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Dental-Related Stem Cells and Their Potential in Regenerative Medicine

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1. Introduction

Stem cells have been opening a promising future in clinical therapies because of their two remarkable features known as self-renewal and multi-lineage differentiation. These cells can be classified in terms of their origin (embryonic, prenatal and postnatal stem cells) as well as the differentiation commitments (pleuripotent, multipotent and unipotent). Postnatal stem cells, also known as the adult stem cells (ASCs), normally exist in almost every adult tissues, including bone marrow, skin, neural tissues, and dental epithelium, acting as supportive cells by their regeneration capacity. Among different stem cell types, ASCs seem to be more applicable in stem cell-mediated therapies and regenerative medicine because these cells lack ethical concerns, and possesses less tumorgenic potency than their embryonic counterparts.

Recently, human dental stem cells (DSCs), a subtype of ASCs, have drawn worldwide attention for future therapies due to their both technical and practical superiorities. In addition to having some mesenchymal stem cell (MSC) characteristics, including plastic adherent ability with formation of colonies in vitro, and also immunoprivileged properties, DSCs are easily-accessible cells with higher proliferation capacity than ordinary marrow-derived MSCs. Currently, there are six types of stem/progenitor cells determined in dental-related tissues. 1) dental pulp stem cell (DPSCs), 2) stem cells from human exfoliated deciduous teeth (SHED), 3) periodontal ligament stem cells (PDLSC), 4) stem cells from apical papilla (SCAP) of developing tooth, 5) dental follicle stem/progenitor cells (DFPCs) and 6) gingiva stem cells (GSCs). DPSCs, SHEDs and SCAPs are referred to as dental pulp-related stem cells, and PDLSCs & DFPCs as periodontium-related stem cells [1, 5].

This chapter focuses on different aspects of dental-derived adult stem cells, such as their classification, biological characterization, initiating culture, cultivation systems, cryopreservation and potential applications in tissue engineering and regenerative medicine. The data are organized as three main parts, including: 1) Dental-related stem cell biology: from the classification to the characterization and differentiation potential 2) Dental-related stem cell initiation culture, culture systems and cryopreservation 3) Dental-related stem cell- based tissue regeneration.

2. Dental-related stem cell biology: From the classification to the characterization & differentiation potential

According to the literature, there are several types of stem/progenitor cells existed in dental tissue. In this section, each of these cells will be described in terms of their main characteristics.

2.1. Dental Pulp Stem Cells (DPSCs)

The presence of stem cells in dental pulp tissue primarily have been reported in 1985 by Yamamura [3, 4, 6] (Figure 1). Later on, Caplan et al. have demonstrated that these cells presented osteogenic and chondrogenic potential in vitro, and could also differentiate into dentin, in vivo. In 2000, Gronthos et al. have isolated dental pulp stem cells from adult human dental pulp, which had the ability to regenerate a dentin-pulp-like complex [7]. Interestingly, some recent works have found the presence of stem cells in inflamed pulp with capacity to form mineralized matrix both in vitro and in vivo. These findings make dental pulp as an interesting tissue source of putative stem cells, even in diseased form.[8]

DPSCs are similar to MSCs in some ways: they are of fibroblastic morphology with selective adherence to solid surfaces, having good proliferative potential and capacity to differentiate in vitro, and the ability to repair tissues in vivo. It's interesting to note that DPSCs could differentiate into not only osteoblasts, chondrocytes & adipocytes, but also myocytes, neurons and hepatocytes lineages in vitro [4]. DPSCs are characterized by their negative expression of hematopoietic antigens (e.g., CD45, CD34, CD14), and positive expression of stromal-associated markers (e.g., CD90, CD29, CD73, CD105, CD44) (Table 1). They also express multipotent marker (STRO-1) and extracellular matrix proteins, such as collagen, vimentin, laminin, and fibronectin. [9-11]. Interestingly, some of the pluripotent stem cell markers, such as Oct4, Nanog, Sox2, Klf4, SSEA4 & c-Myc have been reported to express on DPSCs [12-14]. More recently, it was demonstrated that core transcription factor of the reprogramming Oct4, Nanog, Klf4 and c-Myc become significantly down-regulated following the DPSC differentiation [4].

Apart from stemness markers, DPSCs are also shown to express bone markers, such as bone sialoprotein, osteocalcin, alkaline phosphates (ALP), and type I collagen. This indicates their differentiation commitment into bone tissue [15]. On the other hand, the expression of dentin sialophosphoprotein (an odontoblast specific protein precursor) is not present in the cultures of hDPSCs implied that these cells represent an undifferentiated pre-odontogenic phenotype [7, 16].

From immunological perspective, it has been reported that DPSCs displayed more immnosuppressive activities than the BM-MSCs. This was obvious in inhibiting T cells response in vitro [17].

Based on some investigations, there is a sub-type of DPSCs referred to as "immature dental pulp stems cells" (IDPSCs), which have promising potential in future stem cell researches. IDPSCs were firstly, isolated from pulp tissue of the human exfoliated deciduous as well as permanent teeth [18]. These cells express both embryonic and MSC markers (see part 2.2). It has been indicated that transferring of human IDPSCs (hDPSCs) into mouse blastocysts resulted in formation of human/mouse chimera which was able to retain proliferation and differentiation capacity [19]. Furthermore, hIDPSCs possess the capacity to rapidly reprog-rammed into induced pleuripotent stem cells (iPSc) which are able to produce primary hIDPSC-iPSC colonies even under feeder-free conditions [20].

2.2. Dental Stem cells from Human Exfoliated Deciduous teeth (SHED)

In 2003, Miura et al. have reported to isolate a stem cell population from the living pulp remnants of exfoliated deciduous teeth. These authors have termed the cells as stem cells from human exfoliated deciduous teeth (SHED) [21] (Figure 1). These cells which are believed to be of the neural crest origin are heterogeneous fibroblast-like population possessing an extensive proliferating capacity than either DPSCs or BM-MSCs [22]. In terms of surface epitopes, it has been found that they express markers of MSCs (STRO-1, CD146, SSEA4, CD90, CD73, CD 105, CD106 and CD 166) and lack of hematopoietic/endothelial markers (CD34, CD31) (Table 1). Under an appropriate culture conditions, SHED are able to differentiate into the variety of cell types, including neural cells, angiogenic endothelial cells, adipocytes, osteoblasts, and odontoblasts [23-25]. In vivo transplantation of SHED have been reported to result in formation of bone and dentin like-tissue. [18, 21, 26-29]. There are some studies suggested that SHED is different from IDPSCs in terms of expression of stem cell markers (see part 1.1)[18, 30]. Moreover, some research works have been reported that SHED would possess immunomo-dulatory function as seen in BM-MSCs [28].

2.3. Periodontal Ligament Stem Cells (PDLSC)

Periodontal ligament stem cells (PDLSCs) have first been introduced by Seo et al. [31] (Figure 1). Like MSCs, PDLSCs have been reported to form adherent clonogenic population of fibroblast-like cells in the culture. They express both early MSC markers such as, STRO-1 and CD146, and other MSC and pluripotent makers, such as CD44, CD90, CD105, CD73, CD26, CD10, CD29 and CD166; meanwhile, they have no expression for CD40, CD80, and CD86[31-33] (Table 1). Some investigations have revealed that PDLSCs may be positive for embryonic stem cell markers, as well, including SSEA1, SSEA3, SSEA4, TRA-1-60, TRA-1-81, Oct4, Nanog, Sox2 and Rex1, and ALP [34]. Based on some research works, SSEA4-positive PDLSCs displayed the potential to generate adipocytes, osteoblasts, chondrocytes (from mesodermal layer), neurons (from ectodermal layer), and hepatocytes (from endodermal lineage) *in vitro* [31, 34, 35]. Furthermore, it has been shown that transplantation of PDLSCs

into immunocompromised rodents resulted in the generation of cementum/PDL-like structure and contributes to periodontal tissue repair [31].

PDLSCs show immunomodulatory activity by up-regulation of soluble immunosuppressive factors (TGF-β1, hepatocyte growth factor (HGF) and indoleamine 2, 3-dioxygenase (IDO) in the presence of activated peripheral blood mononuclear cells (PBMNCs). Similar to the DPSCs, PDLSCs are positive for HLA-ABC (MHC class I antigen) while negative for HLA-DR (MHC class II antigen) [32].

2.4. Dental Follicle Progenitor Cells (DFPCs)

In 2005 & 2007, Morsczeck et al. and Kémoun et al., respectively have identified unique undifferentiated lineage committed cells possessing mesenchymal progenitor features in the human dental follicle (Figure 1). The cells were referred to as "dental follicle precursor cells" (DFPCs) [36, 37]. Characteristically, DFPCs, similar to the bone marrow stem cells, are adherent and colony-forming cells. These cells have been reported to express Notch-1, CD13, CD44, CD73, CD105, and STRO-1 [1, 36] (Table 1). Human DFPCs has been believed to consist of precursor cells for cementoblasts, periodontal ligament cells, and osteoblasts. Under appropriate *in vitro* conditions, they are capable of differentiating into osteoblasts, cementoblasts, chondrocytes and adipocytes. Interestingly, although both DFPCs and SHED are of the neural crest origin, their neural differentiation potentials are different under the same culture conditions. It has been reported that SHED possess good differentiation potential than DFPCs in terms of the expression of Pax6 which is a marker of retinal stem cells [27].

2.5. Stem Cells from the Apical Papilla (SCAP)

Stem cells from dental apical papilla (SCAP) were first identified and characterized by Sonoyama et al. in human permanent immature teeth [38] (Figure 1). These authors described the cells as adherent clonogenic cells with mesenchymal stem cell features, which are expressed STRO-1, CD24, CD29, CD73, CD90, CD105, CD106, CD146, CD166, and ALP, and not expressing CD34; CD45; CD18; and CD150. Among these markers, CD24 would be of a specific marker for SCAP since it's not found in the other dental stem cells (Figure 2). Excitingly, some authors have reported that SCAP display higher telomerase expression than both DPSCs and BM-MSCs [38]. Furthermore, SCAP has been shown to positively stain with several neural markers implying their possible origin from the neural crest [39]. In terms of differentiation, SCAP are capable of generating osteoblasts, odontoblasts and adipocytes in vitro. An in vivo study has demonstrated that these cells form hard tissue when being loaded onto hydroxyapatite (HA) and implanted subcutaneously in immunocompromised rats [38-40]. Moreover, SCAP have been reported to possess a significantly higher mineralization potential as well as proliferation rate than DPSCs. This finding might be of some importance for their use in dental and/or bone tissue engineering and regeneration [41].

About the possibility of immunogenicity of SCAPs, an independent study have reported that swine SCAPs are non-immunogenic and suppressed T cells proliferation in vitro [42].

2.6. Stem Cells derived from Gingiva (GSCs)

The isolation of a stem cell population from gingiva was firstly reported by Zhang et al. in 2009 [43] (Figure 1). These authors derived the cells from the spinous layer of human gingiva and referred to them as gingival stem cells (GSCs). In terms of markers, it has been shown that GSCs are negative for CD45/CD34, but positive for CD29, CD44, CD73, CD90, CD105, CD146, STRO-1 and SSEA4 (Table 1). In addition, extracellular matrix proteins, such as collagen, vimentin, Collagen type-1, and fibronectin have been reported to express in these cells [43, 44]. Like MSCs, GSCs possess a differentiation potential into osteoblasts, adipocytes and chondrocytes in vitro [45]. Moreover, these cells have been found to be able to differentiate along endothelial as well as neural cell lineages. Furthermore, in vivo bone regeneration potential of GSCs was demonstrated by transplantation of GSCs/HA into immunocompro-mised mice [45]. More importantly, in a comparative study, it was demonstrated that GSCs showed stable phenotypes, maintain normal karyotype and telomerase activity in long-term cultures in comparison with BM-MSC [45].

As with other dental related stem cells, GSCs has been found to display immunomodulatory functions; they inhibit lymphocytes proliferation and express a wide range of immunosup-pressive factors, including Interleukin-10 (IL-10), IDO, inducible NO synthase (iNOS), and cyclooxygenase 2 (COX-2) in response to the inflammatory cytokine, IFN- γ [43].
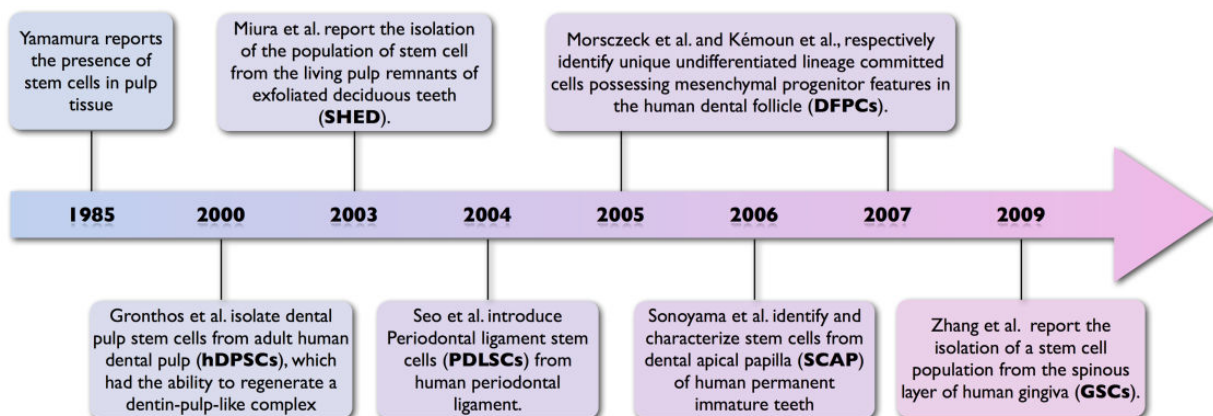

Figure 1. Timeline about the highlights in the history of the isolation of dental-related stem cells (see text).

	DPSCs	SHED	PDLSCs	DFPCs	SCAPs	GSCs
CD (+)	STRO-1	STRO-1	STRO-1	STRO-1	STRO-1	STRO-1
	CD10		CD10	CD10		
	CD13	CD13	CD13	CD13	CD13	CD13
					CD24	
			CD26			
	CD29	CD29	CD29	CD29	CD29	CD29
	CD44	CD44	CD44	CD44	CD44	CD44
	CD59		CD59	CD59		
	CD73	CD73	CD73	CD73	CD73	CD73
	CD90	CD90	CD90	CD90	CD90	CD90
	CD105	CD105	CD105	CD105	CD105	CD105
	CD106	CD106	CD106		CD106	CD106
	CD117					
	CD146	CD146			CD146	CD146
		CD166	CD166		CD166	CD166
CD (-)	CD14	CD14	CD14			
						CD18
	CD19					
	CD24					
	CD34	CD34	CD34	CD34	CD34	CD34
			CD40			
	CD45	CD45	CD45	CD45	CD45	CD45
			CD80			
			CD86			
					CD150	
	HLA-DR		HLA-DR	HLA-DR		HLA-DR

Table 1. Cell Surface Marker Profiles of dental-related stem cells. DPSC; Dental pulp stem cell, SHED; Stem cells from human exfoliated deciduous teeth, PDLSC; Periodontal ligament stem cells, DFPC, Dental follicle precursor cells, SCAP; Stem cells from dental apical papilla, GSC; Gingival stem cells. [1-4]

3. Dental-related stem cell initiation culture, culture systems and cryopreservation

In dental related stem cell researches, the first step is to isolate cells from tissue sources. The next step is to expand the cells into sufficient number. In some occasion, it may be necessary to preserve the cells for future use since tissue sources would not be available on demand. In this section, we will describe the location of the certain teeth tissue from which the stem cell population can be derived and followed by description of common methods by which the isolation culture can be initiated. At the end, culture systems for the cell propagation as well as the main points regarding issue of cell cryopreservation will be explained.

3.1. Anatomical location of teeth tissue from which DSCs can be derived

Most of the human DSCs are come from teeth, which are subjected to the orthodontic treatments. Based on the studies, molars and premolars are mostly used for this purpose. Third molars (wisdom teeth) are the most common teeth for extraction in dental clinical practice. In addition, developing wisdom teeth during the adult life are the excellent candidates as the accessible source of developing tissue similar to those in embryonic development. There are a few studies considering the supernumerary teeth derived from other teeth, such as canine, for the isolation of DPSCs [12] In some cases, such as the isolation of DPSCs from inflamed dental pulps, endodontic treatments are used rather than orthodontics surgeries [46]. In any case, considering the precise location of the dental tissues in tooth anatomy is important to achieve certain types of DSCs with minimum cell contaminations. Hence, here is the brief description of the localization for the specific DSCs isolation.

3.1.1. Tissues contained dental pulp-related stem cells (DPSCs, SHED & SCAP)

Isolation of DPSCs following the pulp extraction could be achieved by either through the root or crown of the dental organ. In the case of SHEDs or DPSCs, which are derived from incompleted root teeth, the exposed pulp is accessible from the root without applying any specific procedure [21]. In other situation (extracted permanent or deciduous teeth), dental pulp extraction is accomplished through the dental crown by cutting the cementum-enamel junction (CEJ) using dental instruments, such as pliers (bone forceps), extirpation needle, Gracey curette, dental fissure burs, etc. [6] Moreover, in the case of inflamed teeth, pulp tissues are removed during the endodontic therapies [46] (Figure 2)

The isolation of SCAP is achieved by gentle separation of root apical papilla from the surface of the root with immature apex (i.e. located in the exterior of the root foramen area before the complete eruption of tooth in the oral cavity) during the extraction of third molars [47]. Root maturation results in the elimination of apical papilla; hence, the maturation period of teeth are important for isolation of SCAP. (Figure 2)

3.1.2. Tissues contained periodontium-related stem cells (PDLSCs & DFPCs) & GSCs isolation

PDLSC can be obtained from the middle third of the root surfaces of extracted PDL tissue, which is a soft connective tissue surrounded between the cementum and the inner wall of the alveolar bone socket. It's accomplished by scrapping surface of the middle third of the root [31].

DFPCs can be isolated by dissecting dental follicle from the upside of the dental crown from impacted teeth. Human dental follicle is an ectomesenchymal tissue that is derived from cranial neural crest. This tissue surrounds developing tooth germ and involves in the coordination of tooth eruption and periodontium formation. This tooth germ's tissue can easily be isolated after wisdom tooth extraction by routine orthodontical related surgeries. Impacted teeth, usually third molars, normally fail to erupt through the gum because of their encasement in the jawbone; therefore, routine surgical procedures are required for the extraction. [36, 37]. (Figure 2)
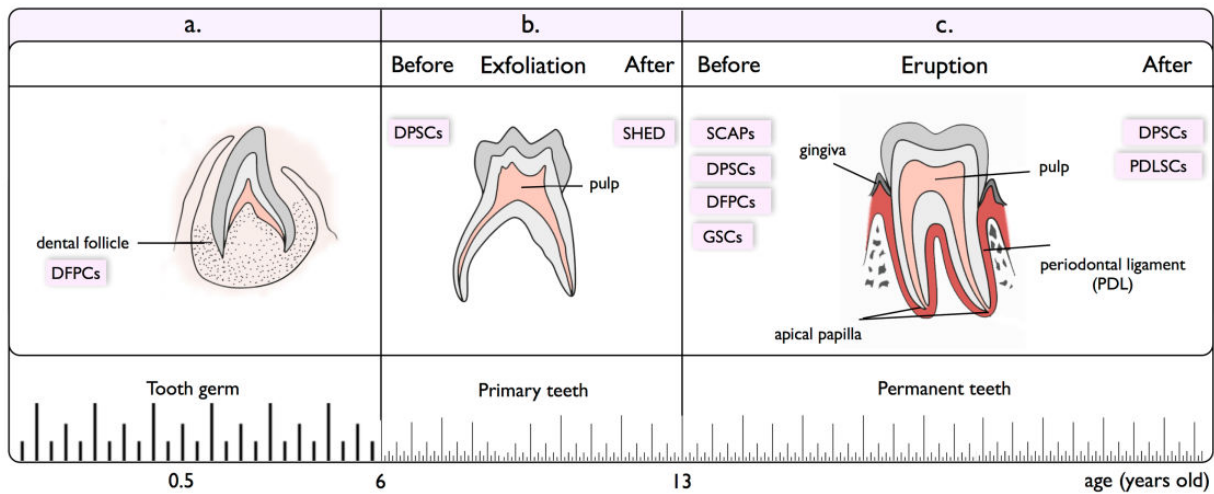
Figure 2. Overall view of dental-related stem cells based on different anatomical locations and stages during the human lifetime in. a. tooth germ, b. primary teeth, c. permanent teeth.

GSCs could be isolated from clinically healthy gingiva, which are obtained as remnant or discarded tissues following routine dental procedures [43]. (Figure 2)

3.2. DSCs culture initiation

In general, dental-related stem cells could isolate by either (1) enzymatic digestion (ED) of tissues or (2) outgrowth (OG) from tissue explant. In the case of enzymatic digestion, after the extraction, tissues are placed into the digestion enzymes, (generally, collagenase type-I & dispase) for about 30-60 minutes at 37 °C to achieve single-cell suspensions. In order to purify DSCs, single-cell suspensions could be subjected into (1) size-sieved isolation (using 3 μm strainer followed by 20 μm strainer), (2) stem cell colony cultivation (single colony culture of stem cells) or (3) magnetic/ fluorescence activated cell sorting (sorting based on surface markers) [48].

In the outgrowth method (OG), tissues are minced into 1-2 mm pieces and placed into the culture dishes to outgrowth [18]. More recently, Lizier and his co-workers established a scaled-up hIDPSCs culture system based on in vitro re-plating of pulp tissue explants followed by 3-4 days expansion [49].

There are some evidences, which suggested different behavior of DSCs according to the ED or OG isolation methods [41, 50, 51]. According to Huang et al. DPSC isolated by ED method (DPSC-ED) from permanent teeth showed higher proliferation rate than those isolated by the OG method (DPSC-OG) [50]. Moreover, STRO-1 & CD34 markers expressed more in DPSC-ED in comparison with DPSC-OG. DPSC-ED derived from deciduous and permanent teeth has been reported to display higher mineralization rate in the defined osteo/odonto medium [51, 52].

3.3. DSCs culture systems

Following the isolation of dental-related stem cells, the next step is to culture-expand the cells into the multiple copies since in the most strategies related to the cell-based-treatment of tissue defects, the copious amount of regenerating cells is needed. Many researchers have been focused on optimizing effective conditions under which DSCs can efficiently be propagated. On the other hand, differentiation potential of the multiplied cells must be determined because discovering the potential commitments of the cells may lead to better selection of them for future organ-targeted treatments [27, 48, 53]. Due to these considerations, this section opens up a brief overview for different DSC culture systems designed for the cell expansion and differentiation.

3.3.1. Serum free vs. serum rich culture systems

Normally, the isolation and expansion of DSC have depend on a high concentration of serum culture media (10%), which provide better cell adhesion during the initial isolation of the cells. Unfortunately, in long-term cultivation, the high level of serum might lead to spontaneous differentiation or malignant transformation of cells. In addition, use of serum in culture may result in contamination of cell culture with bovine pathogen for instance bovine spongiform encephalopathy (BSE). For these reasons, serum free culture systems are highly recommended [54-57]. In this regards, many attempt has been made to optimize DSC cultivation in serum-free or low serum medium. For example, Karbanova et al. have reported that DPSCs cultivated in low-serum medium exhibit less proliferation rate and different expression of stem cell markers compared to those cultivated in serum rich medium [14]. In contrast, Hirata et al have cultivated the cells in serum free media and have found the same survival rate of the cells as those cultivated in the serum containing medium [58].

In the case of DFPCs, studies indicated that applying serum replacement media didn't affect the expression of connective tissue markers, such as collagen type I and type III, and also neural stem progenitor marker, nestin [59]; however, there is no information about the possible changes in other markers in this regards.

It's interesting to note that SHEDs & PDLSCs cultivated in defined serum free media have been reported to display higher proliferation rate than those cultivated in the medium containing serum. Moreover such cells have found to express comparatively higher "stemness" markers [60]. Applying serum free media is one of the critical requirements for the future clinical treatments; therefore, additional works are needed for optimizing conditions to achieve final conclusion.

3.3.2. Neurosphere-forming vs. adherent culture systems

Sphere-forming culture systems are generally applied for neurogenic differentiation of stem cells. This culture system has also been used for DSC cultivation. The idea of applying such a system came from the consideration of neural crest origin of DSCs. It has been well established that neural stem/progenitor cells which are isolated from variety of sources are

grown as neurospheres in defined serum-free culture medium supplemented with EGF and / or bFGF [61-63].

DSC Suspension culture was first suggested by Morita et al. (2007). These authors have cultivated PDLSCs in the sphere culture and found that PDLSC have the ability to form neurospheres in serum-free culture containing epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and leukemia inhibitory factor (LIF) with the ability to differentiate into both neural and mesodermal progeny [64].

In 2008, Sasaki et al. have cultivated DPSCs from the rat incisor in the sphere-culture and found that under these conditions, the cells expressed neural and glial markers. They have also noticed that CD81 positive DPSCs that were localized in odontoblast layer of apical portion of the dental pulp may have more potential to form neurospheres [65] Later on, it was demonstrated that sphere form of SCAP had multi-differentiation potential into mineralized cells, adipocytes and also myocytes under the defined media in vitro. Furthermore, In vivo studies have indicated that sphere SCAP showed more potential to generate mineralized tissues in comparison with the non-sphere SCAP [47].

3.3.3. Co-culture systems

Site-specific tissue interactions are essential for orchestration of proliferation, differentiation and also homeostasis of cells during the tooth germ development. In particular, epithelial-mesenchymal interactions are the most important developmental events, which are involved in reciprocal crosstalk between the ectodermal and mesenchymal tissues. These sequential interactions are critical for tooth morphogenesis and cell differentiation [66]. To imitate these in vivo interactions, co-culture systems have been developed.

Using co-culture systems, some attempts have been made to promote DSCs differentiation in vitro. In this regards different cell types have been examined as co-culture cells. For example, it has been demonstrated that co-culture of DFPCs/SCAP could lead to formation of bone-like structure in vivo and in vitro. This occurs since interaction between the cells stimulate cementogenic/osteogenic differentiation of DFPCs leading to up-regulation of bone intermediate proteins, such as bone morphogenetic protein 2 (BMP2), osteoprotegerin (OPG), bone sialoprotein (BSP) and osteocalcin (OCN), as well as down-regulation of receptor activator of nuclear factor κB ligand (RANKL) [67]. In other experiment, Arakaki et al. have co-cultured DPSCs with dental epithelial cells and found that in such a system, dental epithelial cells secret BMP2 and BMP4, thereby promote odontoblastic differentiation of DPSCs [68]. Interestingly, it has also been reported that co-culture of DPSCs with epithelial cells lead to epithelium invagination, as well [69]. Moreover, Wang et al. have established a co-culture of hDPSCs with osteoblasts and demonstrated that a higher mineralization and up-regulation of osteogenic-related genes in hDPSCs compared with those cultured in the absence of osteoblasts [70]. Furthermore, co-culture of DPSCs with non-dental MSCs, such as amniotic fluid-derived MSCs (AF-MSCs), has been reported to leads to the bone differentiation of AF-MSCs indicating the pre-commitment of DPSCs to induce osteogenic differentiation [71].

3.4. DSCs cryopreservation

According to the diversity of dental-related stem cells and their remarkable features for cell-mediated therapies and tissue engineering, developing a reliable method for cell banking have become a priority for future use. In this regard, cryopreservation could be established at the levels of teeth (organs), dental tissues or cells. In the case of teeth or dental tissues cryopreservation, minimal processing may needed for banking. There are some evidences which are demonstrated the successful cryopreservation of healthy and diseased teeth as well as dental tissues [72-78]. On the other hand, cryopreservation of DSCs still has been considered as an active area of the researches. There are several parameters which need to be considered prior to an establishment of a successful and more efficient protocol. The parameters which need to be determined include (1) DSCs-cultured passage(s), which leads to high-efficiency recovery post thaw, (2) concentration of cryoprotective agent, (3) cell concentration for high-efficient cryopreservation, (4) storage temperature, (5) the process of cryopreservation, and (6) evaluation of growth, surface markers and differentiation properties of DSCs after post thaw. So far, there have been many researches considering the comparative cryopreservation methods for optimizing the best protocols. More recently, it has been suggested that magnetic cryopreservation of DSCs was much better than conventional slow-freezing procedure in terms of cytotoxicity [76, 79, 80].

4. Dental-related stem cell based tissue regeneration

Although DSCs are newborn in the field of stem cell therapy and tissue engineering, they have opened the promising windows not only in tooth repair and regeneration studies but also in other organs. To date, most of DSCs mediated cell therapies and tissue-engineering studies have been focused on the animal models. However, more recently, a few clinical trial studies have also been accomplished. Meanwhile, the immunogenicity of these cells should be more considered for their allogeneic transplantation.

4.1. DSCs-based tooth engineering and regeneration of dental related tissue

In general, in the field of dentistry, the final goal of tissue engineering is to develop tooth-replacement therapies using the whole-bioengineered-tooth technology. To fulfill this, some authors have conducted the remarkable investigations. In this regard, there is some experiment in which either dissociated tooth germs or mesenchymal and epithelial cells derived from the tooth germs were prepared and loaded on to the prefabricated tooth-shaped scaffolds in order to fabricate a bioengineered tooth. Based on these investigations, tooth germ cells possessed a high potential to form dentin, enamel, pulp, and periodontal tissues in vivo [81-84]. However, using embryonic tooth germ and problems related to immunogenicity of animal transplantation, make this an unfeasible approach to clinical application of tooth regeneration [84].

Alternative cells would be DSCs. Using these cells, some preliminary studies have been accomplished to examine whether DSCs in combination with appropriate scaffold are able to promote regeneration of tooth tissues [85, 86]. For instance, transplantation of PDLSCs

loaded onto HA/TCP have been shown to result in the production of cementum in mice while using gelfoam (collagen based gelatin sponge) as the carrier has been reported to fail creating cementum [31, 87]. Sonoyama and co-workers have reported the creation of a root/periodontal complex being capable of supporting a porcelain crown in swine by applying both SCAP and PDLSCs in HA/TCP as a carrier [38]. In a study on canine model, we have recently succeeded to achieve regeneration on an experimentally-created defect in peridontium using autologous DPSCs loaded onto Bio-Oss scaffolds [88]. Furthermore, Nakashima et al. have reported a successful induction of whole-pulp regeneration after pulpectomy in a dog model using autologous DPSCs loaded onto 3D scaffold of collagen-I & II and SDF-1 (stromal-cell-derived factor-1) as the morphogene [89, 90]. Moreover, a culture system termed as organ-engineering methods using PDLSCs have been developed to generate not only tooth root, but also the surrounding periodontal tissues, including PDL and alveolar bone in mouse model [84]. In addition to the above-mentioned animal studies, there is a published retrospective pilot study in human. According to this trial, autologous transplantation of PDL stem/progenitor cells might provide therapeutic improvement for the periodontal defects without any adverse effects during 32–72 months of follow-up [91].

4.2. DSCs-based tissue engineering and regeneration for other organs

Apart from the potential applications in dental tissue engineering and therapy, DSCs have been opened a dynamic field in repair and regeneration of non-dental tissues. In this context, there are many investigations indicating applicability of dental related stem cells in variety of disease models in the animal. For instance, SHED has been reported to exhibit a potential to improve parkinson's disease in rat by differentiating into dopaminergic neuron-like cells. Based on the different investigations, these cells have also the ability to promote wound healing in mice [92-94]. It has been demonstrated that SHED also contribute to repair of the critical-sized calvarial defects in mice model [95].

Transplantation of DPSCs has been shown to improve alzheimer's and parkinson's disease as well as acute myocardial infarction in a rat model [96-98]. Similarly, transplantation of hIDPSCs in canine model with golden retriever muscular dystrophy (GRMD) resulted in some improvement [99]. In addition, it has been reported that transplantation of tissue-engineered hIDPSC sheet was successfully reconstructed the corneal epithelium in rabbits with total limbal stem cell deficiency (LSCD) [100]. Applying PDLSCs with collagen based gelatin sponge carrier have been found to improve facial wrinkles by generating large amounts of collagen fibers in the mouse indicating the potential capacity of PDLSC in the field of plastic surgery. In the case of GSCs, *Intra-peritoneal (ip)* administration of the cells displayed improvement of inflammation-related tissue destruction in experimental colitis [43].

4.3. Immunogenicity of allogeneic transplantation of DSCs

According to the promising place of DSCs in clinical treatments in future, considering the immunogenicity of DSC transplantation is critical. Although autologous DSCs rather than allogenous ones are preferred for repair and regeneration purposes, several limitations such

as inadequate cell numbers and donor site morbidity carry out problems. Thus, more studies will be needed to evaluate immunogenicity of allogenous DSCs for the future clinical applications. There are some evidences suggested that DSCs, as the mesenchymal stem cells, have immunomodulatory properties both in vitro and in vivo. These studies are almost considering in vitro immunogenicity of DSCs by evaluating the expression of pro/anti-inflammatory mediators, such as MHC classes, TGF-β, ODO, HGF, nitric oxide (NO), prostaglandin, immunosuppressive minor H antigen (HLA-G), and interferon (IFN)-γ as well as effect of DSCs in suppression of T cells proliferation [28, 101, 102].

In addition, in vivo studies also showed DSCs immunomodulatory functions. For instance, SHED transplantation up-regulated the ratio of Treg (regulatory T cells) and Th17 in MRL/ lpr mice model compared to the BM-MSCs; meanwhile, no change in the expression of IL-6 & IL-10 was detected. On the other hand, transplantation of human DPSCs into the rat model didn't initiate the immunologic responses in recipients [103]. Moreover, in another study, it was demonstrated that transplantation of hIDPSCs didn't show the immune reaction in the canine [99].

In spite of existing evidences, which suggested the immunomodulatory effect of DSCs, there are some data proposing that there is different expression of immune receptors, such as toll-like receptors (TLRs) on the cells. Presence of this receptor could affect the immumomodulatory phenotypes of DPSCs and DFPCs [101, 104, 105]; On the other hand, since some studies suggested that BM-MSCs may regain immunogenic property upon differentiation [106], the subject of immunogenicity of DSCs must be examined not only in an undifferentiated state but also in differentiated form.

5. Conclusion

Dental-related tissue contained several types of stem cells collectively referred to as dental stem cells (DSCs). In this stem cell family, there are 6 named member so far recognized and described. These include dental pulp stem cell (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAP) of developing tooth, dental follicle stem/progenitor cells (DFPCs) and gingival stem cells (GSCs). All these easily-accessible stem cells can be derived from dental tissue obtained from both young and adult patients. Furthermore the cells are described as having immunomodulatory function. These characteristics make DSCs a unique source for repair and regeneration of injured tissue. In this context, many studies have so far been conducted on the animal models and the reports together indicated the extensive potential of the cells in tissue repair and regeneration not only in teeth but also in other organs. After all these animal studies, some centers have started clinical trials to examine the cell potential in human diseases. To exploit the extensive regenerating potential of DSCs in clinic, many additional clinical trials must be planed and conducted till therapeutic protocols using these cells become established.

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