4):909–911

70. Sykes AP, Kemp GL, Dobbins R et al (2015) Randomized efficacy and safety trial of once-daily remogliflozin etabonate for the treatment of type 2 diabetes. Diab Obes Met 17:98–101. doi:10.1111/dom.12393
71. Eng C, Kramer CK, Zinman B et al (2015) Glucagon-like peptide-1 receptor agonist and basal insulin combination treatment for the management of type 2 diabetes: a systematic review and meta-analysis. Lancet 384(9961):2228–2234
72. Hausenblas HA, Schoulda JA, Smoliga JM (2015) Resveratrol treatment as an adjunct to pharmacological management in type 2 diabetes mellitus—systematic review and meta-analysis. Mol Nutr Food Res 59:147–159. doi:10.1002/mnfr.201400173
73. Govindasamy V, Abdullah AN, Ronald VS et al (2010a) Inherent differential propensity of dental pulp stem cells derived from human deciduous and permanent teeth. J Endod 36(9):1504–1515
74. Gronthos S, Mankani M, Brahim J et al (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Nat Acad Sci U S A 97(25):13625–13630
75. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126(4):663–676
76. Miura M, Gronthos S, Zhao M et al (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Nat Acad Sci U S A 100(10):5807–5812
77. Nakamura S, Yamada Y, Katagiri W et al (2009) Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. J Endod 35(11):1536–1542
78. Bluteau G, Luder HU, De Bari C et al (2008) Stem cells for tooth engineering. Eur Cell Mater 16:1–9
79. Yao S, Pan F, Prpic V et al (2008) Differentiation of stem cells in the dental follicle. J Dent Res 87(8):767–771
80. Estrela C, Holland R, Estrela CRDA et al (2014) Characterization of successful root canal treatment. Bra Dent J 25(1):3–11
81. Ikeda E, Yagi K, Kojima M et al (2008) Multipotent cells from the human third molar: feasibility of cell-based therapy for liver disease. Differentiation 76(5):495–505
82. Ishkitiev N, Yaegaki K, Calenic B et al (2010) Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features in vitro. J Endod 36(3):469–474
83. Ishkitiev N, Yaegaki K, Imai T et al (2012) High-purity hepatic lineage differentiated from dental pulp stem cells in serum-free medium. J Endod 38(4):475–480
84. Ishkitiev N, Yaegaki K, Imai T et al (2015) Novel management of acute or secondary biliary liver conditions using hepatically differentiated human dental pulp cells. Tissue Eng Part A 21(3–4):586–593
85. Okada M, Imai T, Yaegaki K et al (2014a) Regeneration of insulin-producing pancreatic cells using a volatile bioactive compound and human teeth. J Breath Res 8(4):046004
86. Vasanthan P, Gnanasegaran N, Govindasamy V et al (2014) Comparison of fetal bovine serum and human platelet lysate in cultivation and differentiation of dental pulp stem cells into hepatic lineage cells. Biochem Eng J 88:142–153
87. Huang CC, Pelaez D, Bendala JD et al (2009) Plasticity of stem cells derived from adult periodontal ligament. Reg Med 4(6):809–821
88. Gerace D, Martiniello-Wilks R, O'Brien BA et al (2015) The use of β-cell transcription factors in engineering artificial β cells from non-pancreatic tissue. Gene Ther 22(1):1–8
89. Pokrywczynska M, Krzyzanowska S, Jundzill A et al (2013) Differentiation of stem cells into insulin-producing cells: current status and challenges. Arch Immuno Therap Exp 61(2):149–158
90. Govindasamy V, Ronald VS, Abdullah AN et al (2011) Differentiation of dental pulp stem cells into islet-like aggregates. J Dent Res 90(5):646–652
91. Tsaniras SC, Jones PM (2010) Generating pancreatic β-cells from embryonic stem cells by manipulating signaling pathways. J Endocrino 206(1):13–26
92. Sawangmake C, Nowwarote N, Pavasant P et al (2014) A feasibility study of an in vitro differentiation potential toward insulin-producing cells by dental tissue-derived mesenchymal stem cells. Biochem Biophy Res Comm 452(3):581–587

93. Lee JS, An SY, Kwon IK et al (2014a) Transdifferentiation of human periodontal ligament stem cells into pancreatic cell lineage. Cell Biochem Func 32(7):605–611
94. Carnevale G, Riccio M, Pisciotta A et al (2013) In vitro differentiation into insulin-producing β-cells of stem cells isolated from human amniotic fluid and dental pulp. Dig Liv Dis 45(8):669–676
95. Ishkitiev N, Yaegaki K, Kozhuharova A et al (2013) Pancreatic differentiation of human dental pulp CD117+ stem cells. Reg Med 8(5):597–612
96. Di Meglio F, Castaldo C, Nurzynska D et al (2010) Epithelial–mesenchymal transition of epicardial mesothelium is a source of cardiac CD117-positive stem cells in adult human heart. J Mol Cel Cardio 49(5):719–727
97. Miettinen M, Lasota J (2005) KIT (CD117): a review on expression in normal and neoplastic tissues, and mutations and their clinicopathologic correlation. Appl Immunohistochem Mol Morpho 13(3):205–220
98. Okada M, Ishkitiev N, Yaegaki K et al (2014b) Hydrogen sulphide increases hepatic differentiation of human tooth pulp stem cells compared with human bone marrow stem cells. Int Endod J 47(12):1142–1150
99. Kubo A, Shinozaki K, Shannon JM et al (2004) Development of definitive endoderm from embryonic stem cells in culture. Development 131(7):1651–1662
100. Yasunaga M, Tada S, Torikai-Nishikawa S et al (2005) Induction and monitoring of definitive and visceral endoderm differentiation of mouse ES cells. Nat Biotechnol 23(12): 1542–1550
101. Yoshida Y, Shimomura T, Sakabe T et al (2007) A role of Wnt/beta-catenin signals in hepatic fate specification of human umbilical cord blood-derived mesenchymal stem cells. Am J Physiol Gastrointest Liver Physiol 293(5):G1089–G1098
102. Kodama Y, Hijikata M, Kageyama R et al (2004) The role of notch signaling in the development of intrahepatic bile ducts. Gastroenterology 127(6):1775–1786
103. DomÃnguez-Bendala J (2009) In: Turksen K (ed) Pancreatic stem cells. Humana Press, SpringerLink, New York
104. Thu KL, Radulovich N, Becker-Santos DD et al (2014) SOX15 is a candidate tumor suppressor in pancreatic cancer with a potential role in Wnt/β-catenin signaling. Oncogene 33(3): 279–288
105. McFarlane MR, Brown MS, Goldstein JL et al (2014) Induced ablation of ghrelin cells in adult mice does not decrease food intake, body weight, or response to high-fat diet. Cell Metabolism 20(1):54–60
106. Sarkar SA, Kobberup S, Wong R et al (2008) Global gene expression profiling and histochemical analysis of the developing human fetal pancreas. Diabetologia 51(2):285–297
107. García-Jiménez C, García-Martínez JM, Chocarro-Calvo A et al (2014) A new link between diabetes and cancer: enhanced WNT/β-catenin signaling by high glucose. J Mol Endocrino 52(1):R51–R66
108. Kim JT, Liu C, Zaytseva YY et al (2015) Neurotensin, a novel target of Wnt/β-catenin pathway, promotes growth of neuroendocrine tumor cells. Int J Cancer 136(6):1475–1481
109. Harvey AE, Lashinger LM, Hays D et al (2014) Calorie restriction decreases murine and human pancreatic tumor cell growth, nuclear factor-κB activation, and inflammation-related gene expression in an insulin-like growth factor-1– dependent manner. PloS One 9(5), e94151
110. O'Reilly S, Ciechomska M, Cant R et al (2014) Interleukin-6 (IL-6) trans signaling drives a STAT3-dependent pathway that leads to hyperactive transforming growth factor-β (TGF-β) signaling promoting SMAD3 activation and fibrosis via Gremlin protein. J Biol Chem 289(14):9952–9960
111. Gaarenstroom T, Hill CS (2014) TGF-signaling to chromatin: how Smads regulate transcription during self-renewal and differentiation. Semin Cell Dev Biol 32:107–118
112. Li Z, Shangguan Z, Liu Y et al (2014) Puerarin protects pancreatic β-cell survival via PI3K/Akt signaling pathway. J Mol Endocrino 53(1):71–79
113. Abel EV, Kim EJ, Wu J et al (2014) The Notch pathway is important in maintaining the cancer stem cell population in pancreatic cancer. PloS One 9(3), e91983

114. Lee JJ, Perera RM, Wang H et al (2014b) Stromal response to Hedgehog signaling restrains pancreatic cancer progression. Proc Nat Acad Sci U S A 111(30):E3091–E3100
115. Thomas MM, Zhang Y, Mathew E et al (2014) Epithelial Notch signaling is a limiting step for pancreatic carcinogenesis. BMC Cancer 14(1):862
116. Derynck R, Muthusamy BP, Saeteurn KY (2014) Signaling pathway cooperation in TGF-β-induced epithelial–mesenchymal transition. Curr Opin Cell Bio 31:56–66
117. Mathew E, Zhang Y, Holtz AM et al (2014) Dosage-dependent regulation of pancreatic cancer growth and angiogenesis by hedgehog signaling. Cell Rep 9(2):484–494
118. Hebrok M, Kim SK, St Jacques B et al (2000) Regulation of pancreas development by hedgehog signaling. Development 127(22):4905–4913
119. Honselmann KC, Pross M, Jung CMF et al (2015) Regulation mechanisms of the hedgehog pathway in pancreatic cancer: a review. JOP 16(1):25–32
120. Kanafi MM, Rajeshwari YB, Gupta S et al (2013) Transplantation of islet-like cell clusters derived from human dental pulp stem cells restores normoglycemia in diabetic mice. Cytotherapy 15(10):1228–1236
121. Chelluboina B, Veeravalli KK (2015) Application of human umbilical cord blood-derived mononuclear cells in animal models of ischemic stroke. J Stem Cell Res Transplant 2(1):1014–1021
122. Govindasamy V, Ronald VS, Totey S et al (2010b) Micromanipulation of culture niche permits long-term expansion of dental pulp stem cells—an economic and commercial angle. Vitro Cel Dev Biol-Anim 46(9):764–773
123. Lohmann M, Walenda G, Hemeda H et al (2012) Donor age of human platelet lysate affects proliferation and differentiation of mesenchymal stem cells. PLoS One 7(5), e37839
124. Vecchiatini R, Penolazzi L, Lambertini E et al (2014) Effect of dynamic three-dimensional culture on osteogenic potential of human periodontal ligament-derived mesenchymal stem cells entrapped in alginate microbeads. J Perio Res. doi:10.1111/jre.12225
125. Talens-Visconti R, Bonora A, Jover R et al (2007) Human mesenchymal stem cells from adipose tissue: differentiation into hepatic lineage. Toxicol In Vitro 21(2):324–329
126. Truong W, Hancock WW, Anderson CC et al (2006) Coinhibitory T-cell signaling in islet allograft rejection and tolerance. Cell Transplant 15(2):105–119
127. Elseberg CL, Salzig D, Czermak P (2014) Bioreactor expansion of human mesenchymal stem cells according to GMP requirements. Methods Mol Biol. doi:10.1007/7651_2014_117
128. Lu SJ, Kelley T, Feng Q et al (2013) 3D microcarrier system for efficient differentiation of human induced pluripotent stem cells into hematopoietic cells without feeders and serum. Reg Med 8(4):413–424
129. De Santa BP, Van Den Brink GR, Roberts DJ (2003) Development and differentiation of the intestinal epithelium. Cell Mol Life Sci 60(7):1322–1332
130. Gnanasegaran N, Govindasamy V, Musa S et al (2014) ReNCell VM conditioned medium enhances the induction of dental pulp stem cells into dopaminergic like cells. Cytotech 68(2):343–353
131. Kanai-Azuma M, Kanai Y, Gad JM et al (2002) Depletion of definitive gut endoderm in Sox17-null mutant mice. Development 129(10):2367–2379
132. Norris DP, Brennan J, Bikoff EK et al (2002) The Foxh1-dependent autoregulatory enhancer controls the level of Nodal signals in the mouse embryo. Development 129(14):3455–3468
133. Richardson SM, Hoyland JA, Mobasheri R et al (2010) Mesenchymal stem cells in regenerative medicine: opportunities and challenges for articular cartilage and intervertebral disc tissue engineering. J Cell Physiol 222(1):23–32
134. Korbutt GS, Mallett AG, Ao Z et al (2004) Improved survival of microencapsulated islets during in vitro culture and enhanced metabolic function following transplantation. Diabetologia 47(10):1810–1818
135. Shen C, Meng Q, Zhang G (2011) Chemical modification of polysulfone membrane by polyethylene glycol for resisting drug adsorption and self-assembly of hepatocytes. J Memb Sci 369(1):474–481

136. Chiang CH, Wu WW, Li HY et al (2015) Enhanced antioxidant capacity of dental pulp-derived iPSC-differentiated hepatocytes and liver regeneration by injectable HGF-releasing hydrogel in fulminant hepatic failure. Cell Transplant 24(3):541–559
137. Moritz W, Meier F, Stroka DM et al (2002) Apoptosis in hypoxic human pancreatic islets correlates with Hif-1α expression. Faseb J 16(7):745–747
138. Krol S, del Guerra S, Grupillo M et al (2006) Multilayer nanoencapsulation. New approach for immune protection of human pancreatic islets. Nano Lett 6(9):1933–1939
139. Nolan K, Millet Y, Ricordi C et al (2008) Tissue engineering and biomaterials in regenerative medicine. Cell Transplant 17(3):241–243
140. Guimarães ET, Cruz GDS, Farias de Almeida T et al (2013) Transplantation of stem cells obtained from murine dental pulp improves pancreatic damage, renal function, and painful diabetic neuropathy in diabetic type 1 mouse model. Cell Transplant 22(12):2345–2354
141. Gu G, Dubauskaite J, Melton DA (2002) Direct evidence for the pancreatic lineage: NGN3$^+$ cells are islet progenitors and are distinct from duct progenitors. Development 129(10):2447–2457
142. Harrison KA, Thaler J, Pfaff SL et al (1999) Pancreas dorsal lobe agenesis and abnormal islets of Langerhans in Hlxb9-deficient mice. Nat Gen 23(1):71–75
143. Brocard J, Feil R, Chambon P et al (1998) A chimeric Cre recombinase inducible by synthetic, but not by natural ligands of the glucocorticoid receptor. Nuc Acids Res 26(17): 4086–4090
144. Feil R, Brocard J, Mascrez B et al (1996) Ligand-activated site-specific recombination in mice. Proc Nat Acad Sci U S A 93(20):10887–10890

Chapter 13
Dental Stem Cells in Regenerative Medicine: Clinical and Pre-clinical Attempts

Ferro Federico and Renza Spelat

13.1 Dental Stem Cells (DSCs) Origin and Types

Mammalian tooth tissues, which share a number of morphogenetic similarities with many ectodermal organs such as hairs and glands, derive from reciprocal interactions of oral ectoderm and neural crest mesenchyme [1, 2]. The first sign of tooth morphogenesis is the establishment of dental lamina, a structure that represents a condensation of the oral epithelium, with relative thickening corresponding to the future dental region. Therefore, dental crown organogenesis proceeds through three different stages known as bud, cap, and bell, and when the crown has almost completely formed, root formation starts to differentiate from the dental follicle and the dental papilla mesenchyme [1, 2].

Epithelial and mesenchymal reciprocal interactions as result of a conserved three-dimensional molecular signaling environment are crucial not just during tooth morphogenesis but also for the stem cell's niche establishment and maintenance. In fact, it has been confirmed that stem cells reside in adult dental tissues, and that Morsczeck et al. [3] demonstrated their neural crest origin. At present, five types of DSCs have been isolated from adult, both mature and immature, and embryonic-like dental tissues. These stem cells can be simply divided into two groups depending on the dental tissue derivation and dental developmental stage reached (Fig. 13.1).

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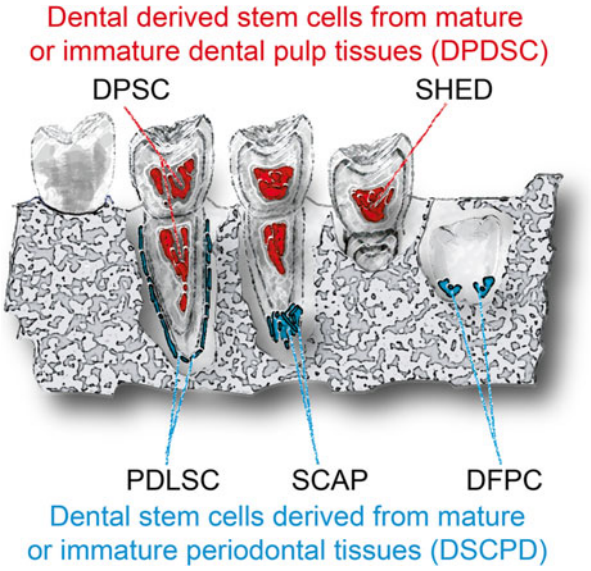

Fig. 13.1 Sources of dental stem cells (DSCs). Five types of DSCs have been isolated from adult, both from mature or immature, and embryonic-like dental tissues. Acronyms: DPSCs, Dental Pulp Stem Cells; SHEDs, Stem cells from Human Exfoliated Deciduous teeth; PDLSCs, Periodontal Ligament Stem Cells; SCAPs, Stem Cells of the Apical Papilla; DFSCs, Dental Follicle Stem Cells

13.1.1 DSC Classification

13.1.1.1 DSCs Derived from Mature or Immature Dental Pulp Tissues (Dental Pulp Derived Stem Cells-DPDSCs)

Dental pulp stem cells (DPSCs) were isolated for the first time by Gronthos et al. [4] from extracted adult dental tooth pulp tissues. It is generally believed that these cells are mainly present or derive from the perivascular regions [5] or peripheral nerve-associated glia [6] of the pulpal cavity, migrating from there to the site of injury when required.

Stem cells from exfoliated deciduous teeth (SHEDs) have been isolated for the first time by Miura et al. [7] reporting that even exfoliated/loosen organs contain stem cells. More recently, a subgroup of highly immature stem cells have been isolated both from dental pulps of supernumerary teeth as well as from exfoliated deciduous teeth [8]. Data derived from their characterization studies confirmed that those cells express not only mesenchymal stem cell (MSC) markers but also embryonic stem cell (ESC) markers, suggesting that these cells are precursors of DPSCs and SHEDs. However, it was recently demonstrated that DPSCs and SHEDs also express those markers [9, 10].

13.1.1.2 DSCs Derived from Mature or Immature Periodontal Tissues (DSCs Periodontium Derived-DSCPD)

Recent evidence suggests that stem cells are present in the dental follicle (DFSCs) of the tooth germs at various stages of development [11]. Dental follicle is a loose embryonic-like connective tissue, part of the dental sac, which surrounds the developing tooth and is involved in periodontium formation as well as in tooth eruption.

DSCs derived from apical papilla (SCAPs); apical papilla is a cell mass at the tip of developing tooth roots, which is rich in progenitor cells involved in root formation [12]. Both DFSCs and SCAPs are present and isolable only for a limited time because dental follicle and apical papilla become a part of the dental periodontium and dental root during tooth maturation and formation [11–14].

Another source of DSCs is that of mature periodontal tissues, *i.e.* periodontal ligaments, alveolar bone, cementum, and all those tissues involved in tooth fixation to dental alveolus. These tissues cover the whole tooth root's outer surface and presence of stem cells within those fully differentiated tissues has been proven, referred to as periodontal ligament stem cells (PDLSCs) [15].

13.2 In Vitro Properties of DSCs

DSCs are an easily accessible source of adult stem cells, and do not involve the ethical issues associated with stem cells derived from embryonic tissues. They have also been shown to maintain their stemness and self-renewal capabilities after cryopreservation and thawing [16, 17]. In general, cell characterization studies revealed that DSCs express mesenchymal markers along with embryonic stem cell markers as summarized in Table 13.1 [8, 9, 19, 20].

Many in vitro studies verified that DSCs (DPDSC and DSCPD) are multipotent because of having differentiation potential towards at least three distinct cell lineages: osteo/odontogenic, adipogenic and neurogenic [3, 7, 9, 14, 17, 18, 21]. However, it was also evidenced that DPDSCs are capable of chondrogenic, myogenic [17], endothelial [22], hepatocytic [9, 23, 24] in vitro differentiation capacities, and can also be used for derivation of induced pluripotent stem cells (iPSC) [25]. Siqueira da Fonseca et al. [26] also proved that human DPDSCs, contributing to the formation of mouse/human chimaera embryos, are also able to differentiate into cell types derived from ectoderm and endoderm despite their ecto-mesenchymal origin. Those results have confirmed that DPDSCs are pluripotent and biologically compatible with the mouse embryonic environment, capable of being 'reprogrammed' to a cell type similar to the embryonic phenotype by the host milieu, without any human external modification [26]. In addition, many studies have reported the immunomodulatory and immunosuppressive properties of DSCs by three main action of mechanisms: (i) cell cycle arrest of immune cells at the G1 phase, (ii) direct interaction with immune cells, and (iii) paracrine effect through secretion of various factors including human leukocyte

Table 13.1 Summary of the mesenchymal and embryonic stem cell markers expressed in dental derived stem cells (DSCs). Kerkis et al. [8], Ferro et al.[18]), Bojic et al. [19], Huang et al. [20], Ferro et al. [9]

		DPDSC		DSCPD		
Name	Alternative name	DPSCs	SHEDs	DFPCs	SCAPs	PDLSCs
CD 13	Aminopeptidase N	+	+	+	+	+
CD 29	Integrin β1	+	+	+	+	+
CD 44	Hyaluronate receptor	+	+	+	+	+
CD 73	Ecto-5'-nuclotidase	+	+	+	+	
CD 90	Thy-1	+	+	+	+	+
CD 105	Endoglin	+	+	+	+	+
CD 146	Mel-CAM	+	+		+	+
Oct4 (octamer-binding transcription factor 4)	POU5F1	+	+	+	+	+
Nanog	Homeobox protein NANOG	+	+			
Sox2	SRY - box 2	+	+			
SSEA4 (stage specific embryonic antigen 4)		+	+			
SSEA3 (stage specific embryonic antigen 3)		+	+			
TRA 1-60 (tumor recognition antigen 1-60)		+	+			

Acronyms: DPDSCs, Dental Pulp Derived Stem Cells; DSCPDs, Dental Stem Cells Periodontium Derived; DPSCs, Dental Pulp Stem Cells; SHEDs, Stem cells from Human Exfoliated Deciduous teeth; PDLSCs, Periodontal Ligament Stem Cells; SCAPs, Stem Cells of the Apical Papilla; DFSCs, Dental Follicle Stem Cells; CD, Cluster of Differentiation

antigen G (HLA-G), prostaglandin E2, various cytokines (TGFβ, IL6, IL10, HGF, VEGF, etc.) and enzymes (indoleamine 2,3-dioxygenase and inducible nitric oxide synthase) [27–29].

13.3 Clinical and Pre-clinical Trials of DSCs

Because of their relatively simple recovering methodology, availability, tolerogenic properties and differentiation capabilities, DSCs have also been tested in various in vivo trials. However, the vast majority of these in vivo studies have been conducted on animal models, and to date very few clinical trials have been completed or are currently being performed.

Because stem cells derived from embryonic-like dental tissues (DFSCs) are not a readily available cell source, we are not going to discuss them; instead we prefer to highlight those DSCs which are most suitable for clinical trials due to being easier to obtain such as DPDSC (DPSCs and SHEDs), DSCPD) (SCAPs and PDLSCs) (Fig. 13.2).

13.3.1 Clinical and Pre-clinical Trials of DPDSCs

DPDSCs have been proven to be useful not only for dental tissue engineering and periodontal tissue regeneration but also for regeneration of structures of the craniofacial region.

13.3.1.1 Odontogenic and Osteogenic Differentiation

Different attempts to obtain de novo pulp regeneration have been made by recellularizing tooth mineralized fragments seeded with DPDSCs, followed by their in vivo implantation. Six weeks after implantation, results confirmed successful regeneration of dental pulp-like tissue but not odontoblast-like cells [30]. A vascularized pulp tissue, as well as a dentin-like mineral tissue, were obtained by Huang et al. [31] when a section of human tooth root, modified with synthetic scaffold, and seeded with DPDSCs, was transplanted in vivo. Similarly, another research demonstrated that transplantation of DPDSCs cultured in aggregate condition or DPDSCs seeded on collagen scaffold in a dog model were both able to regenerate a dental pulp/odontoblastic-like tissue [32, 33] (Fig. 13.2).

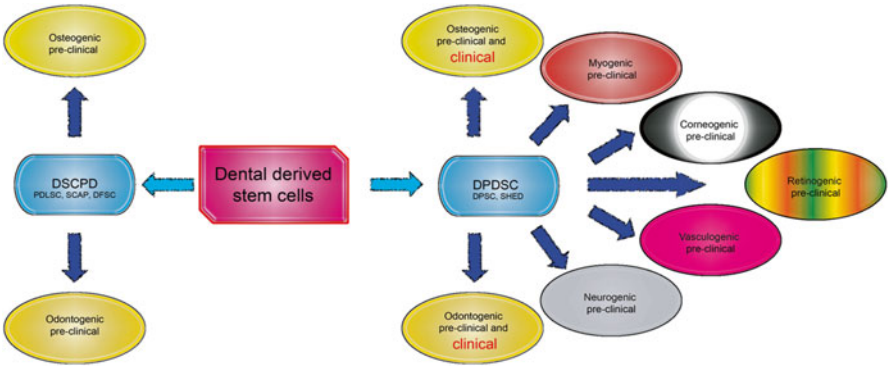

Fig. 13.2 Summary of pre-clinical and clinical dental stem cell (DSC) studies. Pre-clinical studies verified DSCs (DPDSCs and DSCPDs) stemness, by testing their differentiation capabilities. Completed and ongoing clinical trials have already or are being performed in order to prove DPDSCs (DPSCs and SHEDs) clinical effectiveness in human. Acronyms: DPDSCs, Dental Pulp Derived Stem Cells; DSCPDs, Dental Stem Cells Periodontium Derived

In a pre-clinical study, Graziano et al. used a scaffold system seeded with DPDSCs, previously pre-differentiated into osteoblastic cells, to test the bone regeneration capability of stem cells after transplantation into immunocompromised rats (2008). Hydroxyapatite nano-crystals, as well as bone specific markers in the explanted constructs, were confirmed by using X-ray diffraction and immunohistochemical analysis, respectively [34]. Furthermore, in yet another study, rats with large cranial defects were cured by using DPDSCs implanted a collagen membrane [35].

In one of the few attempted clinical trials, human DPDSCs isolated from third molars were seeded onto collagen scaffolds. After that, both mandibular third molars of the patients were extracted at the same time, and one of the two sockets was then filled with the collagen scaffold seeded with DPSCs, while the other one, filled with collagen scaffold alone, was used as negative control. After 3 months period, radiographic and histological analysis evidenced a higher amount of bone restoration in patients treated with DPSCs than in those treated with collagen scaffold alone [36]. In addition, after a 3 year follow-up, novel synchrotron radiation-based holo-tomographic (HT) imaging analysis confirmed that regenerated bone was homogeneously vascularized and qualitatively compact. This clinical trial shed light on the possibility of using DPSCs to restore bone defect in humans, indicating that DPSCs can have a future leading role at least in bone regenerative therapy [37] (Fig. 13.2).

Two other ongoing clinical trials are summarized in the U.S. National Institutes of Health ClinicalTrials.gov site. The first one is conducted by Daniela Franco Bueno at the hospital sirio-libanes in Brazil, which aims to show positive effects of DPSCs seeded in a collagen and hydroxyapatite biomaterial system on patients with cleft lip and palate alveolar bone defects through prospective qualitative and quantitative investigation of bone de-novo formation (NIH [38] ClinicalTrials.gov).

The second trial is being directed by Jin Yan at the fourth military medical university in China. The main goal of this clinical trial is both to clarify the efficiency of autologous SHEDs to regenerate pulp and apical tissue in the patients with immature permanent teeth and pulp necrosis and to confirm the safety of using autologous stem cells in clinical endodontic regenerative medicine (NIH [39] ClinicalTrials.gov).

13.3.1.2 Non-osteo/Odontogenic Pre-clinical Trials

Besides being used in dentin, periodontal, and craniofacial tissue regeneration approaches, DPDSCs have also displayed pluripotent capabilities in pre-clinical treatment of myocardial infarction, muscular regeneration and corneal reconstructions along with vascular and neural restorations in various animal models.

Myocardial Infarction Therapy and Muscular Tissue Regeneration

It has been evidenced that after transplantation into the infarction zone of nude rats, DPDSCs favor cardiac repair. The restoration of cardiac functionality was confirmed by the change in the frontal wall thickening, change in left ventricular

fractional region, reduction in the infarct size, and increased angiogenesis. Researchers established that the cardiac restorative function was due to the DPDSCs' proangiogenic and antiapoptotic activities rather than their direct differentiation into cardiac cells [40, 41] (Fig. 13.2). By contrast, Kerkis et al. [42] proven that transplanted DPDSCs can differentiate into dystrophin-producing multinucleated cells and engraft into dog muscles, thus confirming the potential of DPDSCs for muscular dystrophy clinical therapy.

Corneal and Retinal Regeneration Potential of DPSCs

DSCs have also been used for corneal reconstruction, and for this purpose, a de-epithelialized human amniotic membrane uniformly covered with DPDSCs was transplanted onto corneal bed in in vivo conditions. Histological analysis after 3 months confirmed that those cells were able to regenerate a new tissue-engineered corneal epithelium [43]. Additionally, Mead and co-workers [44] transplanted DPSCs or bone marrow mesenchymal stem cells (BMMSCs) into the rat's vitreous body of the eye in which optic nerve had previously been surgically damaged. Twenty one days after transplantation, a higher rate of retinal ganglion cells survival in DPSCs treated rats was noted compared to those treated with BMMSCs [44] (Fig. 13.2).

Vasculogenic Differentiation of DPDSCs (Treatment of Ischemia)

Vasculogenic DPDSCs were isolated and tested for their ability to engraft and improve the blood flow by secreting pro-angiogenic proteins such as vascular endothelial growth factor-A (VEGF-A) as well as favoring the formation of capillary network in a rat model with hind limb ischemia [33].

Neural Differentiation of DPDSCs

Damaged spinal cords have been positively influenced by the DPDSCs transplantation. During this in vivo pre-clinical trial on rats, human DPDSCs were shown to induce regeneration of nerves. Results showed that human DPDSCs promoted the regeneration of transected axons by both directly inhibiting multiple axon growth inhibitors and preventing the apoptosis of neurons, astrocytes, and oligodendrocytes as well as differentiating and substituting oligodendrocytes that have been lost [45].

Another in vivo pre-clinical trial on mice with induced compressive spinal cord injury confirmed that human DPDSCs were able to recover damaged neural tissues by integration with axonal myelination and a high degree of trophic factor expression [46]. DPDSC neural integration was also confirmed by Király et al. [47] who injected labeled human DPDSCs into the cerebrospinal fluid of rats. They proved that DPDSCs differentiated towards neuronal-like cells expressing neuron-specific proteins and voltage-dependent sodium and potassium channels.

13.3.2 Clinical and Pre-clinical Trials of DSCPD

Comparison of osteogenic potentials of human DSCPDs and human DPDSCs suggested that DSCPD is a better osteogenic/odontogenic stem cell source than DPDSC. In fact, DSCPDs have been proven more likely to be multipotent rather than pluripotent, and thus specifically useful for dental (odontoblastic, cementoblastic and periodontal tissues) and bone tissues regeneration [48]. The findings were confirmed by labeling DSCPDs derived from ovine periodontal ligament tissue, and implanting them into immunodeficient mice. Results revealed that transplanted cells exhibiting an osteoblastic/cementoblastic phenotype were able to survive and also produce mineralized tissue as well as a ligament structure similar to Sharpey's fibers with concomitant vasculature. Furthermore, DSCPDs' potential role in dental tissue regeneration was tested on an ovine animal model with a periodontal defect. Histological examinations at 8 weeks post-implantation evidenced surviving labeled DSCPDs, regenerated periodontal tissues, cementum and bony structures [49].

Jin Yan and co-workers at the fourth military medical university in China are also conducting a clinical trial aiming to clarify the effectiveness of autologous PDLSCs to regenerate periodontal tissue in periodontitis affected patients with deep intraosseous defects (>5 mm), and to confirm the safety of using autologous DSCPDs in clinical periodontal regenerative medicine (NIH [50]) ClinicalTrials.gov).

13.4 Why to Choose DSCs for Clinical Trials?

As already suggested, DPDSCs have displayed pluripotent capabilities in the preclinical treatment of myocardial infarction, muscular regeneration, corneal and retina reconstructions as well as in vascular and neural restorations in various animal models. Thus, DPDSCs have literally the capability to be used in clinical trials involving tissues phenotypically very different from their dental origin. However, at present DSCs have been tested preferentially in clinical trials which involved hard tissues (dental or bone related tissues) because of their origin. It seems that the research community is following an unwritten rule or more likely a precaution, which tries to avoid possible complications, that adult tissue-derived stem cells should be clinically tested, in the first instance, to regenerate damaged tissues phenotypically closer to their tissue origin ("first choice" stem cell source). This way of thinking, without doubt, reflects the willingness of the research community to proceed correctly and with caution. However, this will not reveal all the DSC potential and will not advance tissue engineering. Thus, the question, which could also be applied to other adult stem cell sources, is: "*Why DSCs should be chosen to be tested in clinical trials*".

There are direct motivations, or cell specific properties, which will prompt us to choose DSCs as a stem cell source suitable for clinical trials different than those already attempted. DPDSCs (DPSCs and SHEDs) and DSCPDs (PDLSCs, SCAPs

and DFSCs) are great autologous stem cell sources being also useful for relatives closer to the donor because they can be easily collected starting at childhood. In addition, they are not subjected to the same ethical concerns associated with embryonic derived cells. Within DSCs, SHEDs have more advantages with respect to DPSCs, PDLSCs, SCAPs, DFPCs because they are simpler to isolate, do not cause pain, are rapidly proliferating cells, and are also complementary to stem cells derived from the umbilical cord blood [51].

The comparison between DSCs and stem cells of "first choice", phenotypically most similar or related to the damaged or non-functional tissue to be treated, is and will be extremely helpful to understand if DSCs are suitable for testing in clinical trials involving tissues different from either dental or bone related ones.

Recent papers have discussed how DPSCs' neural and epithelial differentiation capabilities are better than those of the BMMSCs [44, 52], and adipose tissue derived stem cells (ADSCs) [53], most probably because of their neuro-ectodermal derivation. In addition, it has also been demonstrated that expression profile for 15 of the most important pluripotent stem cell proteins between BMMSCs, ADSCs DPSCs and umbilical cord blood derived stem cells (UCBSCs) are almost identical [51].

On the vasculogenic side, DPSCs are able to secrete pro-angiogenic factors, improving the blood flow in rat models with hind limb ischemia [33], as well as to regenerate a well vascularized dental pulp after their transplantation [54, 55], suggesting that the vascular regenerative field can also significantly benefit from the pro-angiogenic properties of DPSCs. Those data strongly support the fact that adult stem cells are almost "interchangeable", indicating that DPDSCs can be a suitable stem cells source at least in clinical trials involving neural, epithelial and vascular damaged tissues and organs.

A wider use of DSCs is also supported by the fact that DPDSCs, in addition to their direct therapeutic action by differentiating and replacing damaged host's cells function, secrete many different molecules which indirectly provide the desired therapeutic action [27–29, 33, 40, 41, 44, 46]. In fact, the secretome of DPDSCs includes molecules and extracellular vesicles which could have paracrine as well as systemic effects. Summarizing those indirect therapeutic effects, which are regenerative, immune modulatory, anti-inflammatory, anti-tumoral, suggest the use DPDSCs' secretome in clinical trials including tissue regeneration, autoimmune, inflammatory, and malignant diseases.

A recent review on stem cells clinical trials proposed that there are many novel clinical trials conducted to cure cardiovascular diseases or attempt to regenerate vascular or cardiac tissues by using different types of stem cells (skeletal myoblasts, bone marrow cells, peripheral blood cells or autologous cardiac stem cells derived from heart biopsies) [56]. However, those clinical outcomes are often not convincing because results displayed only limited cardiac tissue regeneration and re-functionalization effects. Thus, "first choice" stem cells failure is a typical indirect motivation that invites the assessment of other stem cell sources in clinical trials. However, this must be carried out alongside valuable pre-clinical tests aimed to evaluate their suitability. DPDSCs have already been demonstrated to have cardiac

restorative function because of their pro-angiogenic activity rather than having direct differentiation capacity [40, 41], as well as displaying myogenic differentiation capability [42], and thus represent a valid alternative source. In addition, ethical concerns related to the stem cell sources (*i.e.* cells from fetal or cadaveric tissues), and the difficulty in isolating the more specific or already tested source, could reasonably lead us to search for alternatives. Thus, because of the relatively simple and painless harvest process and their high proliferation rate, DSCs can represent a valid and alternative stem cell source to be tested in clinical trials requiring cardiovascular diseases/regeneration or vascular tissue regeneration.

The fact is that DPSCs are an extremely valuable source which has similar or higher potential than other well-known stem cell sources (*i.e.* BMMSCs, ADSCs and UCBSCs), and many researchers are considering the use of these stem cell sources in clinical trials which do not involve the regeneration of bone or tooth related tissues. However, it is good to keep in mind that we need to find a clearer understanding of the healing/regenerative processes triggered by DPSCs and today, more than ever, focused pre-clinical studies will help us to achieve this.

13.5 Stem Cells and "Good Practice"

In the last 22 years since the term "tissue engineering" was coined by Langer and Vacanti [57], tissue engineering has not advanced as expected even though important achievements have been made.

Adult or ESC therapy and tissue engineered products have been classified as advanced therapy medicinal products (ATMPs) [58] and justifiably have to satisfy stringent requirements for use in human beings. It has been established that clinical trials must be conducted in compliance with the ethical principles originating from the declaration of Helsinki, as well as consistently following good clinical practices (GCP), and the applicable regulatory requirements (Official Journal of the European Union 2010/C 82/01 [59]).

Ethical and scientific quality standard for the design, conduct and recording of research involving humans is regulated by international GCP. GCP comprise 13 core principles, which are applied to all clinical investigations that could affect the safety and well-being of human participants. GCP principles were developed by the regulatory authorities of the EU, Japan and US in 1996, becoming effective in 1997. These rules provide an international guarantee that data and reported results of clinical trials are reliable and accurate, and rights, safety and confidentiality of participants in clinical research are respected and protected.

Initially, the GCP was not enforced by law, however, it was internationally recognized as best practice, and thus, with the advent of the Medicines for Human Use (Clinical Trials) Regulations and the EU Directive on Good Clinical Practice, GCP compliance became a legal obligation for all trials of investigational medicinal products [60]. In addition, to be in compliance with GCP, the stem cell clinical manufacturing process should follow the principles of good

manufacturing practice (GMP) [61], and clinical testing requires strict observance of good clinical laboratory practice (GCLP) [62]. In summary, to satisfy GMP protocols, cells must be strictly controlled starting from the collection and manipulation of raw materials (isolation), through the intermediate product processing (expansion process), to the quality controls (characterization, functionality, multipotency, and safety), storage (storage, labeling, packaging) and therapeutic use (transplantation, infusion into the patient) [63] (Fig. 13.3).

Researchers and clinicians are gaining awareness of the need to share, standardize and recognize GMPs for the possible use of stem cells for clinical applications. Nevertheless, animal derivatives (*e.g.* serum) are widely used in basic and pre-clinical experimentation, and use of serum represents a major obstacle to stem cells clinical application [64] since they are associated with many problems related to the presence of viruses, mycoplasmas, prions or other pathogenic, toxic or immunogenic agents [65–68]. Because of such safety risks, regulatory authorities discourage or prohibit the use of animal derivatives for the manufacture of biological products intended for clinical trials [69]. Therefore, it will be important to determine if DSCs, or stem cells in general, can be harvested, isolated, characterized, stored and transplanted without ever being directly exposed to animal sera or animal derivatives. Many studies are currently being planned and performed with the aim of addressing this question and developing an animal serum-free media capable of supporting the expansion and maintaining the stemness of DSCs [70]. Platelet-rich plasma (PRP), which contains various growth factors, has been positively tested as a natural substitute of animal serum to support the expansion and multipotency of DSCs [71]. Recently, it was demonstrated that a chemically defined culture medium to which a small amount of human sera had been added, was able to select a fast proliferating population of DPSCs, which expressed ESC and MSC markers as good as in a medium containing higher volume of animal serum [9, 18]. For the same reason, other authors prefer to use autologous serum when they are conducting pre-clinical studies and planning to perform clinical trials by using stem cells [54].

DPDSCs (DPSCs and SHEDs) and DSCPD (PDLSCs and SCAPs) can be collected almost without any trauma starting from childhood, and thus they could be stored and conserved for a considerable length of time, before being used. Therefore, it is extremely important and necessary to improve long-term storage and cryopreservation protocols to safely and reliably store those DSCs. In general, a variable percentage of dimethylsulfoxide (DMSO) and animal serum is employed as cryoprotectant for the cryopreservation and storage of hematopoietic, MSCs, ESCs and iPCSs. DMSO is used as standard following a 1 to 2 °C/min controlled rate freezing and a rapid thawing protocol [72]; however, it has been demonstrated to be toxic to tissues and cells. Toxicity is time-, temperature- and concentration-dependent, and DMSO affects the epigenetic profile of the stem cell by inducing differentiation and thus loss of stemness [73, 74]. Therefore, the use of DMSO, as well as animal serums (mostly fetal bovine serum-FBS), as components of the cryopreserving solution for cells to be used in clinical trials is not advisable. Many researchers investigated the possibility of developing or improving the cryopreserving solutions. Attempting to reduce and relieve adverse effects of DMSO on cell recovery and

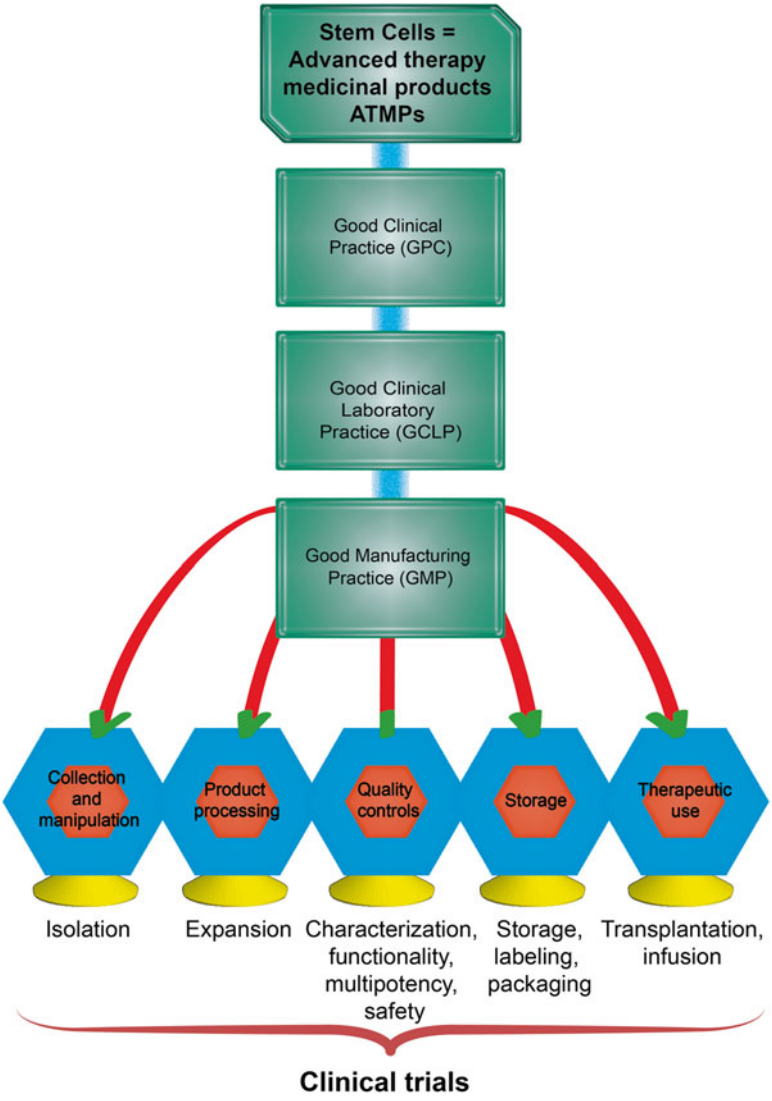

Fig. 13.3 The road to clinical trials. Adult or embryonic stem cell therapy and tissue engineered products have been classified as advanced therapy medicinal products (ATMPs) and their clinical trials should be conducted in accordance with good clinical practice (GCP). To be in compliance with GCP, stem cells clinical manufacturing process should follow the principles of good manufacturing practice (GMP), and clinical testing requires strict observance of good clinical laboratory practice (GCLP). Thus, stem cell must be strictly controlled starting from the collection and manipulation of the raw materials (isolation), through the intermediate products processing (expansion process), to the quality controls (characterization, functionality, multipotency, and safety), storage (storage, labeling, and packaging) and therapeutic use (transplantation, infusion) into the patients

engraftment, many researchers successfully lowered its concentration to as low as 5 % [75, 76], and even to 2 %, leading to optimized the cooling rates [72]. Alternatives to DMSO containing cryogenic solutions (*i.e.* polyvinylpyrrolidone, methylcellulose), as well as animal serum (*i.e.* human serum or human serum albumin) have also been developed and positively tested over recent years to increase stem cells' cryopreservation safety and their clinical utility [77, 78]. Along with those attempts to reduce or substitute DMSO in storage protocols, Gioventù et al. [79] tested the possibility of cryoconserving DPSCs inside the whole tooth. In their opinion, avoiding the purification step will reduce the possibility of stemness loss due to the cryopreservation period; moreover, it will reduce the initial cost and assist the efforts required to set up a DSC bank [79].

Stem cell expansion and banking need to be as safe as possible, reliable and effective if intended for clinical trial purposes. For this reason, it is also a good practice to avoid, as much as possible, direct human interaction with stem cells since handling could lead to contamination. However, even if GCPs applied to validate the advanced medicinal products used today in clinical trials have been established, most cells are still obtained and cultured by using typical handling methods, which are subjected to the personnel's technical ability [80].

A highly reproducible and reliable handling of stem cells can be achieved by automating those processes. Fortunately, many effective automatic cell culture systems have been developed in today's technology [81–87]. In summary, those automated "GCPs-following" devices will improve large-scale culture and expansion of the stem cells by automating most of the manual cell culture steps (*e.g.* media exchange, cells centrifugation and pelleting, cells splitting and passaging), thus, allowing safe handling of stem cells to be used for clinical purposes. In addition, automation in stem cells culture or generally stem cells handling will allow tight control over multiple growth parameters and sterility, ensuring a higher rate of uniformity to classic culture methods.

13.6 Stem Cells Clinical Trials: Suggestions and Concerns

DSCs are relatively easy to obtain ecto-mesenchymal stem cells with already tested for their stemness and differentiation capabilities in vitro and in vivo. Our scientific literature search revealed that to date the vast majority of in vivo applications have been performed on animal models and very few clinical trials have been attempted. A recent review reasonably stated that a cause of this delayed clinical translation is primarily the lack of clear good manufacturing practices (GMP) or common methods to isolate and characterize DSCs [88]. In a different point of view, La Noce and coworkers proposed that because clinical trials are long, difficult and expensive, even the most dedicated researcher could be discouraged from pursuing stem cell clinical transition. They, therefore, suggested that whenever possible, and without compromising patient safety, bureaucratic procedures should be simplified. Nevertheless, even if the use of DSCs in basic research and medical therapy does not bring up any ethical and legal issues with respect to ESCs, those cells will not be free of any risk [89].

The reality is that any cell has a latent risk of becoming a cancer cell and cancer is not the only risk derived from transplants, even from autologous adult stem cell transplants [90]. Some of other risks are heart attack, thrombosis (blood clotting) [91], infection or autoimmune response [92], which can be fatal to patients. Those adverse effects during clinical trials represent just a small number of reports. Thus, alongside our efforts to translate stem cell therapy from the bench to the bed-side, we must also ensure that these therapies are safe and in this area a lot of work remains to be done.

To comprehend and decrease any potential danger associated with stem cells therapies, improved in vitro and in vivo techniques are required to guarantee gene aberration-free expansion of cells, tumorigenic-free potential, increased population and differentiation purity, and identify those risk factors that can be routinely screened before transplantation (*i.e.* infections). Furthermore, we need to more reliably predict those adverse immunological responses, and better track transplanted cells by developing improved immunological models and tracking techniques, and thus expand our knowledge and reduce those risks [89] (Fig. 13.4).

It is good to keep in mind that we also need to find a way to provide a better understanding of the healing/regenerative process elicited by stem cells; today, more than ever, focused pre-clinical studies will help us to answer to those questions prior to their clinical testing. Indeed, pre-clinical studies should help to understand

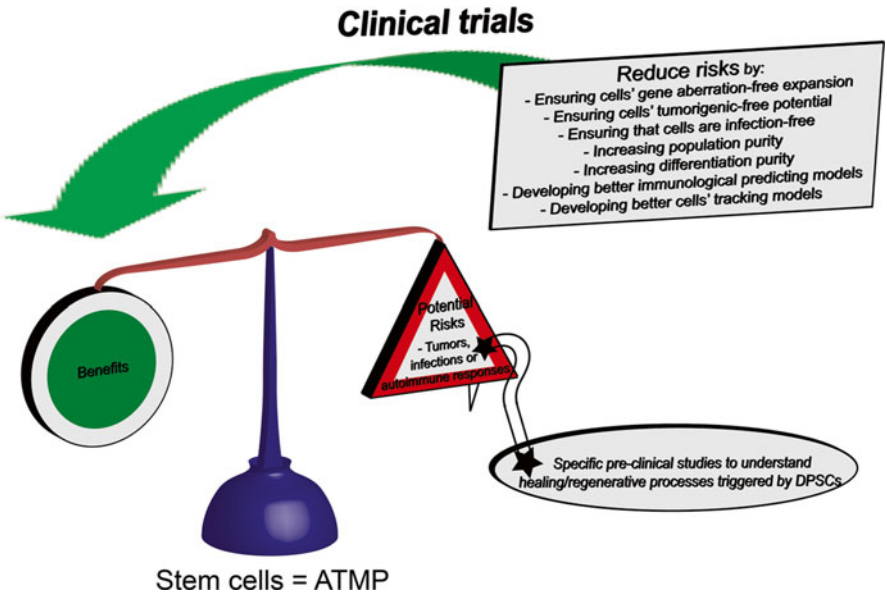

Fig. 13.4 Potential risks and solutions. Any transplanted cell has a potential risk to form a tumor, as well as they might cause infections or catastrophic autoimmune reactions. Thus, risks associated with stem cells therapies would be reduced by ensuring gene aberration-free expansion, testing cells' tumorigenic-free potential, increasing population and differentiation purity, and identifying those risk factors that can be routinely screened before transplantation. In addition, it may be helpful to develop a more effective predictive immunological response model and cell tracking techniques. Acronyms: ATMPs, advanced therapy medicinal products

the "possible" direct negative specific effects of the adult stem cells directly related to their stemness/differentiation capacity. This also relates to the indirect negative therapeutic action resulting from their paracrine and systemic action and thus related to their secretome [27–29, 33, 40, 41, 44, 46]. To conclude, we are fully aware that stem cells therapy, as with many drug treatments, may not be perfectly safe. This knowledge should make one main clinical priority that the benefit to the patient will outweigh the risk.

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References

1. Mikkola ML (2007) Genetic basis of skin appendage development. Semin Cell Dev Biol 18:225–236
2. Pispa J, Thesleff I (2003) Mechanisms of ectodermal organogenesis. Dev Biol 262:195–205
3. Morsczeck C, Schmalz G, Reichert TE et al (2008) Somatic stem cells for regenerative dentistry. Clin Oral Invest 12:113–118
4. Gronthos S, Mankani M, Brahim J et al (2000) Postnatal human dental pulp stem cells (DPSC) in vitro and in vivo. Proc Natl Acad Sci USA 97:13625–13630
5. Tecles O, Laurent P, Zygouritsas S et al (2005) Activation of human dental pulp progenitor/stem cells in response to odontoblast injury. Arch Oral Biol 50:103–108
6. Kaukua N, Shahidi MK, Konstantinidou C et al (2014) Glial origin of mesenchymal stem cells in a tooth model system. Nature 513:551–554
7. Miura M, Gronthos S, Zhao M et al (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA 100:5807–5812
8. Kerkis I, Kerkis A, Dozortsev D et al (2006) Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs 184:105–116
9. Ferro F, Spelat R, D'Aurizio F et al (2012) Dental pulp stem cells differentiation reveals new insights in Oct4A dynamics. PLoS One 7, e41774. doi:10.1371/journal.pone.0041774
10. Huang AH, Chen YK, Lin LM et al (2008) Isolation and characterization of dental pulp stem cells from a supernumerary tooth. J Oral Pathol Med 37:571–574
11. Honda MJ, Imaizumi M, Tsuchiya S et al (2010) Dental follicle stem cells and tissue engineering. J Oral Sci 52:541–552
12. Sonoyama W, Liu Y, Fang D et al (2006) Mesenchymal stem cell-mediated functional tooth regeneration in Swine. PLoS One 1:e79
13. Huang GT, Sonoyama W, Liu Y et al (2008) The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod 34:645–651
14. Sonoyama W, Liu Y, Yamaza T et al (2008) Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. J Endod 34:166–171
15. Seo BM, Miura M, Sonoyama W et al (2005) Recovery of stem cells from cryopreserved periodontal ligament. J Dent Res 84:907–912
16. Laino G, Carinci F, Graziano A et al (2006) *In vitro* bone production using stem cells derived from human dental pulp. J Craniofac Surg 17:511–515
17. Zhang W, Walboomers XF, Shi S et al (2006) Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation. Tissue Eng 12:2813–2823
18. Ferro F, Spelat R, Beltrami AP et al (2012) Media containing low human serum as clinical grade substitute for bovine serum. PLoS One 7(11), e48945. doi:10.1371/journal.pone.0048945

19. Bojic S, Volarevic V, Ljujic B et al (2014) Dental stem cells-characteristics and potential. Histol Histopathol 29:699–706
20. Huang GTJ, Gronthos S, Shi S (2009) Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine. Dent Res 88:792–806
21. Morsczeck C, Vollner F, Saugspier M et al (2009) Comparison of human dental follicle cells (DFCs) and stem cells from human exfoliated deciduous teeth (SHED) after neural differentiation in vitro. Clin Oral Invest 14:433–440
22. d'Aquino R, Graziano A, Sampaolesi M et al (2007) Human postnatal dental pulp cells codifferentiateinto osteoblasts and endotheliocytes: a pivotalsynergy leading to adult bone tissue formation. Cell Death Differ 14:1162–1171
23. Ishkitiev N, Yaegaki K, Calenic B et al (2010) Deciduous and permanent dental pulp mesenchymal cells acquire hepatic morphologic and functional features in vitro. J Endod 36:469–474
24. Ishkitiev N, Yaegaki K, Imai T et al (2012) High-purity hepatic lineage differentiated from dental pulp stem cells in serum-free medium. J Endod 38:475–480
25. Yan X, Qin H, Qu C et al (2010) iPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. Stem Cells Dev 19:469–480
26. Siqueira da Fonseca SA, Abdelmassih S, de Mello Cintra Lavagnolli T et al (2009) Human immature dental pulp stem cells' contribution to developing mouse embryos: production of human/mouse preterm chimaeras. Cell Prolif 42:132–140
27. Tomic S, Djokic J, Vasilijic S et al (2011) Immunomodulatory properties of mesenchymal stem cells derived from dental pulp and dental follicle are susceptible to activation by toll-like receptor agonists. Stem Cells Dev 20:695–708
28. Wada N, Menicanin D, Shi S et al (2009) Imunnunomodulatory properties of human periodontal ligament stem cells. J Cell Physiol 219:667–676
29. Yamaza T, Kentaro A, Chen C et al (2010) Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. Stem Cell Res Ther 1:5
30. Prescott RS, Alsanea R, Fayad MI et al (2008) In vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice. J Endod 34:421–426
31. Huang GT, Yamaza T, Shea LD et al (2010) Stem/progeni tor cel l-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng Part A 16:605–615
32. Iohara K, Nakashima M, Ito M et al (2004) Dentin regeneration by dental pulp stem cell therapy with recombinant human bone morphogenetic protein 2. J Dent Res 83:590–595
33. Iohara K, Zheng L, Wake H et al (2008) A novel stem cell source for vasculogenesis in ischemia: subfraction of side population cells from dental pulp. Stem Cells 26:2408–2418
34. Graziano A, d'Aquino R, Laino G et al (2008) CD34+ stem cells produce bone nodules *in vivo*. Cell Prolif 41:1–11
35. de Mendonça CA, Bueno DF, Martins MT et al (2008) Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. J Craniofac Surg 19:204–210
36. d'Aquino R, De Rosa A, Lanza V et al (2009) Human mandible bone defect repair by the grafting of dental pulp stem / progenitor cells and collagen sponge. Eur Cell Mater 12:75–83
37. Giuliani A, Manescu A, Langer M et al (2013) Three years after transplants in human mandibles, histological and in-line holotomography revealed that stem cells regenerated a compact rather than a spongy bone: biological and clinical implications. Stem Cells Transl Med 2:316–324
38. NIH (2015) ClinicalTrials. https://clinicaltrials.gov/ct2/show/NCT01932164?term=dental+stem+cells&rank=1. Accessed 4 Apr 2015
39. NIH (2015) ClinicalTrials. https://clinicaltrials.gov/ct2/show/NCT01814436?term=dental+stem+cells&rank=3. Accessed 4 Apr 2015
40. Gandia C, Arminan A, Garcia-Verdugo JM et al (2008) Human dental pulp stem cells improve left ventricular function, induce angiogenesis and reduce infarct size in rats with acute myocardial infarction. Stem Cells 26:638–645

41. Iohara K, Zheng L, Ito M et al (2009) Regeneration of dental pulp after pulpotomy by transplantation of CD31(-)/CD146(-) side population cells from a canine tooth. Regen Med 4:377–385
42. Kerkis I, Ambrosio CE, Kerkis A et al (2008) Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic? J Transl Med 6:35. doi:10.1186/1479-5876-6-35
43. Gomes JA, Geraldes Monterio B, Melo GB et al (2010) Corneal reconstruction with tissue-engineered cell sheets composed of human immature dental pulp stem cells. Invest Ophthalmol Vis Sci 1:1408–1414
44. Mead B, Logan A, Berry M et al (2013) Intravitreally transplanted dental pulp stem cells promote neuroprotection and axon regeneration of retinal ganglion cells after optic nerve injury. Invest Ophthalmol Vis Sci 54:7544–7556
45. Sakai K, Yamamoto A, Matsubara K et al (2012) Human dental pulp-derived stem cells promote locomotor recovery after complete transaction of the rat spinal cord by multiple neuro regenerative mechanism. J Clin Invest 122:80–90
46. de Almeida FM, Marques SA, Bdos RS et al (2011) Human dental pulp cells: a new source of cell therapy in a mouse model of compressive spinal cord injury. J Neurotrauma 28:1939–1949
47. Király M, Kádár K, Horváthy DB et al (2011) Integration of neuronally predifferentiated human dental pulp stem cells into rat brain *in vivo*. Neurochem Int 59:371–381
48. Chadipiralla K, Yochim JM, Bahuleyan B et al (2010) Osteogenic differentiation of stem cells derived from human periodontal ligaments and pulp of human exfoliated deciduous teeth. Cell Tissue Res 340:323–333
49. Menicanin D, Mrozik KM, Wada N et al (2014) Periodontal-ligament-derived stem cells exhibit the capacity for long-term survival, self-renewal, and regeneration of multiple tissue types in vivo. Stem Cells Dev 23:1001–1011
50. NIH (2015) ClinicalTrials. https://clinicaltrials.gov/ct2/show/record/NCT01357785. Accessed 6 Apr 2015
51. Stanko P, Kaiserova K, Altanerova V et al (2014) Comparison of human mesenchymal stem cells derived from dental pulp, bone marrow, adipose tissue, and umbilical cord tissue by gene expression. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub 158:373–377. doi:10.5507/bp.2013.078
52. Karaoz E, Demircan PC, Saglam O et al (2011) Human dental pulp stem cells demonstrate better neural and epithelial stem cell properties than bone marrow-derived mesenchymal stem cells. Histochem Cell Biol 136:455–473
53. Mead B, Logan A, Berry M et al (2014) Paracrine-mediated neuroprotection and neuritogenesis of axotomised retinal ganglion cells by human dental pulp stem cells: comparison with human bone marrow and adipose-derived mesenchymal stem cells. PLoS One 9:e109305. doi:10.1371/journal.pone.0109305
54. Iohara K, Murakami M, Takeuchi N et al (2013) A novel combinatorial therapy with pulp stem cells and granulocyte colony-stimulating factor for total pulp regeneration. Stem Cells Transl Med 2:521–533
55. Rosa V, Zhang Z, Grande RH et al (2013) Dental pulp tissue engineering in full-length human root canals. J Dent Res 92:970–975
56. Li MD, Atkins H, Bubela T (2014) The global landscape of stem cell clinical trials. Regen Med 9:27–39. doi:10.2217/rme.13.80
57. Langer R, Vacanti JP (1993) Tissue engineering. Science 260:920–926
58. Regulation (EC) N. 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) N. 726/2004
59. European Commission. Detailed guidance on the request to the competent authorities for authorisation of a clinical trial on a medicinal product for human use, the notification of substantial amendments and the declaration of the end of the trial (CT-1). Official Journal of the European Union 2010/C 82/01

60. Imperial College London (2015) http://www3.imperial.ac.uk/clinicalresearchgovernanceoffice/researchgovernance/goodclinicalpractice. Accessed 4 Apr 2015
61. EudraLex (2010). Volume 4: Good manufacturing practice (GMP) Guidelines for medicinal products for human and veterinary use, laid down in Commission Directives 91/356/EEC, as amended by Directive 2003/94/EC, and 91/412/EEC. Brussels, SANCO/C8/AM/sl/ares(2010)1064597. Accessed 4 Apr 2015
62. Stevens W (2003) Good Clinical Laboratory Practice (GCLP): The need for a hybrid of Good Laboratory Practice and Good Clinical Practice guidelines/standards for medical testing laboratories conducting clinical trials in developing countries. Quality Assurance 10:83–89
63. Torre ML, Lucarelli E, Guidi S et al (2014) Ex vivo expanded mesenchymal stromal cell minimal quality requirements for clinical application. Stem Cells Dev 24(6):677–685
64. Ulloa-Montoya F, Verfaillie CM, Hu WS (2005) Culture systems for pluripotent stem cells. J Biosci Bioeng 100:12–27
65. Eloit M (1999) Risks of virus transmission associated with animal sera or substitutes and methods of control. Dev Biol Stand 99:9–16
66. Shah G (1999) Why do we still use serum in the production of biopharmaceuticals? Dev Biol Stand 99:17–22
67. Van der Valka J, Mellorb D, Brandsc R (2004) The humane collection of fetal bovine serum and possibilities for serum-free cell and tissue culture. Toxic in Vitro 18:1–12
68. Wessman SJ, Levings RL (1999) Benefits and risks due to animal serum used in cell culture production. Dev Biol Stand 99:3–8
69. Asher DM (1999) Bovine sera used in the manufacture of biologicals: current concerns and policies of the US. Food and drug administration regarding the transmissible spongiform encephalopathies. Dev Biol Stand 99:41–44
70. Tarle SA, Shi S, Kaigler D (2011) Development of a serum-free system to expand dental-derived stem cells: PDLSCs and SHEDs. J Cell Physiol 226:66–73
71. Lee JY, Nam H, Park YJ et al (2011) The effects of platelet-rich plasma derived from human umbilical cord blood on the osteogenic differentiation of human dental stem cells. In Vitro Cell Dev Biol Anim 47:157–164
72. Berz D, McCormack EM, Winer ES et al (2007) Cryopreservation of hematopoietic stem cells. Am J Hematol 82:463–472
73. Adler S, Pellizer C, Paparella M et al (2006) The effects of solvents on embryonic stem cell differentiation. Toxicol In Vitro 20:265–271
74. Iwatani M, Ikegami K, Kremenska Y et al (2006) Dimethyl sulphoxide has an impact on epigenetic profile in mouse embryoid body. Stem Cells 24:2549–2556
75. Hunt CJ, Armitage SE, Pegg DE (2003) Cryopreservation of umbilical cord blood: 2. tolerance of CD34+ cells to multimolar dimethyl sulphoxide and the effect of cooling rate on the recovery after freezing and thawing. Cryobiology 46:76–87
76. Woods EJ, Liu J, Pollok K et al (2003) A theoretically-optimised method for cord blood stem cell cryopreservation. J Hematother Stem Cell Res 12:341–350
77. Thirumala S, Wu X, Gimble JM et al (2010) Evaluation of polyvinylpyrrolidone as a cryoprotectant for adipose tissue-derived adult stem cells. Tissue Eng Part C Methods 16:783–792
78. Thirumala S, Wu X, Gimble JM (2010) Evaluation of methylcellulose and dimethyl sulphoxide as cryoprotectants in a serum-free freezing media for the cryopreservation of adipose derived adult stem cells. Stem Cells Dev 19:513–522
79. Gioventù S, Andriolo G, Bonino F et al (2012) A novel method for banking dental pulp stem cells. Transfus Apher Sci 47:199–206. doi:10.1016/j.transci.2012.06.005
80. Soncin S, Lo Cicero V, Astori G et al (2009) A practical approach for the validation of sterility, endotoxin and potency testing of bone marrow mononucleated cells used in cardiac regeneration in compliance with good manufacturing practice. J Transl Med 7:78
81. Joannides A, Fiore-Heriche C, Westmore K et al (2006) Automated mechanical passaging: a novel and efficient method for human embryonic stem cell expansion. Stem Cells 24:230–235
82. Kami D, Watakabe K, Yamazaki-Inoue M et al (2013) Large-scale cell production of stem cells for clinical application using the automated cell processing machine. BMC Biotechnol 13:102. doi:10.1186/1472-6750-13-102

83. Kino-Oka M, Ogawa N, Umegaki R et al (2005) Bioreactor design for successive culture of anchorage-dependent cells operated in an automated manner. Tissue Eng 11:535–545
84. Koike H, Kubota K, Sekine K et al (2012) Establishment of automated culture system for murine induced pluripotent stem cells. BMC Biotechnol 12:81
85. Terstegge S, Laufenberg I, Pochert J et al (2007) Automated maintenance of embryonic stem cell cultures. Biotechnol Bioeng 96:195–201
86. Thomas RJ, Chandra A, Liu Y et al (2007) Manufacture of a human mesenchymal stem cell population using an automated cell culture platform. Cytotechnology 55:31–39
87. Thomas RJ, Anderson D, Chandra A et al (2009) Automated, scalable culture of human embryonic stem cells in feeder-free conditions. Biotechnol Bioeng 102:1636–1644
88. La Noce M, Paino F, Spina A et al (2014) Dental pulp stem cells: state of the art and suggestions for a true translation of research into therapy. J Dent 42:761–768
89. Heslop JA, Hammond TG, Santeramo I et al (2015) Concise review: workshop review: understanding and assessing the risks of stem cell-based therapies. Stem Cells Transl Med 4:389–400
90. Martin-Padura I, Gregato G, Marighetti P et al (2012) The white adipose tissue used in lipotransfer procedures is a rich reservoir of CD34+ progenitors able to promote cancer progression. Cancer Res 72:325–334. doi:10.1158/0008-5472.CAN-11-1739
91. Jonsson TB, Larzon T, Arfvidsson B et al (2012) Adverse events during treatment of critical limb ischemia with autologous peripheral blood mononuclear cell implant. Int Angiol 31:77–84
92. Alderazi YJ, Coons SW, Chapman K (2012) Catastrophic demyelinating encephalomyelitis after intrathecal and intravenous stem cell transplantation in a patient with multiple sclerosis. J Child Neurol 27:632–635. doi:10.1177/0883073811422831

Chapter 14
Future Perspectives in Dental Stem Cell Engineering and the Ethical Considerations

Naohisa Wada, Atsushi Tomokiyo, and Hidefumi Maeda

14.1 Introduction

In early periods of dentistry, patients first expected pain relief from their dentists, which led to dentists identifying the cause of the problem and eliminating it by tooth extraction, pulpectomy, and dental scaling. As the field of dentistry advanced, the trends of dental treatment shifted to the functional restoration of the oral cavity by artificial materials such as composite resin fillings, metal and ceramic restorations, dentures and dental implants. More recently, the restoration of the original shape and function of the oral cavity using regenerative medicine and biomaterials has become a major focus of study. Especially, regenerative treatments in dental implantology and periodontology have achieved great progress. For example, platelet-rich plasma (PRP) has been applied with dental implants to promote osseointegration, though the effects are still controversial [1], while enamel matrix derivative (EMD) has also been used as a major growth factor to regenerate periodontal tissue [2]. However, in both of these approaches, as the treatment effects depend on host cells near the damaged site, there is insufficient tissue regeneration when the extent of damage to the periodontal and bone tissues is relatively large. As current therapies for dental tissue regeneration have shown limited potential, the development of alternative strategies to reconstruct damaged or destroyed dental tissue is greatly needed. In recent years, tissue engineering strategies incorporating stem cells has

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been proposed for the development of new dental tissue therapies, such as tissue engineering of alveolar bone, periodontal tissue, dentin/pulp complex and ultimately whole tooth. In this chapter, the future perspectives of dental stem cell (DSC) engineering and ethical considerations are described.

14.2 Future Dental Tissue Engineering

14.2.1 Dental stem cells (DSCs)

14.2.1.1 Cell Source

Stem cells, including somatic stem cells, embryonic stem (ES) cells and induced pluripotent stem (iPS) cells, commonly possess a self-renewal capacity that allows them to actively undergo cell division, and multipotency that allows them to differentiate into various types of cells. Thus, the use of stem cell populations in tissue engineering has become a major focus in recent years. However, each stem cell type has different properties with respect to the tissue that they are derived from. The stage of maturity of certain stem cells can affect their capacity for differentiation into multiple lineages. Thus, each stem cell population has different advantages and disadvantages for various tissue engineering applications. Furthermore, there remains much to be learned about the suitability of different stem cell types for their particular applications.

Human ES cells, derived from blastocysts produced by in vitro fertilization, were first established by Thomson et al. in 1998 [3]. As ES cells possess the potential to differentiate into derivatives of the three embryonic germ layers (endoderm, mesoderm and ectoderm), these cells were initially anticipated as playing a leading role in regenerative medicine. However, because of ethical issues associated with their derivation, the use of ES cells for tissue engineering was very restricted. ES cells have been used as a tool for examining the mechanisms of tissue and organ development and regeneration.

Somatic stem cells such as hematopoietic stem cells and mesenchymal stem cells (MSCs) are found in different organs or tissues. These stem cells exhibit limited differentiation potential in comparison with ES cells. The advantages of somatic stem cells compared with ES cells for tissue engineering are the availability of autologous transplantation, low risk of cancer formation and ease of controlling their differentiation into target cells. Among somatic stem cells, MSCs have been identified in various tissues throughout the human adult body, including bone marrow, adipose tissue, placenta and muscle [4–7]. Human MSCs were originally isolated from aspirates of adult bone marrow [8], and they displayed the potential to differentiate into mesodermal lineage cells, such as adipocytes, chondrocytes and osteoblasts [6], endodermal lineage cells [9], and even neuroectodermal lineage cells [10]. Regarding dental tissue, MSCs were first isolated from dental pulp tissue in 2000 [11], and since then other dental tissue-derived MSCs

Table 14.1 Human dental stem cells (DSCs)

Human dental mesenchymal stem cells (MSCs)	Origin	Reference
Dental pulp stem cells (DPSCs)	Pulp tissue from permanent teeth	[11]
Stem cells from human exfoliated deciduous teeth (SHEDs)	Pulp tissue from exfoliated deciduous teeth	[12]
Periodontal ligament stem cells (PDLSCs)	Periodontal ligament tissue from permanent teeth	[13]
Precusor cells from dental follicle (DFPCs)	Dental follicle tissue from immature wisdom teeth	[14]
Stem cells from appical papilla (SCAPs)	Appical papilla tissue from immature wisdom teeth	[15]
Gingiva-derived mesenchymal stem cells (GMSCs)	Gingival tissue	[16, 17]

have been reported [12–17] (Table 14.1). Given that most dental and oral tissues, except for enamel, are mesenchymal tissues, MSCs could be a promising cell source for dental and oral tissue engineering. In particular, MSCs originating from gingival and dental pulp tissue are believed to be strong candidate cell sources for tissue engineering, as gingival tissue is rich and easily obtainable, and dental pulp tissue can be retrieved from extracted teeth, which are usually discarded. It is also believed that MSCs derived from original tissue possess the potential to regenerate damaged tissue more effectively than other sources. Thus, future studies should aim to identify the factors controlling the differentiation of MSCs derived from other tissues into the cells of damaged tissues.

One limitation, however, is that MSCs obtained from the patients vary in number and quality depending on the patient's health. In 2007 and 2008, pluripotent stem cell populations, termed iPS cells, were generated from neonatal and adult human dermal fibroblasts by the overexpression of four transcription factors [18–20]. iPS cells display comparable properties to ES cells in terms of their potential to generate cells from the three embryonic germ layers. Therefore, iPS cells are expected to be an alternative source of large quantities of pluripotent cells for regenerative research, because of not only their differentiation potential but also the use of patient-derived autologous cells. iPS cells derived from dental tissues, such as dental pulp, periodontal ligament and gingival tissue, have been already established, and are believed to be a good source of seed cells for dental tissue engineering [21–24] (Table 14.2). In addition, recent studies have reported factors that can induce cell dedifferentiation from various tissue-derived cells into undifferentiated mesenchymal cell types. Activin receptor-like kinase-2 (ALK2) in endothelial cells and octamer-binding transcription factor 4 (OCT4) in cord or peripheral blood CD34-positive cells cause induced conversion into MSC-like cells [25, 26], while Notch reprograms epidermal-derived melanocytes into neural crest stem-like cells [27]. Such factors may become available to induce the formation of MSCs from differentiated cells.

Table 14.2 iPS cells derived from dental tissues

Origin	Author	Reference
DPSCs	Yan et al.	[21]
SHEDs	Yan et al.	[21]
SCAPs	Yan et al.	[21]
Dental pulp cells	Tamaoki et al.	[22]
Gingival fibroblasts	Egusa et al.	[23]
Periodontal ligament cells	Wada et al.	[24]

14.2.1.2 Autologous *vs.* Allogeneic Cell Transplantation

Autologous stem cell transplantations display good promise for future tissue engineering applications. However, it is often difficult to generate sufficient, healthy and high quality cells from a single donor. To circumvent these problems, it is hoped that a cell transplantation method using allogeneic stem cells will be established, as an alternative cell source to autologous stem cells. Allogeneic sources could generate sufficient numbers of healthy stem cells with defined regenerative capacity for transplantation. One of the most important issues is that the use of allogeneic cells may lead to their rejection by the host immune system because of major histocompatibility complex (MHC)-mismatching.

MSCs derived from dental tissue have been reported to possess immunosuppressive properties similar to bone marrow stem cells [17, 28]. Wada et al. demonstrated that periodontal ligament stem cells (PDLSCs), dental pulp stem cells (DPSCs) and gingival fibroblasts lack the expression of immune costimulatory factors, such as MHC class II, CD40, CD80 and CD86 antigens, and suppress peripheral blood mononuclear cell proliferation following stimulation [28]. Gingiva-derived mesenchymal stem cells (GMSCs) have also been shown to be capable of immunomodulatory functions through the expression of immunosuppressive factors, including interleukin (IL)-10, IDO, inducible nitric oxide synthase and cyclooxygenase-2 [17]. In addition, another potential mechanism of immunomodulation by DPSCs and stem cells derived from exfoliated deciduous teeth (SHED) has been reported to occur via the induction of apoptosis in activated T-cells [29, 30]. Furthermore, several studies have reported that intravenous administration of allogeneic dental MSCs results in marked suppression of host immune reactions in preclinical animal models of transplant rejection and immune-mediated diseases, such as murine colitis, systemic lupus erythematosus and allergic contact dermatitis models [17, 29–31]. In a lacZ transgenic mouse model, periodontal tissue was regenerated after allogeneic tooth transplantation by replacement with host cells, without any host immune responses [32]. In an ovine periodontal defect model, allogeneic PDLSC implantation into periodontal defects induced regeneration of alveolar bone, cementum and periodontal ligament with Sharpey's fibers, without inflammation and infection [33]. These findings have elicited further interest in allogeneic dental MSCs as alternative cell sources that have the potential to modulate alloreactivity and tissue regeneration following transplantation into human leukocyte antigen-mismatched donors.

14.2.2 Growth Factors

Although tissue engineering using cell transplantation is considered to be an attractive method to regenerate damaged tissue, there are still some aspects, such as technical and ethical issues, that have not been resolved in preparation for clinical application. In addition, we have to consider the cost of treatment for clinical application of dental tissue engineering. Hence, tissue engineering using growth factors may be a more realistic, feasible and economical method with respect to cell transplantation for clinical application at present. Thus, the combination of DSCs and growth factors which can accelerate DSC functions such as proliferation, migration, adhesion and differentiation potential are critical to realize dental stem cell engineering in clinics.

In 1997, porcine EMD was reported to induce new bone and cementum formation in periodontal defects by mimicking the biological processes in periodontium development [34]. EMD, which contains a mixture of a variety of growth factors, has so far been the most powerful material for promoting regeneration of periodontal tissues clinically. EMD has been shown to regulate the growth, proliferation, migration, adhesion, bone-related gene expression, and protein synthesis of PDL fibroblasts, osteoblasts and cementoblasts [35]. Suzuki et al. demonstrate that an EMD gel containing transforming growth factor beta 1 (TGF-β1) and bone morphogenetic protein 2 (BMP2) could regulate cell behavior in periodontium [36]. Treatment of PDL cells with TGF-β1 increased gene expression and protein synthesis of collagen [37, 38]. Interestingly, our current study revealed that exogenous application of TGF-β1 induced collagen type I alpha 1 (COLIA1) and alpha smooth muscle actin (α-SMA) gene transcriptions, and α-SMA protein expression in a PDL stem/progenitor cell line; however, two different human PDL cells showed no significant change in gene and protein expression of COLIA1 and α-SMA [39]. These results suggested crucial roles of TGF-β1 in collagen and α-SMA production in predominantly immature PDL cells. BMP2 has been reported to stimulate alveolar bone regeneration of experimental periodontal defect models in animal experiments [40, 41]. Chen et al. generated BMP2-overexpressing bone marrow-derived MSCs using an adenovirus, and implanted them into the periodontal defect models [42]. Intriguingly, they showed enhanced regeneration of the periodontium, including the cementum and Sharpey's fibers, compared with the untreated bone marrow-derived MSCs. In addition, an in vitro study has reported that amelogenin included in EMD enhanced proliferation and migration, but BMP included in EMD induced osteoblastic differentiation of PDL progenitor cells, suggesting the combination of DSCs and EMD may be effective for dental tissue engineering [43].

Recent reports showed the effect of the combination of basic fibroblast growth factor (bFGF) and TGF-β1 on DSCs; whereby bFGF and TGF-β1 synergistically promoted alkaline phosphatase (ALP) activity, the formation of mineralized nodules and odontoblast-related gene expression in DPSCs [44]. Our current study using two different PDL stem/progenitor stem cell lines also revealed that bFGF and TGF-β1 significantly down-regulated ESC-related marker genes, while simultane-

ously upregulating PDL-related genes [45]. These results indicated that bFGF and TGF-β1 treatment could promote pulp and PDL regeneration through the induction of stem cell differentiation.

PRP is generated from autologous blood and includes various growth factors such as platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and epidermal growth factor (EGF). PRP upregulated colony formation, proliferation, mineralization, and odontoblast-related gene expression of DPSCs [46]. PRP also enhanced extracellular matrix protein production and bone-related gene expression in PDLSCs [47]. Moreover, concentrated growth factors from a platelet-type concentrate dose-dependently increased the proliferation and osteoblastic differentiation of PDLSCs [48].

Connective tissue growth factor (CTGF), a cysteine-rich secretory protein belonging to the CCN family, enhanced the synthesis of collagen type I and III, biglycan, and periostin and the formation of mineral deposits in PDLSCs and dental follicle stem/progenitor cells (DFSCs) [49]. Our recent study demonstrated that CTGF up-regulated proliferation, migration, bone/cementum-related gene expression and mineralization in a PDLSCs [50]. Interestingly, the combination of CTGF and bFGF or TGF-β1 down-regulated mineralization of the PDLSCs, while significantly upregulating the expression of connective tissue-related genes. Wnt5a may also be a promising growth factor for controlling periodontal tissue regeneration, as we have recently reported that Wnt5a induced collagen production by PDLSCs through TGFβ1 mediated upregulation of periostin expression, while it also suppressed the osteoblastic differentiation. These results suggest that Wnt5a expression in PDL tissue plays important roles in collagen fibrillogenesis and the suppression of ankyloses [51]. Therefore, the effects of certain growth factor application on periodontal tissue or dental pulp regeneration are based on the induction of DSC proliferation, differentiation, migration and tissue formation. Another approach using growth factors is to induce stemness of cells effectively because the recruitment of MSCs from their niche to the damaged area is an important step in the regeneration of tissue. We have recently reported that semaphorin 3A (Sema3A)-treated PDL cells upregulated the expression of stem cell markers such as NANOG, OCT4, E-cadherin, CD73, CD90, CD105, CD146 and CD166, and promoted cell division and enhanced multipotency [52]. These results suggest that Sema3A may be capable of inducing the dedifferentiation of PDL cells into mesenchymal-stem-like cells, and could be promising for periodontal tissue regeneration; however, further studies are required to clarify the in vivo effects using animal models.

To date, the application of exogenous individual or combinations of growth factors to damaged tissues have provided good results in promoting periodontal regeneration, and the cell transplantation combined with growth factors may be a more effective method for tissue regeneration than cell transplantation alone. Further studies are needed to determine the optimal concentrations and combinations of growth factors and to confirm their safety, especially regarding immune rejection and tumorigenesis.

14.2.3 Scaffolds

14.2.3.1 Bioactive Scaffolds

Scaffolds are important components for tissue engineering because they provide structural support for cell attachment, and have the capability of acting as delivery vehicles for growth factors, while subsequently integrating into the tissue. A huge variety of scaffolds, ranging from natural polymers and ceramics to synthetic polymers, have been reported to induce stem cell proliferation and differentiation, and consequently promote dental tissue regeneration. Natural polymers, such as collagen and chitosan, were the first to be used as scaffolds for dental tissue regeneration. A collagen scaffold seeded with DPSCs, and dentin matrix protein 1 (DMP1) induced the formation of a matrix similar to dentin and pulp [53]. A collagen scaffold stimulated PDLSCs to attach, proliferate, exhibit a PDL spindle-like morphology and subsequently generate PDL-like tissue [54]. DPSCs seeded on a chitosan scaffold survived and differentiated into neural cells [55]. In addition, a scaffold derived from a combination of collagen and chitosan promoted the migration, proliferation, and differentiation of DPSCs and HAT-7 cells [56]. Hydroxyapatite (HA) and tricalcium phosphate (TCP) are two of the most biocompatible ceramics because of their properties of resorption, biocompatibility, low immunogenicity, osteoconductivity, bone bonding capacity and similarity to bone and tooth. Sonoyama et al. transplanted an artificial tooth root fabricated from HA/TCP with root apical papilla stem cells (SCAPs) and PDLSCs into the tooth socket [15]. Three months after transplantation, new dentin and PDL tissues were formed, and the treatment consequently resulted in normal tooth function. Synthetic polymers, such as polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL), polyethylene glycol (PEG) and poly(lactic-co-glycolic) acid (PLGA), have also been used as scaffold materials for various tissue regeneration studies [57, 58].

Although both bioactive ceramics and polymers have been claimed to be the most optimal scaffolds for tissue engineering, they both have advantages and disadvantages for regenerating dental tissues (*e.g.*, periodontal tissue) as dental tissues include both soft tissue (*e.g.* periodontal ligament and gingival), and hard tissues (*e.g.* alveolar bone and cementum). Bioactive ceramics such as HA and β-TCP are well known to show high biocompatibility and osteoconductivity [59–62], while they are not substantially degraded and remain in living organisms under physiological conditions [63, 64]. DPSCs and PDLSCs transplanted with β-TCP scaffolds subcutaneously into the dorsal surfaces of mice have been reported to form dentin/pulp complex-like tissue and periodontal-like tissue [11, 13]. Meanwhile, polymers, including natural and synthetic polymers that form porous structures, are ideal for the engraftment and survival of cells; however, they do not have enough strength to create spaces for regenerating tissue, especially hard tissues that are found in the periodontium. Thus, composites of bioactive ceramics and polymers, which are biomimetic scaffolds, are currently being developed in hope of taking advantage of each component's characteristics to increase the mechanical stability of the scaffold

and improve tissue interaction and their degradation [65]. The fabrication of composites from bioactive ceramics and polymers was generally prepared by simple techniques, whereby bioactive ceramics are immersed in a polymer solution, or polymers are mineralized in saturated bioactive ceramic matrix solutions or in simulated body fluid, followed by drying or freeze-drying. More exact techniques, like electrospinning and thermally induced phase separation, have also been introduced for the fabrication of the composite scaffolds [19, 66, 67].

A number of studies using composites of bioactive ceramics with natural or synthetic polymers have been reported to show favorable results for the regeneration of periodontal tissue. Among various combinations of bioactive ceramics and polymers, the combination of HA and collagen (HA/Col) has frequently been investigated for bone tissue engineering because of its similar structure to bone tissue [68–71]. β-TCP was also studied by many researchers as a bioactive ceramic other than HA for periodontal tissue regeneration, because β-TCP is known to be biocompatible, and osteoconductive similar to HA. Furthermore, the degradation rate of β-TCP is higher than that of HA, which is an important feature for a scaffold to be used in tissue regeneration applications [72]. An in vivo study of a rabbit model of a proximal tibial and distal femoral bone defect revealed that 85 % of β-TCP and only 5.4 % of HA were resorbed at 3 months post-implantation [73]. Hence, the potential of composite scaffolds of β-TCP and polymers has been widely investigated in tissue regeneration.

Composites of bioactive ceramics and polymers are also expected to function as efficient biomimetic scaffolds for the delivery of growth factors and stem cells to implant sites because of their high protein absorption capacity and biocompatibility [74]. A biodegradable polymer, gelatin hydrogel, has been reported to be a promising carrier material for growth factor or drug delivery in various forms such as a sponge, sheet, and liquid [75]. More recently, the combined nanosized β-TCP and collagen scaffold (nβ-TCP/Col) were reported to show good biocompatibility and osteoconductivity in vitro and in vivo [76]. The advantage of using nanosized β-TCP in biomimetic scaffolds is that it is believed to undergo easier resorption compared with the microsized form, while still performing its primary role as a cell attachable scaffold. Thus, the transplantation of DSCs seeded in nanosized β-TCP combined with collagen scaffolds could be more effective method for dental tissue engineering although further study is needed.

14.2.3.2 Custom-Made Scaffold

A rapid prototyping (RP) system has been developed to fabricate scaffolds for tissue engineering applications [77]. The system reads data from computer-aided design images and automatically produces three-dimensional objects according to the virtual design. The application of RP to tissue engineering enables us to produce three-dimensional scaffolds with complex geometries and very fine structures. In addition, RP can control features of scaffolds such as porosity, surface design and mechanical properties, so it allows us to fabricate applications of a desired structural integrity.

Park et al. applied RP to a rat model of PDL fenestration defects, where they fabricated two types of customized defect-fit hybrid scaffolds (random-porous scaffolds and PDL fiber-guiding scaffolds) and transplanted them with PDLSCs into the defects [78]. The fiber-guiding scaffolds significantly enhanced the formation of PDL, bone and cementum in the fenestration defects compared with the random-porous scaffolds, suggesting that well-designed custom-made scaffolds with controlled and oriented fiber channels are important to regenerate functional periodontium.

14.2.4 Dental Tissue Engineering

14.2.4.1 Bone

Although growth factors such as EMD and BMP2 do induce bone formation, their ability to regenerate bone of certain sizes is limited. In the case of large bone defects, DSCs, especially DPSCs and SHEDs, are believed to be promising cell sources to regenerate bone tissue. The implantation of both human stem cells with scaffolds into artificial cranial bone defects in non-immunosuppressed rats resulted in the formation of more new mature bone than cell-free implants [79–81]. Seo et al. reported that SHEDs not only induced recipient cells to differentiate into osteogenic cells for the formation of new bone, which had been previously reported [12], but also actively contributed to bone formation by themselves. The other group demonstrated that DPSC implantation with dental implants in a dog model showed increased osseointegration of dental implants compared with the implantation of bone marrow stem cells and periosteal cells [82]. iPS cells are also believed to be an ideal candidate cell source because of their pluripotency and ability for autologous transplantation. The osteoblast lineage can be differentiated from iPS cell-derived embryoid bodies by culturing with differentiation medium supplemented with dexamethasone, ascorbic acid and β-glycerophosphate in vitro [83, 84]. Osteoblastic differentiation-induced iPS cells were transplanted into calvarial bone defects in SCID mice, and bone formation was confirmed in soft X-ray images and tissue specimens [84]. As iPS cells derived from dental tissue have been well established, the use of iPS cells derived from dental tissue for bone regeneration is of great interest.

14.2.4.2 Dentin/Pulp Complex

Once dentin caries occur, pulp tissue can subsequently become infected with bacteria. The pulp tissue can therefore be difficult to regenerate because of the small volume of the pulp tissue and limited blood supply compared with other connective tissues. Thus, the establishment of stem cell-based therapies to regenerate the damaged dentin/pulp complex can be challenging. However, recently Iohara et al. reported that autologous transplantation of CD31-negative side population cells or CD105-positive cells, which exhibited DPSC-like characteristics, with stromal-cell-derived factor-1 and a collagen

scaffold into the empty root canals of dogs showed dentin/pulp complex regeneration, accompanied by neoangiogenesis and reinnervation [85, 86]. More recently, mobilized DPSCs induced by granulocyte-colony stimulating factor (GCSF) stimulation were examined for their potential for clinical application [87]. The preclinical safety, feasibility and efficacy of pulp regeneration by these human mobilized DPSCs were confirmed in vitro and in vivo in an animal model. In the future, it may be possible to regenerate dentin/pulp complex using DPSCs for endodontic treatment in the clinic. First, however, a major limitation must be addressed in the decontamination of the pulp cavity prior to regeneration of dentin/pulp complex.

14.2.4.3 Periodontal Tissue

Many reports have demonstrated successful PDL tissue regeneration using a combination of stem cells, growth factors and scaffolds in animal models. BMP2-transduced bone marrow derived MSCs combined with a polyethylene and polypropylene oxide copolymer scaffold promoted the formation of PDL-like soft tissue that inserted into both the new cementum and the new bone in rat models [42]. Adipose derived stem cells combined with a PRP gel, acting as growth factor reservoir and a scaffold, filled class III periodontal defects with newly formed PDL, cementum and bone in canine models [88]. Human foreskin-derived iPS cells combined with EMD and an apatite-coated silk fibroin scaffold promoted the repair of mouse periodontal defects by inducing the formation of new PDL, cementum, and bone [89]. Avulsed teeth reimplanted to the tooth sockets with a PDLSCs sheet in combination with platelet-rich fibrin granules showed the induction of PDL tissue healing and a reduction of ankylosis and inflammatory tooth resorption in canine models [90]. More recently, a human clinical trial was performed to investigate the efficacy of a combination of stem cells with growth factors and scaffolds for the healing of PDL disease. Yamada et al. transplanted autologous PDLSCs combined with PRP and atelocollagen scaffolds into bone defects adjacent to the tooth root surface in 17 periodontitis patients with deep intraosseous defects [91]. After 1 year of treatment, mean levels of clinical attachment, probing depth, and alveolar bone were significantly improved, compared with pre-treatment values [91]. The transplantation of PDLSC sheets have also attracted attention in the regeneration of periodontal tissue. Iwata et al. reported PDLSC sheet technology for periodontal tissue regeneration of alveolar bone defects in a beagle dog model [92, 93]. Currently, there is an ongoing clinical trial using human PDLSC sheet for periodontal tissue regeneration in Japan [94]. These reports suggest that tissue-engineering technology has the potential to become an effective method to realize the regeneration of functional PDL tissue.

14.2.4.4 Tooth

One of the ultimate goals of dental tissue regeneration is to construct an artificial whole tooth that performs the complete function of natural tooth. Tissue engineering technology should play crucial roles in achieving functional tooth reconstruction. The

progression of current bioengineering technologies for regenerating three-dimensional organs has involved replicating tissue development through epithelial–mesenchymal interactions that occur in the developing embryo. In the dental field, Honda et al. transplanted cell-scaffold constructs into the omentum of rats that were formed by plating DSCs onto a collagen scaffold, followed by seeding dental epithelial cells on top of the DSCs [95]. The results of their study demonstrated small tooth structures containing all of the tooth tissue components, including enamel, dentine and pulp. Interestingly, when mesenchymal and epithelial cells were cultured in the same dish divided by a microporous membrane, they were unable to form tooth tissue components. This result indicated that the direct interaction of epithelial–mesenchymal cells would be essential for successful generation of bioengineered teeth. In addition, Nakao et al. formed a bioengineered tooth germ that contained epithelial and mesenchymal cells, isolated from a mouse tooth germ, in a collagen gel drop [96]. When the bioengineered tooth germ was cultured in vitro, incisor and molar tooth germ-like tissues were observed in the gel drop. Interestingly, when these tooth germ-like tissues were transplanted into the tooth socket, they generated a correct tooth structure, including enamel, dentin, cementum, pulp, blood vessels, bone, sensory nerves and PDL. More recently, this research group reported that the bioengineered tooth formed by the tooth germ-like tissue successfully erupted and reached occlusion with an opposing tooth [97]. These results suggest that bioengineered teeth generated using tissue engineering technology could have several functions of natural teeth. Although tissue engineering technology should be useful for the formation of artificial teeth, some fundamental problems still remain, including the irregularity of the tooth size and shape, the long period required for regeneration, and the difficulty of acquiring a sufficient number of epithelial mesenchymal cells. For example, an erupted bioengineered tooth was generated from a mouse molar tooth germ, but its size was approximately half of the original molar tooth when it erupted [97]. Thus, further studies by many researchers are required to address these problems.

14.3 Ethical Issues

During recent years, tissue engineering and regenerative medicine using stem cells have been investigated worldwide, and a number of scientists, researchers, clinicians and companies have been competitively engaged in research. Tissue engineering using cell transplantation is shifting from in vitro and animal models to human clinical trials. Information on clinical trials already performed throughout the world can be found on the United States National Institutes of Health Trials website (https://www.clinicaltrials.gov/). It is well known that one of the biggest challenges in tissue engineering using cell transplantation is the ethical issues associated with obtaining cells. The fundamental principle for researchers using stem cells for tissue engineering studies is to respect bioethical guidelines. To protect the life, health, privacy and dignity of research participants, donors of cells and organs and recipients, researchers have to ensure safety and efficacy of stem cell

transplantation. In particular, the safety of the tools, drugs and reagents used for treating cells must be confirmed, and all concerns regarding cell transplantation must be explained to participants, donors and recipients prior to obtaining informed consent. Furthermore, the confidentiality of personal information must always be protected. Notably, with the use of ES cells, there are some distinct ethical issues associated with the embryo from which the cells are obtained, including its right to live. There are also ethical issues with the use of iPS cells and whether these can be used to form an embryo for human cloning.

The use of MSCs for tissue engineering are characterized by less ethical issues as MSCs are not treated with genetic reprogramming unlike iPS cells, and don't normally form teratoma different from ES and iPS cells. In particular, DPSCs, which can be retrieved from pulp tissue of extracted teeth such as wisdom and deciduous teeth, have no ethical tissue because extracted teeth are usually discarded. Nonetheless, there are various risks in stem cell transplantation such as infection, genetic modification, switched cells, and so on. Thus, researchers have the responsibility of adhering to the ethical guidelines of each country, especially regarding clinical trials.

14.4 Future Directions

To date, it is believed that three elements are essential to achieve effective tissue engineering, including a combination of cells, growth factors and scaffolds [98]. In dental tissue engineering, for example, EMD application into marginal bone defects caused by periodontitis is so far one of most effective treatments to regenerate periodontal tissue including alveolar bone. However, in this case, only one element essential to regenerate the tissue is incorporated: EMD, which acted as the growth factors, but the other elements are endogenous host cells and no scaffold. Therefore, it is unsurprising that EMD application has shown limited potential for periodontal tissue regeneration in spite of good clinical performance. To achieve more effective regeneration, novel regenerative methods using stem cells or other cell types are currently being developed. At present, research in regenerative medicine using stem cells is developing at an accelerating pace. To establish alternative effective treatments for regenerating dental tissue using stem cells in clinics, we faces a host of challenges; isolation of effective stem cell populations, identification of stem cell markers and genes, and the development of effective bio scaffold for stem cell maintenance and activity. Future directions of dental tissue engineering using stem cells are illustrated in Fig. 14.1. There is still no definite method to efficiently identify DSCs but this could be avoided by the use of iPS cells. In particular, dental tissue-derived iPS cells might be useful for dental tissue regeneration as they have been shown to maintain an epigenetic memory of their tissue of origin, which influences the subsequent differentiation potential of specific iPS cells [99]. However, the clinical application of MSCs could occur sooner than iPS cells, as there are still some problems associated with iPS clinical applications such as teratoma formation, control of differentiation and cost of reprogramming cells. One of the main issues

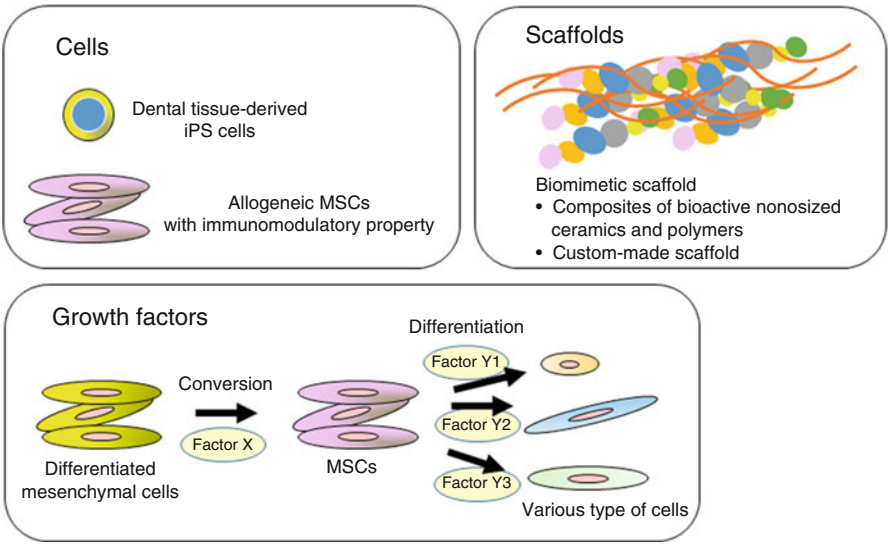

Fig. 14.1 Future directions of dental tissue engineering using stem cells. Three elements essential to achieve effective dental tissue engineering, including a combination of cells, growth factors and scaffolds are currently being developed. Cells; dental pulp stem cells (DPSCs), gingiva derived stem cells (GMSCs), dental tissue-derived iPS cells or allogeneic mesenchymal stem cells (MSCs) which possess immunomodulatory property may be useful. Growth factors; growth factor X which can convert differentiated cells to MSCs, and growth factors Y which can control cell differentiation might be identified. Scaffolds; Custom-made biomimetic scaffold could be useful for dental tissue engineering. By using these elements and methods, more effective dental tissue engineering treatment might be established in the future

regarding the use of autologous MSCs is the technical difficulty associated with the isolation of large enough quantities of MSCs with high quality from one patient, especially as the growth and differentiation potentials of MSCs vary between individuals although DPSCs and GMSCs are relatively overcoming these issues. One way to address this issue is to establish a cell transplantation method using allogeneic MSCs as an alternative cell source. Because of the immunomodulatory properties of MSCs, further study is required to investigate the possibility of transplanting allogeneic MSCs. If possible, selected good quality MSCs can then be stored in a cell bank for widespread use. Another method of potential application is the more efficient induction of MSC populations. If factors which can induce the conversion of differentiated cells into MSCs or neural crest cells are identified, they may be useful for dental stem cell engineering and the efficient regeneration of dental tissue. As the application of growth factors alone is an easy method for clinical treatment, studies focused on the identification of essential growth factors for the effective regeneration of tissues are also of importance. With regard to scaffolds for dental tissue regeneration, an ideal scaffold should be investigated that will allow for sufficient space for tissue regeneration, and exhibit high biocompatibility, good cell attachment, appropriate degradation and be capable of delivering growth factors

and stem cells. Furthermore, we must consider additional factors such as time, cost and risk for the patient. It is important to accumulate solid evidence for not only the regenerative effects but also their mechanisms and safety, so that tissue engineering can be correctly conveyed and applied to patients. Whereby, the dentists and researchers must make more efforts in the studies of regenerative medicine and need to bring together our wisdom.

References

1. Simonpieri A, Del Corso M, Vervelle A, Jimbo R, Inchingolo F, Sammartino G, Dohan Ehrenfest DM (2012) Current knowledge and perspectives for the use of platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) in oral and maxillofacial surgery part 2: bone graft, implant and reconstructive surgery. Curr Pharm Biotechnol 13(7):1231–1256
2. Venezia E, Goldstein M, Boyan BD, Schwartz Z (2004) The use of enamel matrix derivative in the treatment of periodontal defects: a literature review and meta-analysis. Crit Rev Oral Biol Med 15(6):382–402
3. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391):1145–1147
4. Baroffio A, Hamann M, Bernheim L, Bochaton-Piallat ML, Gabbiani G, Bader CR (1996) Identification of self-renewing myoblasts in the progeny of single human muscle satellite cells. Differentiation 60(1):47–57
5. Fukuchi Y, Nakajima H, Sugiyama D, Hirose I, Kitamura T, Tsuji K (2004) Human placenta-derived cells have mesenchymal stem/progenitor cell potential. Stem Cells 22(5):649–658
6. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284(5411):143–147
7. Zannettino AC, Paton S, Arthur A, Khor F, Itescu S, Gimble JM, Gronthos S (2008) Multipotential human adipose-derived stromal stem cells exhibit a perivascular phenotype in vitro and in vivo. J Cell Physiol 214(2):413–421
8. Friedenstein AJ, Chailakhyan RK, Latsinik NV, Panasyuk AF, Keiliss-Borok IV (1974) Stromal cells responsible for transferring the microenvironment of the hemopoietic tissues. Cloning in vitro and retransplantation in vivo. Transplantation 17(4):331–340
9. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP (1999) Bone marrow as a potential source of hepatic oval cells. Science 284(5417):1168–1170
10. Kopen GC, Prockop DJ, Phinney DG (1999) Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci U S A 96(19):10711–10716
11. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S (2000) Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 97(25):13625–13630
12. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, Shi S (2003) SHED: stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 100(10):5807–5812
13. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S (2004) Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 364(9429):149–155
14. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Shi S, Wang S (2006) Mesenchymal stem cell-mediated functional tooth regeneration in swine. PLoS One 1:1–8
15. Morsczeck C, Gotz W, Schierholz J, Zeilhofer F, Kuhn U, Mohl C, Sippel C, Hoffmann KH (2005) Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 24(2):155–165

16. Mitrano TI, Grob MS, Carrion F, Nova-Lamperti E, Luz PA, Fierro FS, Quintero A, Chaparro A, Sanz A (2010) Culture and characterization of mesenchymal stem cells from human gingival tissue. J Periodontol 81(6):917–925
17. Zhang Q, Shi S, Liu Y, Uyanne J, Shi Y, Le AD (2009) Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. J Immunol 183(12):7787–7798
18. Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA, Lerou PH, Lensch MW, Daley GQ (2008) Reprogramming of human somatic cells to pluripotency with defined factors. Nature 451(7175):141–146
19. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131(5):861–872
20. Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA (2007) Induced pluripotent stem cell lines derived from human somatic cells. Science 318(5858):1917–1920
21. Yan X, Qin H, Qu C, Tuan RS, Shi S, Huang GT (2010) iPS cells reprogrammed from human mesenchymal-like stem/progenitor cells of dental tissue origin. Stem Cells Dev 19(4):469–480
22. Tamaoki N, Takahashi K, Tanaka T, Ichisaka T, Aoki H, Takeda-Kawaguchi T, Iida K, Kunisada T, Shibata T, Yamanaka S, Tezuka K (2010) Dental pulp cells for induced pluripotent stem cell banking. J Dent Res 89(8):773–778
23. Egusa H, Okita K, Kayashima H, Yu G, Fukuyasu S, Saeki M, Matsumoto T, Yamanaka S, Yatani H (2010) Gingival fibroblasts as a promising source of induced pluripotent stem cells. PLoS One 5(9):1–12
24. Wada N, Wang B, Lin NH, Laslett AL, Gronthos S, Bartold PM (2011) Induced pluripotent stem cell lines derived from human gingival fibroblasts and periodontal ligament fibroblasts. J Periodontal Res 46(4):438–447
25. Medici D, Shore EM, Lounev VY, Kaplan FS, Kalluri R, Olsen BR (2010) Conversion of vascular endothelial cells into multipotent stem-like cells. Nat Med 16(12):1400–1406
26. Meng X, Su RJ, Baylink DJ, Neises A, Kiroyan JB, Lee WY, Payne KJ, Gridley DS, Wang J, Lau KH, Li G, Zhang XB (2013) Rapid and efficient reprogramming of human fetal and adult blood CD34+ cells into mesenchymal stem cells with a single factor. Cell Res 23(5):658–672
27. Zabierowski SE, Baubet V, Himes B, Li L, Fukunaga-Kalabis M, Patel S, McDaid R, Guerra M, Gimotty P, Dahmane N, Herlyn M (2011) Direct reprogramming of melanocytes to neural crest stem-like cells by one defined factor. Stem Cells 29(11):1752–1762
28. Wada N, Menicanin D, Shi S, Bartold PM, Gronthos S (2009) Immunomodulatory properties of human periodontal ligament stem cells. J Cell Physiol 219(3):667–676
29. Yamaza T, Kentaro A, Chen C, Liu Y, Shi Y, Gronthos S, Wang S, Shi S (2010) Immunomodulatory properties of stem cells from human exfoliated deciduous teeth. Stem Cell Res Ther 1(1):5
30. Zhao Y, Wang L, Jin Y, Shi S (2012) Fas ligand regulates the immunomodulatory properties of dental pulp stem cells. J Dent Res 91(10):948–954
31. Su WR, Zhang QZ, Shi SH, Nguyen AL, Le AD (2011) Human gingiva-derived mesenchymal stromal cells attenuate contact hypersensitivity via prostaglandin E2-dependent mechanisms. Stem Cells 29(11):1849–1860
32. Kim E, Cho SW, Yang JY, Cai J, Lee SL, Ohshima H, Jung HS (2006) Tooth survival and periodontal tissues healing of allogenic-transplanted teeth in the mice. Oral Dis 12(4):395–401
33. Mrozik KM, Wada N, Marino V, Richter W, Shi S, Wheeler DL, Gronthos S, Bartold PM (2013) Regeneration of periodontal tissues using allogeneic periodontal ligament stem cells in an ovine model. Regen Med 8(6):711–723
34. Hammarstrom L (1997) Enamel matrix, cementum development and regeneration. J Clin Periodontol 24(9 Pt 2):658–668
35. Carinci F, Piattelli A, Guida L, Perrotti V, Laino G, Oliva A, Annunziata M, Palmieri A, Pezzetti F (2006) Effects of Emdogain on osteoblast gene expression. Oral Dis 12(3):329–342
36. Suzuki S, Nagano T, Yamakoshi Y, Gomi K, Arai T, Fukae M, Katagiri T, Oida S (2005) Enamel matrix derivative gel stimulates signal transduction of BMP and TGF-β. J Dent Res 84(6):510–514

37. Ritzenthaler JD, Goldstein RH, Fine A, Lichtler A, Rowe DW, Smith BD (1991) Transforming-growth-factor-beta activation elements in the distal promoter regions of the rat alpha 1 type I collagen gene. Biochem J 280(Pt 1):157–162
38. Matsuda N, Lin WL, Kumar NM, Cho MI, Genco RJ (1992) Mitogenic, chemotactic, and synthetic responses of rat periodontal ligament fibroblastic cells to polypeptide growth factors in vitro. J Periodontol 63(6):515–525
39. Fujii S, Maeda H, Tomokiyo A, Monnouchi S, Hori K, Wada N, Akamine A (2010) Effects of TGF-beta1 on the proliferation and differentiation of human periodontal ligament cells and a human periodontal ligament stem/progenitor cell line. Cell Tissue Res 342(2):233–242
40. Kinoshita A, Oda S, Takahashi K, Yokota S, Ishikawa I (1997) Periodontal regeneration by application of recombinant human bone morphogenetic protein-2 to horizontal circumferential defects created by experimental periodontitis in beagle dogs. J Periodontol 68(2):103–109
41. Ripamonti U (2007) Recapitulating development: a template for periodontal tissue engineering. Tissue Eng 13(1):51–71
42. Chen YL, Chen PK, Jeng LB, Huang CS, Yang LC, Chung HY, Chang SC (2008) Periodontal regeneration using ex vivo autologous stem cells engineered to express the BMP-2 gene: an alternative to alveolaplasty. Gene Ther 15(22):1469–1477
43. Kemoun P, Gronthos S, Snead ML, Rue J, Courtois B, Vaysse F, Salles JP, Brunel G (2011) The role of cell surface markers and enamel matrix derivatives on human periodontal ligament mesenchymal progenitor responses in vitro. Biomaterials 32(30):7375–7388
44. He H, Yu J, Liu Y, Lu S, Liu H, Shi J, Jin Y (2008) Effects of FGF2 and TGFbeta1 on the differentiation of human dental pulp stem cells in vitro. Cell Biol Int 32(7):827–834
45. Kono K, Maeda H, Fujii S, Tomokiyo A, Yamamoto N, Wada N, Monnouchi S, Teramatsu Y, Hamano S, Koori K, Akamine A (2013) Exposure to transforming growth factor-beta1 after basic fibroblast growth factor promotes the fibroblastic differentiation of human periodontal ligament stem/progenitor cell lines. Cell Tissue Res 352(2):249–263
46. Lee UL, Jeon SH, Park JY, Choung PH (2011) Effect of platelet-rich plasma on dental stem cells derived from human impacted third molars. Regen Med 6(1):67–79
47. Xu Q, Li B, Yuan L, Dong Z, Zhang H, Wang H, Sun J, Ge S, Jin Y (2014) Combination of platelet-rich plasma within periodontal ligament stem cell sheets enhances cell differentiation and matrix production. J Tissue Eng Regen Med. doi:10.1002/term.1953
48. Yu B, Wang Z (2014) Effect of concentrated growth factors on beagle periodontal ligament stem cells in vitro. Mol Med Rep 9(1):235–242
49. Dangaria SJ, Ito Y, Walker C, Druzinsky R, Luan X, Diekwisch TG (2009) Extracellular matrix-mediated differentiation of periodontal progenitor cells. Differentiation 78(2-3):79–90
50. Yuda A, Maeda H, Fujii S, Monnouchi S, Yamamoto N, Wada N, Koori K, Tomokiyo A, Hamano S, Hasegawa D, Akamine A (2014) Effect of CTGF/CCN2 on osteo/cementoblastic and fibroblastic differentiation of a human periodontal ligament stem/progenitor cell line. J Cell Physiol 230(1):150–159
51. Hasegawa D, Wada N, Maeda H, Yoshida S, Mitarai H, Tomokiyo A, Monnouchi S, Hamano S, Yuda A, Akamine A (2015) Wnt5a induces collagen production by human periodontal ligament cells through TGFbeta1-mediated upregulation of periostin expression. J Cell Physiol. doi:10.1002/jcp.24950
52. Wada N, Maeda H, Hasegawa D, Gronthos S, Bartold PM, Menicanin D, Fujii S, Yoshida S, Tomokiyo A, Monnouchi S, Akamine A (2014) Semaphorin 3A induces mesenchymal-stem-like properties in human periodontal ligament cells. Stem Cells Dev 23(18):2225–2236
53. Prescott RS, Alsanea R, Fayad MI, Johnson BR, Wenckus CS, Hao J, John AS, George A (2008) In vivo generation of dental pulp-like tissue by using dental pulp stem cells, a collagen scaffold, and dentin matrix protein 1 after subcutaneous transplantation in mice. J Endod 34(4):421–426
54. Luan X, Dangaria S, Ito Y, Walker CG, Jin T, Schmidt MK, Galang MT, Druzinsky R (2009) Neural crest lineage segregation: a blueprint for periodontal regeneration. J Dent Res 88(9):781–791
55. Feng X, Lu X, Huang D, Xing J, Feng G, Jin G, Yi X, Li L, Lu Y, Nie D, Chen X, Zhang L, Gu Z, Zhang X (2014) 3D porous chitosan scaffolds suit survival and neural differentiation of dental pulp stem cells. Cell Mol Neurobiol 34(6):859–870

56. Ravindran S, Song Y, George A (2010) Development of three-dimensional biomimetic scaffold to study epithelial-mesenchymal interactions. Tissue Eng Part A 16(1):327–342
57. Gebhardt M, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F (2009) Cell survival within pulp and periodontal constructs. J Endod 35(1):63–66
58. Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, Smith AJ, Nor JE (2008) Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 34(8):962–969
59. de Bruijn JD, van Blitterswijk CA, Davies JE (1995) Initial bone matrix formation at the hydroxyapatite interface in vivo. J Biomed Mater Res 29(1):89–99
60. Yang Z, Yuan H, Tong W, Zou P, Chen W, Zhang X (1996) Osteogenesis in extraskeletally implanted porous calcium phosphate ceramics: variability among different kinds of animals. Biomaterials 17(22):2131–2137
61. Yuan H, Li Y, de Bruijn JD, de Groot K, Zhang X (2000) Tissue responses of calcium phosphate cement: a study in dogs. Biomaterials 21(12):1283–1290
62. Gaasbeek RD, Toonen HG, van Heerwaarden RJ, Buma P (2005) Mechanism of bone incorporation of beta-TCP bone substitute in open wedge tibial osteotomy in patients. Biomaterials 26(33):6713–6719
63. Lu J, Descamps M, Dejou J, Koubi G, Hardouin P, Lemaitre J, Proust JP (2002) The biodegradation mechanism of calcium phosphate biomaterials in bone. J Biomed Mater Res 63(4):408–412
64. Fujita R, Yokoyama A, Kawasaki T, Kohgo T (2003) Bone augmentation osteogenesis using hydroxyapatite and beta-tricalcium phosphate blocks. J Oral Maxillofac Surg 61(9):1045–1053
65. Wang M (2003) Developing bioactive composite materials for tissue replacement. Biomaterials 24(13):2133–2151
66. Danilchenko SN, Kalinkevich OV, Pogorelov MV, Kalinkevich AN, Sklyar AM, Kalinichenko TG, Ilyashenko VY, Starikov VV, Bumeyster VI, Sikora VZ, Sukhodub LF (2011) Characterization and in vivo evaluation of chitosan-hydroxyapatite bone scaffolds made by one step coprecipitation method. J Biomed Mater Res A 96(4):639–647
67. Holzwarth JM, Ma PX (2011) Biomimetic nanofibrous scaffolds for bone tissue engineering. Biomaterials 32(36):9622–9629
68. Kikuchi M, Itoh S, Ichinose S, Shinomiya K, Tanaka J (2001) Self-organization mechanism in a bone-like hydroxyapatite/collagen nanocomposite synthesized in vitro and its biological reaction in vivo. Biomaterials 22(13):1705–1711
69. Takechi M, Miyamoto Y, Ishikawas K, Momota Y, Yuasa T, Tatehara S, Takano H, Minamiguchi S, Nagayama M (2007) Histological evaluation of apatite cement containing atelocollagen. Dent Mater J 26(2):194–200
70. Minabe M, Sugaya A, Satou H, Tamura T, Ogawa Y, Hori T, Watanabe Y (1988) Histological study of the hydroxyapatite-collagen complex implants in periodontal osseous defects in dogs. J Periodontol 59(10):671–678
71. Sugaya A, Minabe M, Tamura T, Hori T, Watanabe Y (1989) Effects on wound healing of hydroxyapatite-collagen complex implants in periodontal osseous defects in the dog. J Periodontal Res 24(4):284–288
72. Yaszemski MJ, Payne RG, Hayes WC, Langer R, Mikos AG (1996) Evolution of bone transplantation: molecular, cellular and tissue strategies to engineer human bone. Biomaterials 17(2):175–185
73. Eggli PS, Muller W, Schenk RK (1988) Porous hydroxyapatite and tricalcium phosphate cylinders with two different pore size ranges implanted in the cancellous bone of rabbits: a comparative histomorphometric and histologic study of bony ingrowth and implant substitution. Clin Orthop Relat Res (232):127–138
74. Woo KM, Seo J, Zhang R, Ma PX (2007) Suppression of apoptosis by enhanced protein adsorption on polymer/hydroxyapatite composite scaffolds. Biomaterials 28(16):2622–2630
75. Tabata Y (2003) Tissue regeneration based on growth factor release. Tissue Eng 9(Suppl 1):S5–S15
76. Ibara A, Miyaji H, Fugetsu B, Nishida E, Takita H, Tanaka S, Sugaya T, Kawanami M (2013) Osteoconductivity and biodegradability of collagen scaffold coated with nano-beta-TCP and fibroblast growth factor 2. J Nanomater 2013:Article ID 639502

77. Loh QL, Choong C (2013) Three-dimensional scaffolds for tissue engineering applications: role of porosity and pore size. Tissue Eng Part B Rev 19(6):485–502
78. Park CH, Rios HF, Jin Q, Sugai JV, Padial-Molina M, Taut AD, Flanagan CL, Hollister SJ, Giannobile WV (2012) Tissue engineering bone-ligament complexes using fiber-guiding scaffolds. Biomaterials 33(1):137–145
79. de Mendonca CA, Bueno DF, Martins MT, Kerkis I, Kerkis A, Fanganiello RD, Cerruti H, Alonso N, Passos-Bueno MR (2008) Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. J Craniofac Surg 19(1):204–210
80. Seo BM, Sonoyama W, Yamaza T, Coppe C, Kikuiri T, Akiyama K, Lee JS, Shi S (2008) SHED repair critical-size calvarial defects in mice. Oral Dis 14(5):428–434
81. Riccio M, Maraldi T, Pisciotta A, La Sala GB, Ferrari A, Bruzzesi G, Motta A, Migliaresi C, De Pol A (2012) Fibroin scaffold repairs critical-size bone defects in vivo supported by human amniotic fluid and dental pulp stem cells. Tissue Eng Part A 18(9-10):1006–1013
82. Ito K, Yamada Y, Nakamura S, Ueda M (2011) Osteogenic potential of effective bone engineering using dental pulp stem cells, bone marrow stem cells, and periosteal cells for osseointegration of dental implants. Int J Oral Maxillofac Implants 26(5):947–954
83. Bilousova G, du Jun H, King KB, De Langhe S, Chick WS, Torchia EC, Chow KS, Klemm DJ, Roop DR, Majka SM (2011) Osteoblasts derived from induced pluripotent stem cells form calcified structures in scaffolds both in vitro and in vivo. Stem Cells 29(2):206–216
84. Hayashi T, Misawa H, Nakahara H, Noguchi H, Yoshida A, Kobayashi N, Tanaka M, Ozaki T (2012) Transplantation of osteogenically differentiated mouse iPS cells for bone repair. Cell Transplant 21(2-3):591–600
85. Iohara K, Imabayashi K, Ishizaka R, Watanabe A, Nabekura J, Ito M, Matsushita K, Nakamura H, Nakashima M (2011) Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1. Tissue Eng Part A 17(15-16):1911–1920
86. Ishizaka R, Iohara K, Murakami M, Fukuta O, Nakashima M (2012) Regeneration of dental pulp following pulpectomy by fractionated stem/progenitor cells from bone marrow and adipose tissue. Biomaterials 33(7):2109–2118
87. Nakashima M, Iohara K (2014) Mobilized dental pulp stem cells for pulp regeneration: initiation of clinical trial. J Endod 40(4 Suppl):S26–S32
88. Tobita M, Uysal CA, Guo X, Hyakusoku H, Mizuno H (2013) Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. Cytotherapy 15(12):1517–1526
89. Duan X, Tu Q, Zhang J, Ye J, Sommer C, Mostoslavsky G, Kaplan D, Yang P, Chen J (2011) Application of induced pluripotent stem (iPS) cells in periodontal tissue regeneration. J Cell Physiol 226(1):150–157
90. Zhao YH, Zhang M, Liu NX, Lv X, Zhang J, Chen FM, Chen YJ (2013) The combined use of cell sheet fragments of periodontal ligament stem cells and platelet-rich fibrin granules for avulsed tooth reimplantation. Biomaterials 34(22):5506–5520
91. Yamada Y, Nakamura S, Ito K, Umemura E, Hara K, Nagasaka T, Abe A, Baba S, Furuichi Y, Izumi Y, Klein OD, Wakabayashi T (2013) Injectable bone tissue engineering using expanded mesenchymal stem cells. Stem Cells 31(3):572–580
92. Iwata T, Yamato M, Tsuchioka H, Takagi R, Mukobata S, Washio K, Okano T, Ishikawa I (2009) Periodontal regeneration with multi-layered periodontal ligament-derived cell sheets in a canine model. Biomaterials 30(14):2716–2723
93. Washio K, Iwata T, Mizutani M, Ando T, Yamato M, Okano T, Ishikawa I (2010) Assessment of cell sheets derived from human periodontal ligament cells: a pre-clinical study. Cell Tissue Res 341(3):397–404
94. Yoshida T, Washio K, Iwata T, Okano T, Ishikawa I (2012) Current status and future development of cell transplantation therapy for periodontal tissue regeneration. Int J Dent 2012:1–8
95. Honda M, Morikawa N, Hata K, Yada T, Morita S, Ueda M, Kimata K (2003) Rat costochondral cell characteristics on poly (L-lactide-co-epsilon-caprolactone) scaffolds. Biomaterials 24(20):3511–3519

96. Nakao K, Morita R, Saji Y, Ishida K, Tomita Y, Ogawa M, Saitoh M, Tomooka Y, Tsuji T (2007) The development of a bioengineered organ germ method. Nat Methods 4(3):227–230
97. Ikeda E, Morita R, Nakao K, Ishida K, Nakamura T, Takano-Yamamoto T, Ogawa M, Mizuno M, Kasugai S, Tsuji T (2009) Fully functional bioengineered tooth replacement as an organ replacement therapy. Proc Natl Acad Sci U S A 106(32):13475–13480
98. Langer R, Vacanti JP (1993) Tissue engineering. Science 260(5110):920–926
99. Kim JB, Zaehres H, Wu G, Gentile L, Ko K, Sebastiano V, Arauzo-Bravo MJ, Ruau D, Han DW, Zenke M, Scholer HR (2008) Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors. Nature 454(7204):646–650

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