Stem Cells and Cloning: Advances and Applications

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REVIEW

Dental Pulp Stem Cells: Advances to Applications

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Abstract: Dental pulp stem cells (DPSCs) have a high capacity for differentiation and the ability to regenerate a dentin/pulp-like complex. Numerous studies have provided evidence of DPSCs' differentiation capacity, such as in neurogenesis, adipogenesis, osteogenesis, chondrogenesis, angiogenesis, and dentinogenesis. The molecular mechanisms and functions of DPSCs' differentiation process are affected by growth factors and scaffolds. For example, growth factors such as basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β), nerve growth factor (NGF), platelet-derived growth factor (PDGF), and bone morphogenic proteins (BMPs) influence DPSC fate, including in differentiation, cell proliferation, and wound healing. In addition, several types of scaffolds, such as collagen, hydrogel, decellularized bioscaffold, and nanofibrous spongy microspheres, have been used to characterize DPSC cellular attachment, migration, proliferation, differentiation, and functions. An appropriate combination of growth factors and scaffolds can enhance the differentiation capacity of DPSCs, in terms of optimizing not only dental-related expression but also dental pulp morphology. For a cell-based clinical approach, focus has been placed on the tissue engineering triad [cells/bioactive molecules (growth factors)/scaffolds] to characterize DPSCs. It is clear that a deep understanding of the mechanisms of stem cells, including their aging, self-renewal, microenvironmental homeostasis, and differentiation correlated with cell activity, the energy for which is provided from mitochondria, should provide new approaches for DPSC research and therapeutics. Mitochondrial functions and dynamics are related to the direction of stem cell differentiation, including glycolysis, oxidative phosphorylation, mitochondrial metabolism, mitochondrial transcription factor A (TFAM), mitochondrial elongation, and mitochondrial fusion and fission proteins. This review summarizes the effects of major growth factors and scaffolds for regenerating dentin/pulp-like complexes, as well as elucidating mitochondrial properties of DPSCs for the development of advanced applications research.

Keywords: dental pulp stem cell, bioactive molecule, growth factor, scaffold, mitochondria, regenerative therapy

Introduction

Dental pulp stem cells (DPSCs) have great potential for a range of applications in stem cell research and regenerative medicine. In the life science literature, there are numerous reports on DPSC properties from in vitro and in vivo studies, such as cell growth, capacity for differentiation, competence in assays, and potential for pioneering stem cell functions. The first report on DPSCs revealed that their stem cell properties are comparable to those of bone marrow stromal cells (BMSCs) in vitro and in vivo.[1]

The study of DPSCs by Gronthos's group[1] reported an immunophenotype similar to that of BMSCs, along with the formation of a calcified nodule upon treatment with differentiation medium in vitro. This group transplanted DPSCs into

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the dorsal surface of immunocompromised mice with hydroxyapatite/tricalcium phosphate (HA/TCP), with the results showing that DPSCs were able to regenerate a dentin/pulp-like complex. They also showed a difference in the structures formed after transplantation compared with the case for BMSCs. In the literature, they speculated that adult dental pulp tissue might also contain a population of stem cells.[1]

The correlation of the presence of these cells in pulp with reparative dentinogenesis has also been explored.[2,3] Reparative dentin is also referred to as tertiary, reactive, or irregular secondary dentin. Tertiary dentin is produced in response to various irritants (attrition, caries, or a restorative dental procedure) by the stimulus-affected cells. There are two categories of tertiary dentin: reactionary dentin, which is deposited by preexisting odontoblasts; and reparative dentin, which is from newly differentiated odontoblast-like cells.[4] The precursors of odontoblasts have been shown to be regulated by growth factors such as transforming growth factor-β (TGF-β), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), epidermal growth factor (EGF), tumor necrosis factor-α (TNF-α), and insulin-like growth factors (IGF)I and II. PDGF and bFGF were revealed to stimulate [^{3}H] thymidine incorporation into DNA, while TGF-β, EGF, and TNF-α have less of an effect of this kind.[5]

DPSCs exhibit greater proliferation than other stem cells, BMSCs and adipocyte stem cells (ASCs). Analysis of the cellular growth curve demonstrated that DPSCs remained in the log phase from 3 to 5 days and that BMSCs had a longer population doubling time (PDT) than DPSCs during a 10-day period. A BrdU cell proliferation assay also showed that DPSCs had higher proliferative ability than BMSCs.[6]

The immunophenotype of DPSCs has been reported to feature mesenchymal stem cell markers, for example, CD73,[7] CD90,[7] and CD105.[8] Interestingly, another mesenchymal stem cell (MSC) marker, STRO-1, was found to be coexpressed with CD146 and pericyte antigen 3G5 in dental pulp, forming a specific niche.[9] In addition, DPSCs express neural lineage markers including those found on neural stem cells, such as nestin,[10,11] musashi-1,[10] βIII tubulin[11] glial fibrillary acidic protein (GFAP),[11] and neuronal nuclei (NeuN).[11]

DPSCs have the capacity to differentiate into odontoblast-like cells. This differentiation capacity was revealed by the finding that DPSCs mixed with HA/TCP were able to regenerate a dentin/pulp-like structure by transplantation into immunocompromised mice.[1] Other in vivo studies on regenerating dentin formation also reported a dentin/pulp-like structure.[12–15] Moreover, several in vivo studies using DPSC transplantation have shown the capacity for differentiation in animal models (eg, osteogenic,[16] angiogenic,[17] and neurogenic[18] functions). For in vivo models, scaffolds are a key factor for tissue engineering. Several kinds of material for scaffolds that influence DPSC properties have been reported.[19–26] Mitochondria are cytoplasmic organelles that have critical functions in energy metabolism for the regulation of stem cells. Considering analysis of the energy metabolism of stem cells for regenerative research, knowledge of mitochondrial properties is important as it should deepen our understanding of the differentiation of these cells. In one study, the differences in energy metabolism in human MSCs were analyzed over the course of their differentiation.[27] Moreover, dysfunction in mitochondrial membrane potential was observed in cells from the deciduous teeth of a Rett syndrome patient, showing the functional importance of stem cells and the value of mitochondrial analysis to explore the effectiveness of cell-based clinical approaches. This review summarizes major growth factors (bFGF, TGF-β, NDF, BMPs, and PDGF), scaffolds, and mitochondrial properties within the research field of DPSCs.

Growth Factors

Basic Fibroblast Growth Factor (bFGF)

bFGF is a potent modulator of cell proliferation, motility, and differentiation.[5,28] The bFGF locus is on chromosome4[29] and has been reported to present mRNAs of 4.6 kilobases (kb) and additionally 2.2 kb in hypothalamus.[30] The important properties of bFGF include a high affinity for heparin/heparan sulfate (HS) and physiological transfer of glycosaminoglycans to the extracellular matrix (ECM). bFGF derived from endothelial cells and bone cells may function in the ECM.[31,32] Moreover, this high affinity for heparin/HS influences the maintenance of many different target tissues.

According to the literature, bFGF has been detected in DPSCs[6] and after the endodontic procedure of irrigation.[33] Basic FGF receptors (FGFR1 and FGFR2) were also found to be expressed in human dental pulp cells.[34] Moreover, treatment of DPSCs with bFGF led to their proliferation and differentiation during neurogenesis[35,36] and osteogenesis.[37] Furthermore, Lue's group[36] reported functional recovery in a spinal cord injury rat model upon the application of heparin–poloxamer hydrogel containing

DPSCs and bFGF. The duration of treatment with bFGF was also shown to affect osteogenic differentiation to DPSCs. In the literature, it is demonstrated that 1 week of treatment increased osteogenic differentiation. Interestingly, 2 weeks of treatment actually decreased osteogenic differentiation, with similar results for these treatment periods being obtained in vitro and in vivo.[37] Overall, these findings show that bFGF promotes proliferation and is related to osteogenic and neurogenic differentiation.

Transforming Growth Factor-β (TGF-β)

The TGF-β subfamily is divided into three isoforms, TGF-β1, 2, and 3, which are produced as large precursor molecules constituting mature TGF-β and the latency-associated peptide (LAP).[38] LAP is cleaved off by an endoprotease and remains noncovalently bound to TGF-β, constituting the small latency complex (SLC).[39] The SLC is associated with latent TGF-β binding proteins (LTBPs) 1–4.[39,40] Active TGF-β is a potent regulator in biological processes, including development, carcinogenesis, wound healing, hematopoiesis, and immune responses, as well as having specific effects on proliferation, differentiation, migration, and apoptosis in microenvironments related to particular cell types,[41] including stem cells such as bone marrow-derived MSCs (BM-MSCs), adipose tisuue-derived MSCs (A-MSCs), and MSCs from dental pulp (DP-MSCs) producing TGF-β1.[42,43] The TGF-β family induces signaling through transmembrane type I and type II membrane binding serine/threonine kinase receptors; there are seven type I receptors [activin receptor like kinase (ALK)] and five type II receptors.[44] Upon the binding of a ligand to type I and type II receptors, type II receptors phosphorylate type I receptors. This leads to the phosphorylation of R-Smads, which induces a downstream signaling pathway.[45]

Several studies have demonstrated that treatment with recombinant TGF-β1 can enhance BMSC and pulp cell proliferation.[5,46] For example, a three-dimensional (3D) aggregate of DPSCs cultured with TGF-β3 and BMP-2 in serum-free medium induced calcification.[47] In addition, Song's group showed that TGF-β1 induced DPSCs to differentiate into bladder smooth muscle cells (SMCs).[48] Moreover, when DPSCs were exposed to SMC-conditioned medium with TGF-β1 for 14 days, this led to the increased expression of SMC-specific gene and protein markers (alpha-SMA, desmin, and calponin). Furthermore, the mature SMC marker myosin was detected after 11 days of this exposure.[48] TGF-β1 in culture medium was also shown to upregulate alpha-SMA in the differentiation of DPSCs into smooth muscle cells.[10] The supplementation of TGF-β1/β3 in culture medium induced DPSCs to undergo chondro-differentiation.[10] TGF-β treatment induced several types of differentiation of DPSCs, including calcification, SMC-specific gene expression, and chondro-differentiation.

Nerve Growth Factor (NGF)

NGF is an essential regulator in the development, survival, differentiation, and maintenance of neuronal and non-neuronal cells. NGF is a member of the neurotrophin family, which includes brain-derived growth factor (BDNF), glial-cell-derived neurotrophic factor (GDNF), neurotrophic-3 (NT-3), and neurotrophic-4/5 (NT4/5).

Two NGF receptors have been identified: the trk proto-oncogene product $p140^{trk}$ (trkA) and the p75 neurotrophin receptor ($p75^{NTR}$).[49–51] The NGF binding sites of neurons are referred to as high-affinity and low-affinity receptor binding sites.[52] The trkA and $p75^{NTR}$ receptors exhibit low-affinity NGF binding.[52–54] High-affinity binding sites are created when trkA and $p75^{NTR}$ are coexpressed.[52] The complex network of signal pathways of trkA includes the Ras-MAP kinase cascade.[55] TrkA and $p75^{NTR}$ are also expressed in keratinocytes[56] and in bone marrow and lymphoid tissues[57] for cell proliferation, differentiation, and survival. Mitsiadis' group reported that NGF, trkA, and $p75^{NTR}$ are expressed in dental tissue and act in cell proliferation, differentiation, and odontogenesis, while also being expressed in nerve fibers of developing human teeth.[58]

Moreover, a tiny group of DPSCs was shown to express NGF, trkA, and $p75^{NTR}$, the expression of which was affected by the presence of β-glycerophosphate in culture medium, especially in cells forming mineralized nodules.[59] DPSCs have the capacity to differentiate into neurons and to repair injured neural systems. In the case of a rat model of spinal cord injury (SCI), recovery of hindlimb locomotor functions occurred upon the transplantation of DPSCs with chitosan scaffolds.[18] Moreover, in a comparative study of DPSCs and BMSCs, DPSC secretion of NGF, BDNF, and NT-3 was shown to be higher than that of BMSCs. Furthermore, DPSC coculture with $\beta III\text{-tubulin}^{+}$ retinal cells was associated with a decrease in the number of neurite-bearing cells and the duration of treatment with Trk receptor blockers.[60] NGF was found to be expressed in dental tissue undergoing cell proliferation and odontogenesis. Furthermore, NGF expression was shown to affect the differentiation of DPSCs and their

potential to promote recovery from spinal cord injury via differentiation into neurons.

Platelet-Derived Growth Factor (PDGF)

PDGF was identified in cell-free plasma derived from serum, a component of whole blood,[61] and purified from human platelets.[62,63] In terms of its structure, PDGF consists of two polypeptide A and B chains combined in three disulfide-linked dimers (AA, AB, and BB). PDGF-C[64] and PDGF-D consist of domains: CUB and PDGF/VEGF and *N*-linked glycosylation site.[65] The gene encoding the PDGF-A chain is located on chromosome 7,[66] while that for the PDGF-B chain is located on chromosome 22.[66,67] The PDGFC gene and PDGFD gene are located on chromosomes 4 and 11.[68]

PDGF binds two receptor tyrosine kinases, namely, α-receptor and β-receptor, which are located on different chromosomes, 4 and 5. The PDGF α-receptor binds the PDGF-A chain and the PDGF-B chain with high affinity, while the PDGF β-receptor binds the PDGF-B chain with high affinity. PDGF-C binds to the α-receptor but not the β-receptor.[64] PDGF-D interacts with the β-receptor, but not the α-receptor.[65] PDGF signaling is a key regulator in mesenchymal cells. PDGF binding to its receptor induces dimerization and autophosphorylation, as well as activation of a signal transduction molecule containing a cytoplasmic CH2 domain.[69]

Human DPSCs secrete PDGF-AA and other growth factors, and the titers of NGF, BDNF, and VEGF were revealed by ELISA to be greater than those of human bone marrow-derived mesenchymal stem cells and human adipose-derived stem cells.[70] The overexpression of PDGF-BB in human DPSCs increases cell proliferation and odontoblastic differentiation in particular. In addition, the secretion of PDGF-BB by DPSCs can increase the likelihood of stem cell homing via the PI3K/Akt pathway and improve the DPSC-mediated dentin–pulp complex regeneration in vivo.[71] Moreover, separated PDGFRβ$^+$ and PDGFRβ$^+$/c-kit$^+$ dental pulp cells show faster proliferation than whole pulp cells and PDGFRβ$^-$ cells. Furthermore, an in vivo study demonstrated that transplanted PDGFRβ$^+$/c-kit$^+$ dental pulp cells with hydrogel formed globular dentin and pulp-like tissue in rat incisor.[72] According to these findings, PDGF enhances DPSC proliferation, odontoblast differentiation, and regeneration of dentin–pulp complex.

Bone Morphogenic Proteins (BMPs)

BMPs, which have been shown to have the ability to induce bone formation, are important in embryo, heart, neural, cartilage, and tooth development. Many studies have reported the characterization[73] of this protein group, which belongs to the TGF-β superfamily. In terms of the ligands of BMPs, they bind to type I and type II receptors that signal through canonical and noncanonical pathways. Upon ligand binding, the type II receptor activates the type I receptor by phosphorylation and activates smads. This signal plays an important role in early odontogenesis[74] and tooth development, including tooth homeostasis,[75] number, size, and shape.[76]

Shi's group demonstrated BMP signal activation of preodontoblasts/odontoblasts, dental pulp, and a small number of transit-amplifying cells (TAC) in 1-month-old mice. Using 1-month-old Gli1-Cre^{ERT2} ;td Tomato mice, Gil1+(tdTomato+) cells showed that the progeny of Gil1+ cells differentiated into odontoblasts and dental pulp cells and colocalized with phosphorylated Smad1/5/9 (activated BMP signaling) after tamoxifen induction in the preodontoblast region and dental pulp cells in close proximity to this region.[75] These findings suggest that BMP signaling maintains tooth morphology and homeostasis.

BMPs influence DPSCs during the processes of proliferation and differentiation. BMP2-transfected DPSCs isolated by STRO-1 revealed high levels of alkaline phosphatase (ALP) activity in vitro and the enhancement of mineralized tissue upon implantation.[77] BMP4 affects the growth of dental pulp cells and enhances the mRNA expression levels of ALP, DSPP, and DMP-1.[78] BMP7 induction resulted in increases in dentin sialophosphoprotein (DSPP), osteocalcin (OCN), dentin matrix protein 1 (DMP-1), and runt-related transcription factor 2 (RUNX2) mRNA expression levels and the formation of mineralized nodules in DPSCs.[79] Through the p38 mitogen-activated protein kinase (MAPK) and WNT canonical pathway, BMP2 was shown to promote the differentiation and mineralization of human DPSCs.[80] Moreover, the incorporation of BMP2 and VEGF into a three-dimensional culture model (TDM) using human DPSCs enhanced the potency of stem cells to induce angiogenesis and odontogenesis. Specifically, the human DPSCs and VEGF were encapsulated in a fibrin gel, and inserted into BMP2-coated demineralized dentin discs. The qRT-PCR results of this TMD showed higher expression of platelet and endothelial cell adhesion molecule (PECAM), BSP, DMP-1, OCN, and CBFA1 than in a monolayer control

group.[81] In another study, the autogenous transplantation of BMP2-treated three-dimensional (3D) porcine pulp cell pellet culture onto amputated pulp induced reparative dentin formation.[82] According to these reports, BMPs affect DPSC proliferation and differentiation, and increase dentinogenesis-related gene expression; moreover, three-dimensional culture enhances the properties of DPSCs.

Scaffolds

Many different carriers for cells have been reported (Table 1). Scaffolds support appropriate cellular attachment, migration, proliferation, differentiation, and function to produce tissue constructs specific to the particular purpose. One such purpose would be to provide support for replacement by transplanted cells, but for scaffolds there is concern about the nature of their degradation, cytotoxicity, and immune reactions to them by the recipient.

$CD105^+$ DPSCs were transplanted with stromal cell-derived factor-1 (SDF-1) and collagen into the mature teeth of dogs that had undergone pulpectomy. This transplantation resulted in newly regenerated tissue, which expressed angiogenic/neurotrophic factors.[19] Suzuki's group also reported the migration of dental stem cells (DSCs) using collagen gel cylinders. The DSCs were seeded on the surface of these cylinders and cultured with stromal-derived factor-1α(SDF-1), bFGF, and BMP7, which induced the recruitment of the cells into the cylinders. SDF-1 or bFGF recruited more cells into collagen gel than the case without cytokines, and BMP7 also recruited few of them.[20] Regarding other cytokines, an investigation of STRO-1-sorted cells (human pulp cells; immature third molars) treated with growth factors (FGF-2 and TGF-β1) in a biodegradable polymer matrix of lactide and glycolide released using a Matrigel-covered dish was also reported. FGF-2 increased dental pulp proliferation and TGF-β1 was observed to exert chemotactic potential. This Matrigel-covered dish culture showed the controlled release of growth factors upon investigating the early stage of pulp/dentin regeneration.[21] In another study, vascularized pulp-like tissue and osteodentin were analyzed upon the transplantation of DPSCs, human umbilical vein endothelial cells (HUVECs), or co-culture of both types of cell encapsulated in a three-dimensional (3D) PuraMatrix™ in mice. The results showed that transplantation in the co-culture group produced more ECM, vascularization, and mineralization than achieved with the DPSC monocultures in vivo.[22] A further study focused on DPSCs and HUVECs encapsulated in 5% gelatin methacrylate (GelMA) xenogeneic hydrogel and injected into root segments. This transplantation in mice showed neovasculature formation.[23] Another scaffold type in the form of ECM was supplied by decellularized dental pulp from swine as a bioscaffold for pulp regeneration. The swine pulp was decellularized with a mixed solution of 10% sodium dodecyl sulfate and Triton X-100. Transplantation of human DPSCs with decellularized dental pulp into nude mice demonstrated ECM preservation and a pulp-like tissue structure, as revealed by histological

Table 1 Scaffold, Growth Factors, and Bioactive Molecules

Reference No.	Authors	Scaffold	Growth factors, Bioactive molecule	in vitro	in vivo
[19]	Iohara et al	Collgen type I and type III	SDF-1		Dog
[20]	Suzuki et al	Mixing rat tail collagen type I solution and 0.02-N acetic acid, human teeth	SDF-1, bFGF, BMP7	3D collagen scaffold	Rat
[21]	Mathieu et al	Matrigel	FGF-2 and TGF-β1 (encaspulated into a biodegradable polymer of lactide and glycolide)	FGF-2 and TGF-β1 loaded microspher composed Matrigel	
[22]	Dissanayaka et al	PuraMatrix™, human teeth	VEGF (for in vitro)	PuraMatrix™	Mouse
[23]	Khayat et al	GelMA hydrogel		GelMA hydrogel	Rat
[24]	Hu et al.	Decellularized dental pulp ECM, tooth slice			Mouse
[25]	Zhang et al.	Decellularized tooth buds	BMP-2	decellularized tooth buds	Mini-pig
[26]	Ravindranet al.	ECM embedded collagen/ chitsosan scaffold		ECM embedded collagen/chitsosan scaffold	Mouse

analysis.[24] Decellularized natural porcine tooth bud has also been shown to be useful as a bioengineered scaffold for tooth regeneration, when transplanted with porcine dental epithelial cells, human dental pulp cells, and human umbilical vein endothelial cells. The implantation of samples into the mandibles of mini-pigs revealed dentin- and enamel-like tissues.[25] In addition, DPSCs were cultured on a 3D scaffold using a decellularized ECM embedded in a collagen/chitosan scaffold. The subcutaneous implantation of the scaffold with DPSCs into nude mice resulted in dental pulp-like tissue and the expression of dentin sialoprotein (DSP) and DSPP.[26] To obtain a deeper understanding of the stemness of DPSCs, there is a need to analyze their activity including in the presence of scaffolds. A key focus for this analysis should be mitochondria, one of the key organelles during the differentiation of DPSCs, the energy from which is vital for this process.

Research on Prospective Advanced Applications for DPSC

Mitochondria

To understand the mechanisms of stem cells including their aging and self-renewal, the establishment of microenvironmental homeostasis and differentiation should be developed as a new approach for DPSC research and therapeutics. According to the literature, mitochondrial functions and dynamics are particularly related to the direction of stem cell differentiation, including for DPSCs.

The sequence of human mitochondrial DNA (mtDNA) is 16,569 base pairs long, which includes genes for the 12S and 16S rRNAs, 22 tRNAs, cytochrome c oxidase subunits I, II, and III, ATPase subunit 6, cytochrome b, and eight other predicted protein-coding genes.[83] Mitochondria function in energy metabolism, which regulates the homeostasis of cells including stem cells.

Undifferentiated stem cells show higher levels of glycolysis compared with stem cells undergoing differentiation.[27,84] Differentiation for osteogenesis has been shown to be retarded by exogenous H_2O_2 and mitochondrial inhibitors. The transition of mitochondrial energy production from glycolysis to oxidative phosphorylation induces osteogenesis in human MSCs.[27] Adipogenic differentiation is inhibited by mitochondrially targeted antioxidants. During differentiation into adipocytes, there are early increases in mitochondrial metabolism and reactive oxygen species (ROS) generation, which are dependent on mTORC1 signaling in the primary human MSCs.[85] Furthermore, during adipocyte differentiation, PPARγ-dependent transcription is dependent on mitochondrial complex III-generated superoxide.[85] Differentiation for adipogenesis and osteogenesis has been shown to be correlated with mitochondrial elongation and increases in Mfn1 and 2 (mitochondrial fusion proteins) expression. Forni's group reported the use of mouse skin mesenchymal stem cells (msMSCs), which are $CD105^+$ $CD90^+$ $CD73^+$ $CD29^+$ $CD34^-$ mesodermal precursors.[86] In addition, chondrogenesis of msMSCs showed increases of Drp1, Fis1, and Fis2 (fission proteins) expression and mitophagy enhancement.[86] The regulation of processes such as fission/fusion, mitochondrial biogenesis, and oxidative metabolism of mitochondria is thus key for differentiation and homeostasis in MSCs.

Intriguingly, many studies have reported that mitochondria are transferred from MSCs to injured cells through tunneling nanotubes. The introduction of MSCs into an infarcted heart mouse model resulted in increased expression of heme oxygenase-1 (HO-1) and peroxisome proliferator-activator receptor gamma coactivator-1-α (PGC-1-α) genes in MSCs infused in intact myocardium. This suggested that HO-1 or mitophagy inhibition was associated with cardiac apoptosis.[87] Heart muscle expresses a high level of the heart muscle protein (HMP) mitofilin.[88] Mitofilin is anchored in the inner mitochondrial membrane and is a transmembrane protein.[89] The morphology of cristae is maintained by the mitochondrial inner membrane organizing system (MINOS) including mitofilin, which is a core component of it along with Mito10. Mitofilin has been reported to function as a multifunctional regulator of mitochondrial morphology and protein biogenesis.[90] In MSCs derived from bone marrow, mitofilin was shown to regulate their homeostasis and osteogenesis.[91] Earlier induction of osteogenic/dentinogenic markers in DPSCs was also achieved by the depletion of mitofilin/3C4 antigens.[92] Adipose (AD)-MSCs and bone marrow (BM)-MSCs showed higher mitochondrial transfer than dental pulp (DP)-MSCs and Wharton's jelly (WJ)-MSCs. In addition, DP-MSCs and WJ-MSCs had reduced mtROS compared with BM-MSCs and AD-MSCs in cardiomyocyte coculture. Moreover, DP-MSCs and WJ-MSCs revealed higher mitochondrial respiratory abilities.[93] The initiation of the differentiation of human DPSCs to odontoblasts was also observed to involve mitochondrial elongation with developed cristae, enhancement of the mitochondrial oxygen consumption rate, increasing mitochondrial ATP production,

upregulation of mitochondrial glycolytic enzyme activities, and increased glycolytic capacity and glycolytic reserve.[94] Disruption of the differentiation of human DPSCs into odontoblasts was also induced by lipopolysaccharide (LPS), which decreased HO-1 and PGC-1-α levels.[95] LPS simulation is inhibited by Schisandrin C and activates mitochondrial biogenesis, which increased HO-1 and PGC-1-α through the phosphorylated-protein kinase B (p-AKt) and nuclear factor erythroid 2-related factor-2 pathway.[96] The above findings show that mitochondrial dynamics, metabolism, and function are associated with the fate of stem cells including DPSCs. Stem cell differentiation is also related to mitochondrial activity. Dental pulp stem cells from children, another type of stem cell from human deciduous teeth (SHED), differentiate into neuronal cells, which was shown to increase mitochondrial membrane potential, mitochondrial DNA, and elongated mitochondria.[97] In patients with Rett syndrome, loss-of-function mutations in MECP2 have been identified, which is a gene encoding methyl-CpG-binding protein (MeCP2). Using MeCP2-expressing and MeCP2-deficient stem cells from exfoliated deciduous teeth, it was shown that differentiating MeCP2-deficient stem cells exhibited reductions in mitochondrial membrane potential and ATP production, and restricted mitochondrial distribution in neurites compared with MeCP2-expressing cells. In addition, central mitochondrial fission factor (dynamin-related protein1) showed lower expression in MeCP2-deficient cells than in MeCP2-expressing ones.[98] These reports suggest the importance of mitochondrial function in stem cells. Understanding the molecular profile and morphology of mitochondria is thus important to improve the effectiveness of DPSC-based clinical approaches.

Conclusion and Future Challenges

The field of research on DPSCs has great potential because the cells not only have the characteristics of good differentiation potential and being easy to culture, but can also be conveniently obtained from extracted teeth, which are usually discarded. Growth factors and scaffolds strongly affect DPSC proliferation and their direction of differentiation. DPSCs can aid the regeneration of dentin/pulp-like complex or other tissues in the presence of growth factors and scaffolds more efficiently than in their absence. Moreover, upon the combination of DPSCs with other cells and bioactive molecules, enhanced DPSC properties were obtained in vitro and in vivo. Currently, to demonstrate the advantage of combining analyses of the expression of major genes and proteins, as an example, dentinogenesis-related genes and proteins were mainly analyzed. Analysis of DPSC metabolism in the presence of growth factors and scaffolds should also help us to obtain a deep understanding of their stemness. Such analysis of metabolism is important because mitochondria are the main organelles producing the energy not only for the maintenance of homeostasis, but also during differentiation. DPSCs are a promising cell source in the cutting-edge research field of stem cells and for developing regenerative medicine applications. Experiments should be performed to evaluate their clinical application, requiring further exploration and a deeper understanding of various characteristics of DPSCs.

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Disclosure

The author reports no conflicts of interest in this work.

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