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Dental Pulp Stem Cells and Neurogenesis

Ibrahim Mortada, Rola Mortada, and Mohamad Al Bazzal

Abstract

Recent advances in regenerative medicine and cell-based therapy are bringing promising perspectives for the use of stem cells in clinical trials. Stem cells are undifferentiated cells capable of multilineage differentiation and available in numerous sources in the human body. Dental pulp constitutes an attractive source of these cells since collecting mesenchymal stem cells from this site is a noninvasive procedure which can be done following a common surgical extraction of supernumerary or wisdom teeth. Thus tissue sacrifice is very low and several cytotypes can be obtained owing to these cells' multipotency, in addition to the fact that they can be cryopreserved and stored for long periods. Mesenchymal stem cells have high proliferation rates making them favorable for clinical application. These multipotent cells present in a biological waste constitute an appropriate support in the management of many neurological disorders. After a brief overview on the different types of dental stem cells, this chapter will focus on the characteristics of dental pulp stem cells, their handling and applications in neural tissue engineering, as well as neural induction protocols leading to their potential therapeutic use in the management of neurological diseases.

Keywords

Biology • DPSCs • Experimental medicine • Regenerative medicine • Stem cells

I. Mortada (✉)
Faculty of Medicine, American University of Beirut, Beirut, Lebanon
e-mail: ikm03@aub.edu.lb

R. Mortada and M. Al Bazzal
Lebanese University School of Dentistry, Beirut, Lebanon

Abbreviations

a-MEM	Minimum essential medium, alpha modification
ATRA	All-trans retinoic acid
BMMSCs	Bone marrow mesenchymal stem cells
DFPCs	Dental follicle progenitor cells
DFSCs	Dental follicle stem cells
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethyl sulfoxide
DSCs	Dental stem cells
ECM	Extracellular matrix
EGF	Epidermal growth factor
MAP 2	Microtubule-associated protein 2
MSCs	Mesenchymal stem cells
NSE	Neuron-specific enolase
PDLSCs	Periodontal ligament stem cells
rMSCs	Rat bone marrow mesenchymal stem cells
SCAP	Stem cells from apical papilla
SHED	Stem cells from human exfoliated deciduous teeth
TNC	Tenascin C

1 Introduction

In recent years, regenerative medicine has evolved rapidly with the discovery of multiple sources of mesenchymal stem cells (MSCs) (Hemmat et al. 2010). After the bone marrow and the adipose tissue, the oral cavity constitutes an important source of MSCs that exist in various areas such as the periodontal ligament, the dental follicle and mainly the dental pulp (Xiao and Nasu 2014). Dental stem cells (DSCs) are mainly characterized by their ease of availability since they are extracted from erupting primary teeth or extracted teeth. This makes their isolation much simpler and less invasive than aspiration of bone marrow mesenchymal stem cells (BMMSCs), for instance. Some studies even demonstrated the DSCs express-specific pluripotency markers (MYC, SOX2) which are absent in other mesenchymal stem cells (Yalvac et al. 2010). The absence of ethical considerations related to their use when compared to the use of totipotent and pluripotent cells and the absence of any history of cancer formation after their transplantation increase the interest in the DSCs.

The dental pulp constitutes the best source of oral stem cells. Under specific conditions, dental pulp stem cells (DPSCs) are capable of multilineage differentiation, among which is the neural differentiation (Gronthos et al. 2000). Extensive research focusing on DPSC-derived neural lineages showed that these cells have a characteristic innate neurogenic potential since they are originally issued from the embryonic neural crest (La Noce et al. 2014). The ability of DPSCs to generate neurons and glial cells makes them an exciting source in the management of neurodegenerative diseases. For this review, the focus is on the DPSCs, their characteristics, isolation, and their neural induction protocols.

2 Dental Stem Cells (DSCs)

As previously mentioned, teeth constitute a promising source of multipotent stem cells. Different populations of MSCs reside in teeth and they are nominated according to their harvest site (Racz et al. 2014; Ding et al. 2015). They present some variations related to their differentiation potential, cell surface receptors, and proliferative capacity although they share the same elongated fibroblast-like morphology that is characteristic to their mesenchymal character (Ding et al. 2015; Xiao and Nasu 2014; Zhao and Chai 2015). Figure 1 highlights the dental and associated tissues from which different populations of DSCs can be isolated.

Several types of DSCs were described.

2.1 Dental Pulp Stem Cells (DPSCs)

DPSCs were initially isolated from wisdom teeth by Gronthos et al. They were reported to have a high proliferation capacity and the potential to

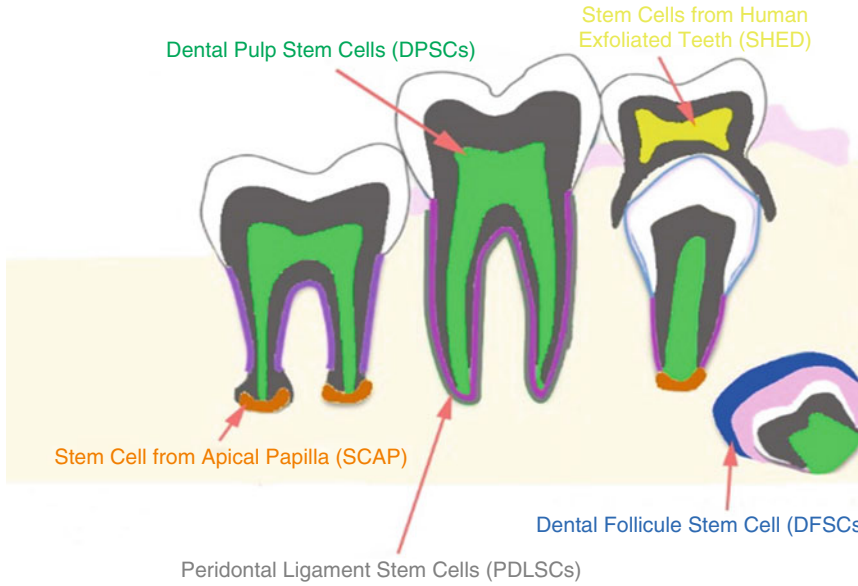

Fig. 1 Tissues of origin of the different populations of DSCs. DPSCs can be extracted from the inner tooth pulp of adult molars (*green*); SHED can be extracted from the pulp of deciduous exfoliated teeth (*yellow*); SCAP can be extracted from the apical papilla (*orange*); PDLSCs can be extracted from the periodontal ligament (*gray*); DFSCs can be extracted from the dental follicle (*blue*)

produce mineralized colonies (Gronthos et al. 2002). DPSCs are located in the dental crown, specifically in the pulp chamber (Baume 1980). The existing cells belong to heterogeneous populations of fibroblasts, osteoprogenitors neural, vascular, and immune cells (Goldberg and Smith 2004). The dental pulp has a high regenerative capacity and helps in the periodontal tissue repair (Dimitrova-Nakov et al. 2014; About 2013).

2.2 Stem Cells from Human Exfoliated Deciduous Teeth (SHED)

These progenitor cells were obtained for the first time from deciduous incisors in 2003. They express various embryonic markers (NANOG and OCT4) and MSCs markers (CD146 and STRO-1) (Sakai et al. 2012). Interestingly, they showed higher capability for differentiation and more proliferation rate than BMMSCs and even DPSCs. They can differentiate into different cell types, such as adipocytes, neural cells, and endothelial cells (Tatullo et al. 2015).

2.3 Periodontal Ligament Stem Cells (PDLSCs)

PDLSCs present similar characteristics to MSCs although they are derived from the neural crest cells (Chai et al. 2000). Those residing in the perivascular wall have similar cell morphology, differentiation potentials, and phenotype (Iwasaki et al. 2013). They resemble BMMSCs in their immunomodulatory ability and can differentiate into osteoblast, cementoblasts, and adipocytes (Wada et al. 2009).

2.4 Stem Cells from Apical Papilla (SCAP)

During dental root formation, SCAP are isolated from the apex. They have similar characteristics to MSCs and can be induced into chondrocytes, adipocytes, and neurons (Guo et al. 2013).

2.5 Dental Follicle Stem Cells (DFSCs)

During the early stages of tooth formation, DFSCs are isolated from dental follicle surrounding tooth germ (Silvério et al. 2012). The dental follicle is an ectomesenchymal condensation of cells. They can differentiate into neurons, osteoblasts, and chondrocytes (Saito et al. 2015).

3 Dental Pulp Stem Cells (DPSCs)

3.1 Dental Pulp as a Source of Multipotent Cells

Teeth are divided into two distinct anatomical entities, the crown and the root, connected to the supporting bone by the periodontal ligament. The crown comprises ameloblasts-generated enamel and odontoblasts-generated dentin in addition to the pulp. After tooth eruption, enamel formation stops occurring naturally due to the disappearance of ameloblasts from the surface. On the other hand, odontoblasts, which are present inside the pulp facing the dentin's inner surface, continue to deposit secondary dentin throughout life. They also form reparative or tertiary dentin in response to various chemical, mechanical, or bacterial stimuli. Interestingly, odontoblasts damage doesn't prohibit tertiary dentin formation in the presence of pulpal disruption. This reparative dentinogenesis was considered to be orchestrated by newly formed odontoblasts emerging from the pulp. Such findings were the starting point to the exploration of stem cell niches that might exist inside the dental pulp (Sonoyama et al. 2007).

DPSC were the first type of isolated DSCs (Gronthos et al. 2000).The pulp is a soft tissue enclosed within mineralized structures, the enamel, the dentin, and the cementum (Nanci 2007). It contains blood vessels, lymphatics, connective tissue, neural fibers (Liu et al. 2006), as well as DPSCs that continuously divide and undergo differentiation into different cell types (Gronthos et al. 2000). Functionally, the pulp detects unhealthy stimuli and assures the homeostasis of the tooth organ (Nanci 2007). It can be divided into four different layers. The external layer contains odontoblasts, the second layer contains mainly collagen fibers, and the third layer comprises undifferentiated cells and progenitor cells including the DPSCs. Undifferentiated cells migrate from this layer to various areas of the body where they can differentiate under specific conditions and various stimulations to give rise to specific cells. The deepest layer of the pulp comprises nerves and blood vessels (Goldberg and Smith 2004).

Dental pulp tissues from third molars, supernumerary teeth, or deciduous teeth constitute an easily accessible origin of MSCs without causing any morbidity to the patient as these teeth are often discarded. DPSCs express embryonic markers of stem cells and display multipotency markers indicating their possible spontaneous neural differentiation. They have higher proliferation rates and are more readily available than BMMSCs. Their multipotential capability and their distinct plasticity are not surprising since these cells come from both mesenchymal and ectodermic components, and they contain cells derived from the neural crest (Alge et al. 2010; D'aquino et al. 2009). The negative immunoreactivity for CD45 antibodies confirms that this cell line is not hematopoietic (D'aquino et al. 2007).

3.2 Neural Markers Expression by DPSCs

Foudah et al. confirmed that DPSCs spontaneously express the neural markers Nestin, NeuN, and β-III-tubulin (Foudah et al. 2014). Feng et al. demonstrated that they spontaneously express Nestin, β-III-tubulin, tyrosine hydroxylase, and microtubule-associated protein 2 (MAP2 (Feng et al. 2013). Govindasamy et al. confirmed that DPSCs exhibit higher spontaneous expression of the neural markers than SHED (PAX6 and Nestin) (Govindasamy et al. 2010). Martens et al. reported that DPSCs express

synaptophysin, β-III-tubulin, and S100 protein with the presence of an eventual positive immunoreaction for galactocerebroside and NGF receptor p75 (Martens et al. 2012). Tamaki et al. confirmed that these undifferentiated mesenchymal stem cells exhibit a positive immune reactivity to antibodies against some neural markers like neurofilament (NF)-200, Nestin, and class β-III-tubulin (Tamaki et al. 2013). Karaoz et al. verified that DPSCs spontaneously express several neural markers associated with both neural stem cells and mature neural lineages of which NES, tenascin C (TNC), GFAP, connexin-43, MAP2ab, c-FOS, NEF-H, NEF-L and TUBB3, SOX2, ENO2 (Karaoz et al. 2010).

The amount of serum used in the medium seems to have an influence on the level of expression of neural markers in undifferentiated DPSCs. A high level of Nestin expression and an absence of β-III-tubulin and S100 are associated with a low serum level of around 2%. However, in high levels of serum of 10%, Nestin expression is relatively reduced (Ranganathan and Lakshminarayanan 2012).

On the other hand, tetrodotoxin, a potent neurotoxin, is quite specific in blocking the sodium ion channel and therefore the flow of sodium ions while having no effect on potassium ions. Therefore it blocks the conduction of nerve impulses along nerve fibers and axons. The patch-clamp recording technique detected a voltage-gated tetrodotoxin-sensitive inward current in undifferentiated DPSCs, which suggests a neural-like sodium conductance. This finding underlines the ability of these cells to exhibit some functions related to mature neurons (Davidson 1994).

4 Handling of Dental Pulp Stem Cells

4.1 Identification

Several techniques are used to identify adult stem cells:

- Identifying their specific surface markers
- Labeling the cells in the living organ with markers and then determining the differentiated cell types to which they give rise
- Removing stem cells from a living animal, labeling them, and transplanting them into a different animal to find out if they would migrate to their original tissue
- Isolating somatic stem cells, culturing them (adding growth factors, introducing genes)
- Infecting adult stem cells with a virus to identify them among others
- Testing their clonogenicity which means their "stemness" capability

4.2 Isolation

The isolation of DPSCs is usually achieved following two methods: the explants method and the enzymatic digestion of pulp tissue method. In the first method, the pulp is surgically removed and the cells are grown from tissue fragments (Roozafzoon et al. 2015), while in the second method, collagenase and dispase digest the dental pulp (Sun et al. 2014; Paschalidis et al. 2014), after which the cells are seeded. Cell proliferation is observed and the MSCs are characterized using flow cytometry based on staining with specific markers (Raoof et al. 2014). Furthermore, other studies noted that the isolation of more immature stem cells necessitates tissue explants in a multistage process in which the progenitor cells are first grown in culture, then enzymatically digested, followed by the expansion of isolated cells (Jung et al. 2012).

In the original protocol of DPSC isolation using impacted wisdom teeth as the harvesting source (Jung et al. 2012), the pulp was extirpated from the tooth, then an enzymatic digestion was performed. The obtained cells formed more colonies and had a higher rate of proliferation when compared to BMMSCs. Since then, numerous studies have explored alternative techniques and improved methods for isolation and culture

of DPSCs (Kawashima 2012). Hilkens et al. (Hilkens et al. 2013) compared the enzymatic digestion and the cell outgrowth methods of isolation of stem cells from wisdom teeth. Results revealed no significant difference in differentiation potentials and markers expression. To date, a specific technique which is superior in terms of karyotypic stability, proliferative ability, or therapeutic use of DPSCs couldn't be reached; however, enzymatic digestion is more frequently employed than outgrowth explants (Ledesma-Martínez et al. 2016).

The type of tooth employed as a donor site of pulp tissue to be isolated was extensively investigated. Impacted third molars are frequently used, although exfoliated deciduous teeth are considered another excellent source of stem cells. Kerkis and Caplan (2012) isolated three distinct stem cell populations from deciduous teeth pulp. Govindasamy et al. (2011) reported higher proliferation rates and higher pluripotent and neuroectodermal markers in the cells isolated from deciduous teeth when compared to those issued from permanent teeth. Isolation of DPSCs derived from supernumerary teeth was also reported in the literature (Huang et al. 2008).

On the other hand, composition of the culture medium is of particular importance especially with the increasing tendency for clinical application of DPSC technologies (Ferro et al. 2012). For instance, bovine serum presents an eventual risk of bovine spongiforme encephalopathy if used in clinical therapy. In order to reduce this risk, a chemically defined medium with decreased levels of human serum can be used (Lizier et al. 2012).

4.3 Culture and Expansion

During their initial growth, self-renewal capacity of MSCs leads to colonies propagating from a unique cell on a plastic surface. These cells have an increased potential of expansion; therefore, the colonies formed in the first culture can be subcultured by several passages (Bakopoulou et al. 2011). Studies reported that DPSCs can be cultured for extended periods of time (6 months) without affecting their markers, their plasticity, or their morphology (Suchanek et al. 2010). In culture, cells were adherent and elongated in shape, with thin expansions (see Fig. 2). After reaching the maximum number of passages prior to entering senescence, these cells still had a normal karyotype and doubling period at up to 40 doublings. The capacity of DPSCs to be expanded to high numbers suggests an important

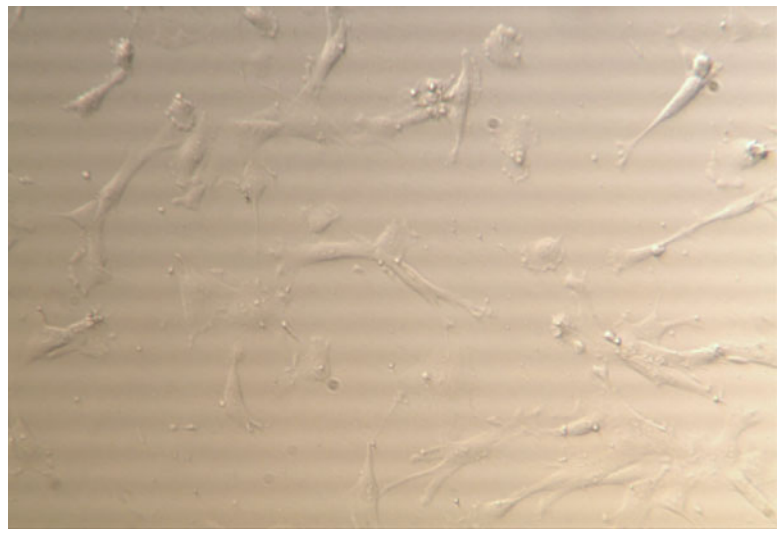

Fig. 2 Expanded cells in culture at day 15. They are spindle-shaped and comprise a homogenous cell population when viewed under the phase-contrast microscope (400×)

advantage for the use of these cells in several therapeutic applications (Laino et al. 2005).

Stem cells are seeded in specific conditions for the purpose of inducing their differentiation into the desired cell type. Growth factors, cell adhesion molecules, and other molecular signals are involved in the initiation and the supporting of the differentiation process.

4.4 Cryopreservation

Cryopreservation allows teeth banking and storage of DPSCs for future use. Cryopreserving cells for clinical use is a very important idea to consider. Studies showed no changes in markers and cell viability when using controlled cooling rates for cryopreservation (Lee et al. 2011). The use of liquid nitrogen allows cells to be cooled. Freezing in the presence of cryopreservative, such as dimethyl sulfoxide (DMSO), is required to inhibit the ice formation inside and around the cells and to prevent cell dehydration that would naturally lead to cell damage and death.

5 In Vitro Applications of DPSCs in Neural Tissue Engineering

5.1 Regulation of Differentiation

DPSCs exhibit a transcriptional change giving rise to progenitor cells without any obvious changes in self-renewal ability. This progression is the first step in the commitment process. At this stage, the progenitor cell has a more limited developmental program. The exit of tooth-derived MSCs from stemness into commitment occurs when the progenitor cell continues its division and acquires distinguishable features of fully committed mature cells with characteristic phenotypes. The differentiation of tooth-derived MSCs and their commitment to mature cell types, for example, neurons, is a well-established process that involves influence of chemical stimuli (forskolin, β-mercaptoethanol) and the activities of cytokines, transcription factors, extracellular matrix molecules, and growth factors (Doi et al. 2004).

5.2 Role of Chemicals

5.2.1 All-Trans Retinoic Acid (ATRA)

ATRA is a preinduction agent used before adding growth factors and a potent differentiation agent in various cells such as neuronal cells. It induces pluripotent cells differentiation into specific lineages among which are neural cell types in a time- and concentration-dependent manner (Kadar et al. 2009).

5.2.2 Dimethyl Sulfoxide (DMSO)

DMSO is a cell-differentiating agent and cryoprotectant. It is also used in the management of brain edema, amyloidosis, and schizophrenia (Santos et al. 2003). DMSO has a major role in the neural differentiation of stem cells, although the mechanism is still unknown. Compared to ATRA, DMSO has lower price and faster action; thus it is recommended as a preinduction factor for the neural differentiation of DPSCs.

5.3 Role of the Extracellular Matrix (ECM)

Differentiation is a well-controlled process highly influenced by the interaction between the cell and its microenvironment. This interaction constitutes the main element that will help conserve the stability of commitment genes. The ECM is in the heart of the cell microenvironment. Its main components are proteoglycans, glycosaminoglycans, and glycoproteins (Blau and Baltimore 1991). One type of interaction between ECM and growth factors is the binding of the basic amino acids of growth factors to heparan sulfate, the negatively charged chain of proteoglycans. Another example of interactions is the important role played by growth factors to regulate the production of the matrix proteins and their respective receptors leading to an

appropriate gene expression regulation (Frescaline et al. 2013).

5.4 Role of Growth Factors

Neurotrophins have an important role in stimulating stem cell neural differentiation. It is therefore necessary to assess the specific role of each of these factors. Stable neural differentiation of stem cells can be achieved by combining chemicals with some growth factors, including NGF, bFGF, PDGF, and BDNF. Osathanon et al. investigated the possible implication of Notch signaling during DPSC neural differentiation induced by growth factor protocol (Osathanon et al. 2014). The authors detected a remarkable increase in mRNA expression level of Notch signaling target gene, *HEY1*, in growth factor-induced DPSC-derived neuronal-like cells. In fact, it was previously demonstrated that Notch signaling target genes, *HEY* family, regulates neural differentiation (Jalali et al. 2011; Mukhopadhyay et al. 2009). In addition, Notch signaling was found to be involved in neurogenic differentiation of PDLSCs (Osathanon et al. 2013). Furthermore, it was found that transfection with Notch intracellular domain and subsequent neurotrophic factors administration was associated with differentiation of mesenchymal stromal cells into neuronal cells (Dezawa et al. 2004). Therefore, current evidence implies the participation of Notch signaling pathway in growth factor-derived DPSC neural differentiation; however, further studies are warranted to elucidate the molecular mechanism of Notch signaling which regulates DPSC fate determination and neurogenic commitment.

We will briefly discuss the characteristics of the neurotrophins.

NGF is an influential factor and has a major role in the development and preservation of the sensory and sympathetic nervous systems. It promotes in vivo and in vitro neurites outgrowth, as well as nerve cell recovery following surgical, ischemic, or chemical injuries. By supporting survival and growth of neural cells, it is able to promote neural differentiation and regulate cell growth. In clinical therapy, NGF exhibits the ability to heal nerve injury. It has an antiapoptotic role in premature neurons and protects axons and myelin from inflammatory damage in order to modulate the immune system. NGF was also found to induce BMMSC differentiation into neural cells, through the generation of neuropeptide signals and receptors. Therefore, NGF is essential for stem cell neural differentiation, and eventually, it could help in treating injured nerves (Aloe et al. 2015; Hu et al. 2016).

bFGF mediates cell proliferation and mitosis and intensifies spinal cord repair and neuronal axon regeneration via the expression of nerve-specific proteins. It is expressed in both embryonic and adult central and peripheral nervous systems, promotes sympathetic and parasympathetic nervous axon growth, maintains neuronal cell survival, and induces damaged nerve repair and neurites outgrowth. Moreover, bFGF can promote differentiation of adrenal pheochromocytoma cells-12. It is a potent growth factor in the neural differentiation of DPSCs by DPSCs-stimulating colony-forming units and upregulation of embryonic stem cell markers Oct4, Nanog, and Rex-1. Increased DPSC neurosphere size and upregulation of neurogenic markers were seen in the presence of bFGF. Hu et al. showed that pretreatment with bFGF promotes neural specification (Hu et al. 2016).

PDGF-BB is an important mitogen for connective tissue cells and is implicated in hyperplasia and embryonic neuron development. PDGF promotes stem cell proliferation and protects them against senescence, apoptosis, and immunomodulatory defects (Hata et al. 2010).

BDNF promotes neuronal differentiation, survival during early development, neural plasticity, and adult neurogenesis. It stimulates and controls oligodendrocyte progenitor cells development in vitro and in vivo (Tsiperson et al. 2015). This signaling molecule regulates neuronal plasticity, proliferation, cell growth, and survival. The

combination of stem cells with BDNF promotes differentiation of primitive cells originating from the bone marrow into the glial lineage. Park et al. used a retroviral vector carrying rats' BDNF cDNA to transduce rat bone marrow mesenchymal stem cells (rMSCs). Following their intravitreal or subretinal injection, these rMSCs were incorporated into the retina, with further production of BDNF (Park et al. 2012).

6 DPSCs: Overview of Protocols for Neural Induction

6.1 Preinduction

DPSCs vary in their differentiation potential. For instance, subpopulations that express the p75 neurotrophin receptor are considered to have superior neural differentiation ability (Dai et al. 2013). To date, a wide variety of protocols leading to in vitro neural differentiation of DPSCs are described in the literature. Many variations exist among these protocols related to the culture medium (basal medium, growth factors, and supplements) but also to the employed surface coating, the culture duration, as well as the existence of a single or multiple culture stages.

Spontaneous neural induction demonstrated by the expression of neural markers was reported in the literature (Gervois et al. 2015; Osathanon et al. 2014). Xiao et al. found that DPSCs cultured in suspension under serum-free conditions could spontaneously differentiate into the neural cells (Xiao and Tsutsui 2013).

6.2 Multistage Culture

Currently, in vitro DPSC neurogenic induction protocols are shifting toward multistage and more complex culture protocols. These are considered to more accurately reproduce the dynamic environment of DPSC niche during neural differentiation. Pretreatment with β-mercaptoethanol was shown to enhance neural progenitors' survival in addition to promoting Nestin expression as shown by Ni et al. (Ni et al. 2001). Lu et al. noted that a 5-h exposure to β-mercaptoethanol (5 mM of concentration) led to the expression of neuron-specific enolase (NSE) and NF-200 by multipotent adipose-derived cells (Lu et al. 2012). 5-azacytidine is a demethylation agent that would consequently erase the epigenetic memory and make DPSCs more pliable to neural lineage differentiation. This agent was also used for the stem cells' preinduction treatment before their exposition to neural cultures (Kiraly et al. 2011).

6.3 Surface Coating

The use of surface coating for cell culture is another variable in the in vitro protocols, and it has an important influence on the neurogenic outcome. The culture of neurosphere suspension occurs in nonadherent culture dishes (Gervois et al. 2015). However, most of the adherent culture occurs on bare tissue culture-treated polystyrene (TCPS). A considerable number of studies reported the culture of DPSCs on different surface coatings such as polyornithine with laminin (Gervois et al. 2015), collagen type IV (Gervois et al. 2015; Lu et al. 2012) and collagen type I (Van Kooten et al. 2004), and chitosan (Osathanon et al. 2014).

Importantly, these surface coatings were chosen for DPSC neural induction based on trial and error of commonly utilized surface coatings for cell culture (Feng et al. 2014). To date, there is no systematic comparison of the various surface coatings used for the neural induction of DPSCs.

6.4 Culture Medium (Basal Medium, Growth Factors, Culture Supplements, and Small Molecules)

The great majority of studies used either Dulbecco's modified Eagle's medium (DMEM)/F12 medium or the neurobasal medium. Few studies used the Eagle's minimum essential medium, alpha modification (a-MEM).

Brewer et al. (Brewer et al. 1993) developed the neurobasal medium. Its formulation was optimized for the survival of in vitro cultured neural cell types. This improvement is best exemplified by its lower osmolarity compared to DMEM and the decrease of glutamine and cysteine concentrations in addition to the removal of toxic ferrous sulfate present in DMEM/F12. An extensive variation exists among the growth factors utilized in the neurogenic induction culture medium. The basic fibroblast growth factor (bFGF) and the epidermal growth factor (EGF) are the two most commonly supplemented growth factors. Research has shown that both bFGF and EGF are potent in stimulating the proliferation of neural stem cells (Santa-Olalla and Covarrubias 1995). The concentrations of bFGF and EGF used for DPSC neurogenic differentiation range from 10 to 50 mg/ml. The last component of the neural induction medium includes a large diversity of small molecules. They include small molecules without any neural inductive effects such as antibiotics (penicillin, streptomycin), antimycotics (amphotericin B), energy supplements (l-glutamine), forskolin, and vitamins such as ascorbic acid. Many combinations of these molecules were utilized in the various published neural inductive protocols of DPSCs, although the optimal combination for inducing neurogenesis is still currently unclear (Kim et al. 2011).

7 Conclusion

The concluding remarks, conceived after a thorough review of published literature, point to an important role for DPSCs in neurogenesis and the advancement of regenerative medicine. The selection of appropriate healthy teeth extracted from young patients with a good medical history, the adoption of ameliorated techniques of stem cell isolation and culture, as well as the employment of the best neural induction protocols constitute the mainstay of a very promising revolution in this field. However, the long-term side effects associated with the use of DPSCs and their potential to transform into tumors over time have not been sufficiently studied thus far. More studies are warranted to clarify possible long-term risks related to the use of these cells, as well as their interactions with the immune system of the host.

Conflicts of Interest The authors declare no conflicts of interest in relation to this article.

References

About, I. (2013). Dentin–pulp regeneration: The primordial role of the microenvironment and its modification by traumatic injuries and bioactive materials. *Endodontic Topics, 28*, 61–89.

Alge, D. L., Zhou, D., Adams, L. L., Wyss, B. K., Shadday, M. D., Woods, E. J., Chu, T. M. G., & Goebel, W. S. (2010). Donor-matched comparison of dental pulp stem cells and bone marrow-derived mesenchymal stem cells in a rat model. *Journal of Tissue Engineering and Regenerative Medicine, 4*, 73–81.

Aloe, L., Rocco, M. L., Omar Balzamino, B., & Micera, A. (2015). Nerve growth factor: A focus on Neuroscience and therapy. *Current Neuropharmacology, 13*, 294–303.

Bakopoulou, A., Leyhausen, G., Volk, J., Tsiftsoglou, A., Garefis, P., Koidis, P., & Geurtsen, W. (2011). Assessment of the impact of two different isolation methods on the osteo/odontogenic differentiation potential of human dental stem cells derived from deciduous teeth. *Calcified Tissue International, 88*, 130–141.

Baume, L. J. (1980). The biology of pulp and dentine. A historic, terminologic-taxonomic, histologic-biochemical, embryonic and clinical survey. *Monographs in Oral Science, 8*, 1–220.

Blau, H. M., & Baltimore, D. (1991). Differentiation requires continuous regulation. *The Journal of Cell Biology, 112*, 781–783.

Brewer, G. J., Torricelli, J. R., Evege, E. K., & Price, P. J. (1993). Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. *Journal of Neuroscience Research, 35*, 567–576.

Chai, Y., Jiang, X., Ito, Y., Bringas, P., Jr., Han, J., Rowitch, D. H., Soriano, P., Mcmahon, A. P., & Sucov, H. M. (2000). Fate of the mammalian cranial neural crest during tooth and mandibular morphogenesis. *Development, 127*, 1671–1679.

Dai, J. W., Yuan, H., Shen, S. Y., Lu, J. T., Zhu, X. F., Yang, T., Zhang, J. F., & Shen, G. F. (2013). p75 neurotrophin receptor positive dental pulp stem cells: New hope for patients with neurodegenerative disease and neural injury. *Shanghai Kou Qiang Yi Xue, 22*, 469–472.

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 and Differentiation, 14*, 1162–1171.

D'aquino, R., De Rosa, A., Laino, G., Caruso, F., Guida, L., Rullo, R., Checchi, V., Laino, L., Tirino, V., & Papaccio, G. (2009). Human dental pulp stem cells: From biology to clinical applications. *Journal of Experimental Zoology. Part B, Molecular and Developmental Evolution, 312b*, 408–415.

Davidson, R. M. (1994). Neural form of voltage-dependent sodium current in human cultured dental pulp cells. *Archives of Oral Biology, 39*, 613–620.

Dezawa, M., Kanno, H., Hoshino, M., Cho, H., Matsumoto, N., Itokazu, Y., Tajima, N., Yamada, H., Sawada, H., Ishikawa, H., Mimura, T., Kitada, M., Suzuki, Y., & Ide, C. (2004). Specific induction of neuronal cells from bone marrow stromal cells and application for autologous transplantation. *Journal of Clinical Investigation, 113*, 1701–1710.

Dimitrova-Nakov, S., Baudry, A., Harichane, Y., Kellermann, O., & Goldberg, M. (2014). Pulp stem cells: Implication in reparative dentin formation. *Journal of Endodontia, 40*, S13–S18.

Ding, G., Niu, J., & Wei, F. (2015). Current understanding of orofacial tissue derived mesenchymal stem cells: An immunological perspective. *Histology and Histopathology, 30*, 255–265.

Doi, M., Nagano, A., & Nakamura, Y. (2004). Molecular cloning and characterization of a novel gene, EMILIN-5, and its possible involvement in skeletal development. *Biochemical and Biophysical Research Communications, 313*, 888–893.

Feng, X., Xing, J., Feng, G., Sang, A., Shen, B., Xu, Y., Jiang, J., Liu, S., Tan, W., Gu, Z., & Li, L. (2013). Age-dependent impaired neurogenic differentiation capacity of dental stem cell is associated with Wnt/beta-catenin signaling. *Cellular and Molecular Neurobiology, 33*, 1023–1031.

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. *Cellular and Molecular Neurobiology, 34*, 859–870.

Ferro, F., Spelat, R., Beltrami, A. P., Cesselli, D., & Curcio, F. (2012). Isolation and characterization of human dental pulp derived stem cells by using media containing low human serum percentage as clinical grade substitutes for bovine serum. *PloS One, 7*, e48945.

Foudah, D., Monfrini, M., Donzelli, E., Niada, S., Brini, A. T., Orciani, M., Tredici, G., & Miloso, M. (2014). Expression of neural markers by undifferentiated mesenchymal-like stem cells from different sources. *Journal of Immunology Research, 2014*, 16.

Frescaline, G., Bouderlique, T., Mansoor, L., Carpentier, G., Baroukh, B., Sineriz, F., Trouillas, M., Saffar, J.-L., Courty, J., Lataillade, J.-J., Papy-Garcia, D., & Albanese, P. (2013). Glycosaminoglycan mimetic associated to human mesenchymal stem cell-based scaffolds inhibit ectopic bone formation, but induce angiogenesis in vivo. *Tissue Engineering. Part A, 19*, 1641–1653.

Gervois, P., Struys, T., Hilkens, P., Bronckaers, A., Ratajczak, J., Politis, C., Brône, B., Lambrichts, I., & Martens, W. (2015). Neurogenic maturation of human dental pulp stem cells following Neurosphere generation induces morphological and electrophysiological characteristics of functional neurons. *Stem Cells and Development, 24*, 296–311.

Goldberg, M., & Smith, A. J. (2004). Cells and extracellular matrices of dentin and pulp: A biological basis for repair and tissue engineering. *Critical Reviews in Oral Biology and Medicine, 15*, 13–27.

Govindasamy, V., Abdullah, A. N., Ronald, V. S., Musa, S., Ab Aziz, Z. A., Zain, R. B., Totey, S., Bhonde, R. R., & Abu Kasim, N. H. (2010). Inherent differential propensity of dental pulp stem cells derived from human deciduous and permanent teeth. *Journal of Endodontia, 36*, 1504–1515.

Govindasamy, V., Ronald, V. S., Abdullah, A. N., Ganesan Nathan, K. R., Aziz, Z. A., Abdullah, M., Zain, R. B., Kasim, N. H., Musa, S., & Bhonde, R. R. (2011). Human platelet lysate permits scale-up of dental pulp stromal cells for clinical applications. *Cytotherapy, 13*, 1221–1233.

Gronthos, S., Mankani, M., Brahim, J., Robey, P. G., & Shi, S. (2000). Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America, 97*, 13625–13630.

Gronthos, S., Brahim, J., Li, W., Fisher, L. W., Cherman, N., Boyde, A., Denbesten, P., Robey, P. G., & Shi, S. (2002). Stem cell properties of human dental pulp stem cells. *Journal of Dental Research, 81*, 531–535.

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*, e62332.

Hata, N., Shinojima, N., Gumin, J., Yong, R., Marini, F., Andreeff, M., & Lang, F. F. (2010). PDGF-BB mediates the tropism of human mesenchymal stem cells for malignant gliomas. *Neurosurgery, 66*, 144–157.

Hemmat, S., Lieberman, D. M., & Most, S. P. (2010). An introduction to stem cell biology. *Facial Plastic Surgery, 26*, 343–349.

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 and Tissue Research, 353*, 65–78.

Hu, Y., Zhang, Y. A. N., Tian, K., Xun, C., Wang, S., & Lv, D. (2016). Effects of nerve growth factor and basic

fibroblast growth factor dual gene modification on rat bone marrow mesenchymal stem cell differentiation into neuron-like cells in vitro. *Molecular Medicine Reports, 13*, 49–58.

Huang, A. H., Chen, Y. K., Lin, L. M., Shieh, T. Y., & Chan, A. W. (2008). Isolation and characterization of dental pulp stem cells from a supernumerary tooth. *Journal of Oral Pathology & Medicine, 37*, 571–574.

Iwasaki, K., Komaki, M., Yokoyama, N., Tanaka, Y., Taki, A., Kimura, Y., Takeda, M., Oda, S., Izumi, Y., & Morita, I. (2013). Periodontal ligament stem cells possess the characteristics of pericytes. *Journal of Periodontology, 84*, 1425–1433.

Jalali, A., Bassuk, A. G., Kan, L., Israsena, N., Mukhopadhyay, A., Mcguire, T., & Kessler, J. A. (2011). HeyL promotes neuronal differentiation of neural progenitor cells. *Journal of Neuroscience Research, 89*, 299–309.

Jung, S., Panchalingam, K. M., Rosenberg, L., & Behie, L. A. (2012). Ex vivo expansion of human mesenchymal stem cells in defined serum-free media. *Stem Cells International, 2012*, 21.

Kadar, K., Kiraly, M., Porcsalmy, B., Molnar, B., Racz, G. Z., Blazsek, J., Kallo, K., Szabo, E. L., Gera, I., Gerber, G., & Varga, G. (2009). Differentiation potential of stem cells from human dental origin - promise for tissue engineering. *Journal of Physiology and Pharmacology, 60*(Suppl 7), 167–175.

Karaoz, E., Dogan, B. N., Aksoy, A., Gacar, G., Akyuz, S., Ayhan, S., Genc, Z. S., Yuruker, S., Duruksu, G., Demircan, P. C., & Sariboyaci, A. E. (2010). Isolation and in vitro characterisation of dental pulp stem cells from natal teeth. *Histochemistry and Cell Biology, 133*, 95–112.

Kawashima, N. (2012). Characterisation of dental pulp stem cells: A new horizon for tissue regeneration? *Archives of Oral Biology, 57*, 1439–1458.

Kerkis, I., & Caplan, A. I. (2012). Stem cells in dental pulp of deciduous teeth. *Tissue Engineering. Part B, Reviews, 18*, 129–138.

Kim, H., Zahir, T., Tator, C. H., & Shoichet, M. S. (2011). Effects of dibutyryl cyclic-AMP on survival and neuronal differentiation of neural stem/progenitor cells transplanted into spinal cord injured rats. *PloS One, 6*, e21744.

Kiraly, M., Kadar, K., Horvathy, D. B., Nardai, P., Racz, G. Z., Lacza, Z., Varga, G., & Gerber, G. (2011). Integration of neuronally predifferentiated human dental pulp stem cells into rat brain in vivo. *Neurochemistry International, 59*, 371–381.

La Noce, M., Mele, L., Tirino, V., Paino, F., De Rosa, A., Naddeo, P., Papagerakis, P., Papaccio, G., & Desiderio, V. (2014). Neural crest stem cell population in craniomaxillofacial development and tissue repair. *European Cells & Materials, 28*, 348–357.

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). *Journal of Bone and Mineral Research, 20*, 1394–1402.

Ledesma-Martínez, E., Mendoza-Núñez, V. M., & Santiago-Osorio, E. (2016). Mesenchymal stem cells derived from dental pulp: A review. *Stem Cells International, 2016*, 12.

Lee, J. H., Lee, D. S., Choung, H. W., Shon, W. J., Seo, B. M., Lee, E. H., Cho, J. Y., & Park, J. C. (2011). Odontogenic differentiation of human dental pulp stem cells induced by preameloblast-derived factors. *Biomaterials, 32*, 9696–9706.

Liu, H., Gronthos, S., & Shi, S. (2006). Dental pulp stem cells. *Methods in Enzymology, 419*, 99–113.

Lizier, N. F., Kerkis, A., Gomes, C. M., Hebling, J., Oliveira, C. F., Caplan, A. I., & Kerkis, I. (2012). Scaling-up of dental pulp stem cells isolated from multiple niches. *PloS One, 7*, e39885.

Lu, Y., Yuan, X., Ou, Y., Cai, Y., Wang, S., Sun, Q., & Zhang, W. (2012). Autophagy and apoptosis during adult adipose-derived stromal cells differentiation into neuron-like cells in vitro. *Neural Regeneration Research, 7*, 1205–1212.

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*, 490–500.

Mukhopadhyay, A., Jarrett, J., Chlon, T., & Kessler, J. A. (2009). HeyL regulates the number of TrkC neurons in dorsal root ganglia. *Developmental Biology, 334*, 142–151.

Nanci, A. (2007). *Ten Cate's oral histology - Pageburst on VitalSource: Development, structure, and function*. Elsevier Health Sciences.

Ni, L., Wen, Y., Peng, X., & Jonakait, G. M. (2001). Antioxidants N-acetylcysteine (NAC) and 2-mercaptoethanol (2-ME) affect the survival and differentiative potential of cholinergic precursors from the embryonic septal nuclei and basal forebrain: Involvement of ras signaling. *Brain Research. Developmental Brain Research, 130*, 207–216.

Osathanon, T., Manokawinchoke, J., Nowwarote, N., Aguilar, P., Palaga, T., & Pavasant, P. (2013). Notch signaling is involved in neurogenic commitment of human periodontal ligament-derived mesenchymal stem cells. *Stem Cells and Development, 22*, 1220–1231.

Osathanon, T., Sawangmake, C., Nowwarote, N., & Pavasant, P. (2014). Neurogenic differentiation of human dental pulp stem cells using different induction protocols. *Oral Diseases, 20*, 352–358.

Park, H. Y., Kim, J. H., Sun Kim, H., & Park, C. K. (2012). Stem cell-based delivery of brain-derived neurotrophic factor gene in the rat retina. *Brain Research, 1469*, 10–23.

Paschalidis, T., Bakopoulou, A., Papa, P., Leyhausen, G., Geurtsen, W., & Koidis, P. (2014). Dental pulp stem cells' secretome enhances pulp repair processes and compensates TEGDMA-induced cytotoxicity. *Dental Materials, 30*, e405–e418.

Racz, G. Z., Kadar, K., Foldes, A., Kallo, K., Perczel-Kovach, K., Keremi, B., Nagy, A., & Varga, G. (2014). Immunomodulatory and potential therapeutic role of mesenchymal stem cells in periodontitis. *Journal of Physiology and Pharmacology, 65*, 327–339.

Ranganathan, K., & Lakshminarayanan, V. (2012). Stem cells of the dental pulp. *Indian Journal of Dental Research, 23*, 558.

Raoof, M., Yaghoobi, M. M., Derakhshani, A., Kamal-Abadi, A. M., Ebrahimi, B., Abbasnejad, M., & Shokouhinejad, N. (2014). A modified efficient method for dental pulp stem cell isolation. *Dent Res J (Isfahan), 11*, 244–250.

Roozafzoon, R., Lashay, A., Vasei, M., AI, J., Khoshzaban, A., Keshel, S. H., Barabadi, Z., & Bahrami, H. (2015). Dental pulp stem cells differentiation into retinal ganglion-like cells in a three dimensional network. *Biochemical and Biophysical Research Communications, 457*, 154–160.

Saito, M. T., Silvério, K. G., Casati, M. Z., Sallum, E. A., & Nociti, F. H., Jr. (2015). Tooth-derived stem cells: Update and perspectives. *World Journal of Stem Cells, 7*, 399–407.

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. *The Journal of Clinical Investigation, 122*, 80–90.

Santa-Olalla, J., & Covarrubias, L. (1995). Epidermal growth factor (EGF), transforming growth factor-alpha (TGF-alpha), and basic fibroblast growth factor (bFGF) differentially influence neural precursor cells of mouse embryonic mesencephalon. *Journal of Neuroscience Research, 42*, 172–183.

Santos, N. C., Figueira-Coelho, J., Martins-Silva, J., & Saldanha, C. (2003). Multidisciplinary utilization of dimethyl sulfoxide: Pharmacological, cellular, and molecular aspects. *Biochemical Pharmacology, 65*, 1035–1041.

Silvério, K. G., Davidson, K. C., James, R. G., Adams, A. M., Foster, B. L., Nociti, F. H., Somermam, M. J., & Moon, R. T. (2012). Wnt/β-catenin pathway regulates Bmp2-mediated differentiation of dental follicle cells. *Journal of Periodontal Research, 47*. doi:10.1111/j.1600-0765.2011.01433.x.

Sonoyama, W., Yamaza, T., Gronthos, S., & Shi, S. (2007). Multipotent stem cells in dental pulp. In *Culture of human stem cells*. Hoboken: Wiley.

Suchanek, J., Visek, B., Soukup, T., El-Din Mohamed, S. K., Ivancaková, R., Mokrý, J., Aboul-Ezz, E. H., & Omran, A. (2010). Stem cells from human exfoliated deciduous teeth--isolation, long term cultivation and phenotypical analysis. *Acta Medica (Hradec Králové), 53*, 93–99.

Sun, H.-H., Chen, B., Zhu, Q.-L., Kong, H., Li, Q.-H., Gao, L.-N., Xiao, M., Chen, F.-M., & Yu, Q. (2014). Investigation of dental pulp stem cells isolated from discarded human teeth extracted due to aggressive periodontitis. *Biomaterials, 35*, 9459–9472.

Tamaki, Y., Nakahara, T., Ishikawa, H., & Sato, S. (2013). In vitro analysis of mesenchymal stem cells derived from human teeth and bone marrow. *Odontology, 101*, 121–132.

Tatullo, M., Marrelli, M., Shakesheff, K. M., & White, L. J. (2015). Dental pulp stem cells: Function, isolation and applications in regenerative medicine. *Journal of Tissue Engineering and Regenerative Medicine, 9*, 1205–1216.

Tsiperson, V., Huang, Y., Bagayogo, I., Song, Y., Vondran, M. W., Dicicco-Bloom, E. & Dreyfus, C. F. 2015. Brain-derived neurotrophic factor deficiency restricts proliferation of oligodendrocyte progenitors following Cuprizone-induced demyelination. ASN Neuro, 7, 1759091414566878.

Van Kooten, T. G., Spijker, H. T., & Busscher, H. J. (2004). Plasma-treated polystyrene surfaces: Model surfaces for studying cell-biomaterial interactions. *Biomaterials, 25*, 1735–1747.

Wada, N., Menicanin, D., Shi, S., Bartold, P. M., & Gronthos, S. (2009). Immunomodulatory properties of human periodontal ligament stem cells. *Journal of Cellular Physiology, 219*, 667–676.

Xiao, L., & Nasu, M. (2014). From regenerative dentistry to regenerative medicine: Progress, challenges, and potential applications of oral stem cells. *Stem Cells and Cloning: Advances and Applications, 7*, 89–99.

Xiao, L., & Tsutsui, T. (2013). Characterization of human dental pulp cells-derived spheroids in serum-free medium: Stem cells in the core. *Journal of Cellular Biochemistry, 114*, 2624–2636.

Yalvac, M. E., Ramazanoglu, M., Rizvanov, A. A., Sahin, F., Bayrak, O. F., Salli, U., Palotas, A., & Kose, G. T. (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. *The Pharmacogenomics Journal, 10*, 105–113.

Zhao, H., & Chai, Y. (2015). Stem cells in teeth and craniofacial bones. *Journal of Dental Research, 94*, 1495–1501.