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Review Article

Enhancing the Plasticity/Stemness of Dental Stem Cells using Growth Factors, Small Molecules and Scaffolds for Tissue Engineering

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Abstract

Dental stem cells (DSCs) are easily obtainable mesenchymal-like stem cells from dental origin exhibiting high plasticity and multipotency. However, the DSCs have enhanced abilities to give rise to mineralized tissues like bones. Also, DSCs can differentiate into dentinogenic, adipogenic, chondrogenic, myogenic, neurogenic and pancreatic β cell lineages that have a possibility of a wide range of applications in regenerative medicine. Moreover, the applicability in regenerative medicine is directly correlated with the plasticity of stem cells, and biocompatibility of the scaffolds. Hence, this paper reviews the recent developments for enhancing the plasticity of dental stem cells using small molecules, growth factors, and scaffolds. This paper also reflects the possibilities of combining several aspects for enhancing the plasticity/stemness of dental stem cells.

Keywords: Dental stem cells (DSCs); Dental pulp stem cells (DPSCs); Bone marrow mesenchymal stem cells (BM-MSCs); Regenerative Medicine; Small molecules; Growth factors; Cell therapy; Scaffolds; Differentiation; Mesenchymal stem cells (MSCs)

Introduction

With the advent of the use of stem cells for regenerative medicine, various stem cells are being explored. Undoubtedly, the pluripotent stem cells match all the criteria for being the best amongst the lot. The criteria for any stem cells to be applicable for regenerative potential are its ability to differentiate into several or all lineages, and their infinite proliferative potential. Pluripotent stem cells meet the criteria above but have issues like teratoma formation; hence alternative stem cells are being explored. Mesenchymal/adult stem cells do not form teratomas and are hence considered safe for cell therapy. Adult stem cells expressing pluripotency factors such as Oct3/4, Nanog, Sox-2, SSEA-4, and Klf4 were earlier perceived to be non-existent in the biological systems. However, various adult/somatic stem cells expressing the pluripotency factors, especially Oct3/4 have now been discovered [1]. Somatic/adult tissues from which high Oct4 expressing cells have been isolated are human ovaries [2], dental pulp [3-6], periodontal tissue [7], neuro-endocrine tissue of prostate [8], peripheral blood [9], bone marrow [10], and amniotic membrane [11]. Bone marrow and peripheral blood harbors very small embryonic-like stem cells [9], whereas multipotent adult progenitor cells were obtained from the bone marrow [10]. Interestingly, most of the adult/mesenchymal stem cells do express low levels of Oct3/4 during early passages, with a gradual decline in the expression of this pluripotency marker [12, 13]. Also, overexpression of the pluripotency genes especially Oct4 and Sox2 provide an added advantage to the mesenchymal stem cells, in terms of, stemness, proliferation and differentiation [14]. A specific example is far-lineage differentiation of

mesenchymal stem cells, such as hepatogenic differentiation of Oct4/Sox2 over expressed adipose tissue stem cells [15]. However, dental pulp stem cells readily differentiate into non-mesenchymal lineages such as hepatocytes because of high levels of Oct4 expression unlike adipose tissue stem cells [16].

As dental stem cells are similar in immunogenic properties as bone marrow mesenchymal stem cells, and express only HLA-class I antigen and not MHC class II antigen, they are likely to elicit a feeble immune response. Hence, the uses of dental stem cells are justified for regenerative medicine. As an applied aspect of regenerative medicine, stemness, proliferation, differentiation and engraftment of dental stem cells can be enhanced by various methods such as using small molecules, growth factors, scaffolds and combinatorial approach.

Dental stem cells, a superior source of mesenchymal stem cells

Various sources of dental stem cells are the dental pulp of permanent teeth (dental pulp stem cells-DPSCs), exfoliated deciduous teeth, periodontal, apical papilla, dental follicle and jaw periosteum [17]. The plasticity of adult stem cells are largely governed by the stem cell niche it occurs and its developmental cues [18-20]. A subfraction of adult stem cells that appears at the top of the hierarchy of mesenchymal stem cells normally possess embryonic/pluripotent stem cell-like characteristics. One example of such a cell type is very small embryonic-like stem cells (VSELs) present in all adult tissues [9]. VSELs express the pluripotent stem cell markers like Oct4, Nanog, SSEA-1, and also the mesenchymal stem cell markers. Similar to VSELs, dental stem cells also exhibit pluripotency markers like Oct4, Nanog, Sox-2, and the MSC markers [3,4,7,21]. Hence, the upstream hierarchical location of dental stem cells and VSELs provide such cells an added advantage of being mesenchymal stem cells with pluripotent characteristics. With regards to, the development of dental stem cells, the pluripotency factors had shown an active involvement. Oct 4, Nanog, Sox-2 and Stat-3 play a major role during the cap and bell stages of early odontogenesis in a developing embryo [6].

Moreover, dental stem cells reportedly differentiate into various lineages like odontogenic, osteogenic, adipogenic, neuronal, pancreatic, hepatic and myogenic [22- 24, 16, 25- 29]. Neural crest origin of dental pulp stem cells, thus, make them attractive targets for neuronal differentiation. Most of the reported work on neuronal differentiation from dental stem cells have focussed on the in-vitro data. However, Király et al. [30] have shown successful integration of neuronally pre-differentiated DPSCs into rat brain. The transplanted DPSCs not only integrated into the host brain, but also exhibited the expression of early neuronal marker N-tubulin, the neuronal specific intermediate filament protein NF-M, the postmitotic neuronal marker NeuN, and glial GFAP. The cells also displayed functional properties such as tetrodotoxin (TTX) sensitive voltage dependent (VD) sodium currents (I(Na)) and TEA sensitive delayed rectifier potassium currents (K(DR) [30] . Also, availability and proliferation of dental stem cells are reportedly greater than BM-MSCs [31]. Hence, due to the influence of pluripotency factors during odontogenesis in a developing embryo DSCs have acquired a greater plasticity, and can be harnessed as a potential source in regenerative medicine.

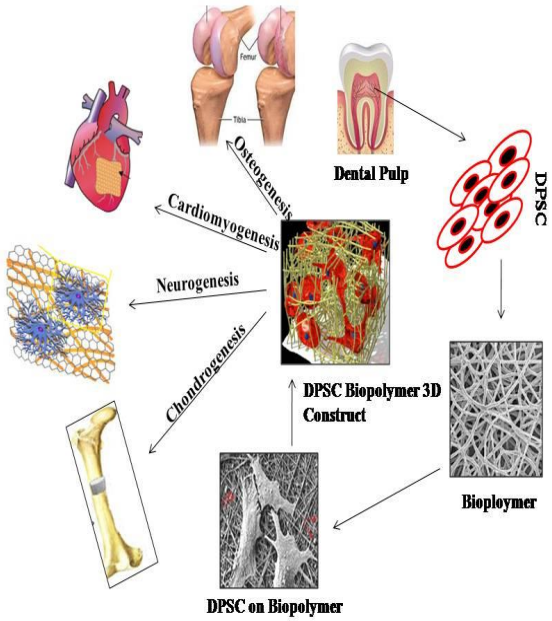
Dental stem cells and Cell Therapy-Present scenario and perspectives

Dental stem cells are multifactorial and multipotent in nature; can be easily obtained from exfoliated or extracted teeth and cultured. Furthermore, these cells exhibit high proliferation rate, multi-differentiation ability, easy accessibility, high viability and easy to be induced to distinct cell lineages. Currently, stem cells are being used in modern medicine for various degenerative diseases such as myocardial infarction, diabetes mellitus, arthritis, spinal cord and Alzheimer's disease [32]. Preclinical assessment of the dental stem cells for tissue engineering applications has also been carried out. Defects of dental origin are yet to be treated using dental pulp stem cells. The ability of dental pulp to regenerate dentin is an old phenomenon; furthermore dentists have also used calcium phosphate and calcium hydroxide that can induce progenitor cells in the pulp to differentiate into odontoblasts. Dental pulp stem cells can create a new revolution in treating dental defects in future [33, 34]. Dental pulp stem cells have also been used for corneal reconstruction [35], myocardial infarction [36], role in treatment for ischemia [37], skeletal muscle regeneration [38], neural regeneration [39], hepatocyte differentiation [16, 40], and differentiation into bone cells [41, 42]. Other reported research using DSCs are for the treatment of infertility [43] and type1 diabetes [44, 45].

Some of the possible applications of DPSCs in combination with biomaterials for tissue engineering purposes are depicted in Figure.1

A dentist can now very well manage periodontal diseases by using stem cell and tissue engineering technology [46, 47]. However, making a new artificial tooth and develop human dental pulp in the laboratory by this technology is a challenge for scientists working in the field dental regenerative therapies. These new strategies provide evidence suggesting that it might be feasible to restore viability in a necrotic young permanent tooth by engineering a new dental pulp. The potential of such therapies is immense and may allow for the completion of the tooth structure by biological regeneration in near future [48]. However, although numerous breakthroughs in stem cell research have been made thus far, their success and

application in clinical trials remains to be ascertained. Strong research into the basic stem cell biology must be performed before scientists move into the clinical trials.



Improving the plasticity of dental stem cells-role of small molecules

As the adult stem cells are prone to undergo a loss of stemness via loss of Oct3/4 during late passages of culturing [12, 13], it is important to preserve their stemness. Also, with regards to dental pulp stem cells, Liu et al. [4] reported the highest expression of Oct4 at passage-2 in primary cultures dental pulp stem cells. Oct4 expression in DPSCs also declined gradually in the later passages [4]. To, obtain a large number of DSCs for cell therapy applications, long-term passaging is mandatory. Hence, the preservation of stemness in DSC becomes essential. DPSCs when treated with small molecules like Pluripotin (SC1), 6-bromoindirubin-3-oxime and rapamycin reportedly preserved the expression of pluripotency markers such as Oct4, Nanog, Sox2 and STRO during later passages of culturing [49]. Indeed, the maintenance of stemness in DPSCs was associated with the decline in the differential potential of the cells into odonto/osteo, adipogenic and neurogenic lineages [49]. All of these small molecules are facilitated stemness via inhibition of major cell signaling pathways [49]. For example, pluripontin (SC1) inhibited RAS-GAP functions via activation of RAS thereby leading to an enhanced activation/phosphorylation of P70S6K (p-P70S6K) of PI3K pathway. Pluripontin was also reported to be helping in maintaining stemness via inhibition of phospho-ERK1 (p44) [49]. Various small molecule inhibitors such as phospho ERK1 inhibitor (S)-14k [50], Mitogen-activated protein/ERK kinase (MEK) inhibitor PD0325901 and glycogen synthase kinase-3 (GSK3) inhibitor CHIR99021 [51] can also be explored for enhancing/preserving the Oct4 expression/ stemness of DSC over several passages. Use of pluripontin to maintain the stemness of DPSCs was inspired by the role of the same in maintaining the pluripotency of mESC in the absence of leukemia inhibitory factor (LIF), feeder cells and BMP4 [52].

Various signaling molecules have been targeted in pluripotent stem cells for maintaining their stemness. The same strategies can also be explored for maintaining the stemness of DSCs. For example, modulation Wnt, ERK, PI3K, GSK3β, LIF-STAT pathways using small molecules in DSCs can be explored, with regards to, the maintenance of stemness. As increased expression of pluripotency markers have been reported with decreased differentiation potential of the cells into odonto/osteo, adipogenic and neurogenic lineage in DPSC [49], further modulations can be achieved by preserving the stemness, as well as, lineage specific propensity. Indeed, there is a great scope for exhaustive screening studies on DSCs using various small-molecule modulators of signaling pathways for maintaining their stemness.

Improving the plasticity of dental stem cells-role of growth factors

Alternate or parallel strategies need to be adopted for maintaining the regenerative potential of DSCs. The role of various growth factors in maintaining the pluripotency and cell fate specifications of embryonic stem cells have been extensively worked out [53-57]. The primary role of growth factors under in-vitro conditions is to either maintain the cell potency or drive the cells into the specific lineage, as desired by the researchers. Growths factors indeed mimic in-vivo like the environment and have been worked out by following the developmental biology. Culturing of DPSCs using various sera conditions for optimal stemness and differentiation have been reported by [21, 58]. Also, isolation methodologies and culture media conditions have been assessed for the stemness and differentiation potential of DPSC [59]. In bone marrow mesenchymal stem cells, certain growth factors such as vascular endothelial growth factor have been used for modulating adipogenesis and osteogenesis [60]. Also in mesenchymal stem cells, inhibition of platelet-derived growth factor has been proven to enhance the stem cell markers Oct4 and Nanog [61]. Use of 5μg/ml of basic Fibroblast growth factor (bFGF) had also resulted in the proliferation and increased stemness (high Oct 4, Nanog, Sox2 and Rex1) in dental stem cells from apical papilla [62]. The short-term treatment of DPSCs with tumor necrosis factor α (TNFα) reportedly increased the expression of pluripotent stem cell markers such as Oct4, Nanog, and SSEA-4 [63]. As DSCs exhibit high levels of pluripotency markers similar to pluripotent stem cells, there remains a vast scope for studying the effects of growth factors in DPSCs that are already established for stemness and lineage commitment in PSCs.

Improving the applicability of dental stem cells –role of scaffolds

The scaffold is a three-dimensional biodegradable porous polymer that serves as a potential support to facilitate delivery of stem cells at a local site. It provides a matrix for cell adhesion and growth; nutrients can be embedded inside the scaffold to promote cell survival. For a material to qualify as a scaffold should have following characteristics such as biodegradability, biocompatibility, malleability, porosity, good physical and mechanical strengths and support vascularity.

Investigators have used DPSCs along with biocompatible three-dimensional spongy materials to assess their differentiation capability for tissue engineering applications. Several polymers like Polylactic acid (PLA), Poly glycolic acid (PGA), Poly-lactic-co-glycolic acid (PLGA) and Polycaprolactone (PCL) reportedly are effective scaffolds for the growth of dental pulp stem cells [64]. PGA Poly (vinyl alcohol) (PVA) was the first synthetic biomaterial used in both implantable and non-implantable medical devices (e.g., contact lenses, artificial meniscus). Important properties of PVA such as low protein adsorption, biocompatibility, high hydrophilicity, easy processability and chemical inertia have promoted its applicability as an excellent synthetic biomaterial [65]. Additionally, PVA can be easily combined with collagen or gelatin to obtain bioartificial scaffold with specific architectural features [66, 67]. These biocomposite hybrid scaffolds promote cell adhesion, proliferation, migration, and differentiation. Spongy hybrid scaffolds like PVA and Gelatin has been successfully tested with gingival fibroblasts, being reported as very promising substrates for tissue engineering [68]. Collagen scaffolds have been popularly used in regenerative studies because of similarities to natural tissues, a combination of polymer and collagen showed significantly more DPSC growth and survival when compared to calcium phosphate and polymer scaffold. [69]. Graphene oxide (GO), Silk fibroin or both in combination with gelatin or hydroxyapatite are extensively studied scaffolds. Such scaffolds have beneficial effects on cell proliferation and differentiation, thus holding promise for bone tissue engineering (BTE). The studied chitosan-based materials, enriched with GO in different proportions, appear to be ideally designed for BTE applications in terms of biocompatibility and properties to promote and support cell growth and proliferation [70, 71]. Some of the extensively used scaffolds are listed in Table 1. Finally, nanomedicine is emerging as a powerful source of innovative cell-targeted-therapies based on nanoengineered materials, whose toxicological risks have yet to be disclosed.

Type of Biomaterial	Application	References
Polymer based Biomaterial	**Biodegradable scaffolds**	
Polyglycolic Acid (PGA)	Tendon, Ligament, Cartilage,	72,73,74,75
poly(lactic-co-glycolic acid) (PLGA)	Adipose, Bone, Cartilage, Muscle	76,77,78,79
poly-D, L-lactic acid (PDLLA)	Cartilage, Nerve	80, 81
Poly(ε-caprolactone) (PCL)	Bone, Cartilage	82, 83
	Non-biodegradable scaffold	
Polymethyl methacrylate (PMMA)	Bone, Cartilage, Dermis, Nerve	84, 85, 86, 87
Polytetrafluoroethylene (PTFE),	Vasculature, Adipose, Cartilage	88, 89, 90
Polydimethylsiloxane (PDMS)	Heart, Bone, Liver, Muscle	91, 92, 93, 94
	Synthetic scaffold	
Polyethylene glycol (PEG)	Nerve, Cardiac, Bone, Liver, Cartilage,	95, 96, 97, 98, 99, 100
	Naturally derived scaffolds	
Alginate	Bone, Cartilage, Dentin	101, 102, 103, 104
Agarose	Bone, Cartilage, Nerve, cornea	105, 106, 107
Chitosan	Skin, Heart, Intestine, CNS	108, 109
Collagen, fibrin, gelatin, hyaluronic acid, Silk and pectin	Cartilage, Bone, Liver, Nerve, vasculature, Dentin, enamel	110, 111, 112, 113, 114, 115, 116, 117, 118, 119
	Bioceramics and Metals	
Titanium/HA	Bone, Dentin, Cartilage	120, 121
tricalcium phosphate	Bone, Cartilage	122, 123

Combinatorial approach for enhancing the regenerative potential of dental stem cells

Translational applications of dental pulp stem cells are beyond just the in vitro manipulations to obtain desired morphologies, marker expression, and lineage differentiation. Rather, as tissue replacement for regenerative medicine, a combinatori-

al approach using scaffolds, growth factors, small molecules, co-culture of DSCs with desired cell types should be incorporated for transplantation. The idea of such a combinatorial approach is to create a 3D microenvironment that replicates in vivo conditions. Various efforts have been taken to create such a 3D microenvironment for DSC transplantations. For example, 3D alginate based microenvironments replicating the shape of gutta-percha that comprises elements of progenitor cells and release of growth factors have been fabricated [124]. DPSCs and human umbilical vein endothelial cells (huVEC) in a ratio of 1:1 were encapsulated by Bhoj et al. [124] in an alginate hydrogel. huVEC cells delivered the growth factors, endothelial growth factor 121 and fibroblast growth factor responsible for the proliferation of DPSCs. Also, the alginate scaffold served as a controlled 3D environment for DPSCs. For regeneration of periodontal tissues, human umbilical cord stem cells, along with growth factor supplementation were applied to root surface scaffolds for the generation of periodontal tissues in vitro [125]. Similarly, DSCs, especially periodontal ligament stem cells from allogeneic sources need to be explored, along with, growth factor supplementation on root surface scaffolds for the generation of periodontal tissues. The combinatorial approach using DPSCs, silk scaffolds and supplementation of the growth factor b-FGF has provided success in generating pulp-like tissues for endodontics for salvaging tooth loss [126].

The combinatorial strategies involving dental pulp regeneration for regenerative endodontics have been extensively researched [125-127]. Also, such combinatorial approaches using DSCs have been studied for making mineralized tissues like bones and cartilages [128, 129]. However, there is a great scope to work on the combinatorial strategies for maintaining the stemness of DSC, as well as, differentiating the DSCs. Desired cell types can thus be obtained by exerting various levels of controls such as alteration of growth factors and types of scaffolds.

Conclusion

Expression of pluripotency markers makes the dental stem cells highly plastic, as compared to their mesenchymal stem cell counterparts. Hence, DSCs can be explored further for their propensity to differentiate into various lineages. As DSCs have been successfully cultured under 3D scaffolds for regenerative medicine applications for endodontics and mineralized tissues, these can further be explored for regenerative applications for neuronal, cardiac disorders and, also in diabetes. Most important, combinatorial approaches using scaffolds, growth factors, small molecules and co-culture with other cell types can be used for regulating the stemness and differentiation of DSCs into desired lineages.

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