

Review Article

Tissue Engineering With Dental Pulp Stem Cells

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Abstract

Mesenchymal stem cell (MSCs) research has played a significant role in field of medicine and dentistry. MSCs of dental origin can be harvested from both deciduous and permanent tooth. These cells can be differentiated into other cell varieties, such as odontoblasts, osteoblasts, chondrocytes, adipocytes, endothelial, neuronal and melanocytes making it a perfect cell source for regeneration of various tissues. These magical cells can be used to treat Parkinson's disease, Myocardial infarction, and Alzheimer's disease etc. It can also provide an attractive solution for hair, bone or tooth loss as well.

Methods: We systematically searched and reviewed articles until July 2020, available on PubMed, Google Scholar and WHO database. Key word used to search the required topic was "dental pulp stem cells", "differentiation potential," "cryopreservation". Suitable full-text articles were selected and data were extracted.

Conclusion: This review defines innovative discoveries in the area of dental pulp stem cell research and their possible application in the tissue regeneration.

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Introduction

Dental Stem Cells (DCS)

In humans, tooth is a complex, rigid, calcified organ in both upper and lower jaws¹. It comprises of both hard and soft tissues that is, enamel, dentin, pulp, cementum and periodontal ligament. Initially, each tooth comprises of three parts, which are enamel organ, dental papilla and dental follicle. Ectodermal cells of Enamel organ produces enamel. Ectomesenchymal cells inside dental papilla are accountable for the synthesis of dentine and pulp tissue. Dental follicle contributes to formation of periodontal ligament, alveolar bone and cementum. Several mesenchymal stem cell (MSCs) populations are present in the tooth called dental stem cells. Dental stem cells

(DSCs) have been obtained from multiple locations within tooth, and are named according to the origin of its source. Human stem cells from exfoliated deciduous teeth (SHED) first studied by Miura *et al* in 2003². Stem cells from apical papilla (SCAP) were discovered by Sonoyama *et al* in 2006³. Dental follicle precursor cells (DFPCs) by Morsczeck *et al* in 2005⁴ and Periodontal ligament stem cells (PDLSCs) discovered by Seo *et al* in 2004⁵. Dental pulp stem cells (DPSCs) were isolated first time in 2000 by Gronthos *et al*^{6,7}

Stem cells isolated from dental tissue comprises less than 1% of the whole stem cells population⁴. DSCs can be isolated from a person's deciduous or permanent teeth³, with extreme ease. These cells can be easily expanded and cultivated, and offer a promising

autologous source of cells for regeneration of not only the bone defects in dentoalveolar and craniofacial regions but also into other tissue types.

Tooth pulp is a part of dentine-pulp complex and this living connective tissue is made up of a non-hematopoietic cells or MSCs and compact fibrous tissue. Two diverse stem cell populations of DPSCs can be identified in pulp organ regarding origin of its source, which are neural crest cells (NCCs) derived and mesenchymal-derived⁴. In 2000 DPSCs were discovered by Gronthos *et al*⁷. All teeth whether primary (deciduous) teeth or secondary (permanent) teeth contains DPSCs. Pulp of wisdom teeth is an ideal source of DPSCs and these cells can be isolated by enzymatic digestion or outgrowth / explant method. Even a recent study showed that damaged pulp still contains stem cells that are competent enough to proliferate and differentiate into other varieties of cells³.

DPSCs, are considered as a variety of MSCs population, as it fulfills the criteria for mesenchymal stem cells (MSC) that is adhesion to plastic, appearance of precise surface antigen, and capacity for in vitro differentiation. Therefore DPSCs possess all markers that have been used in identification of MSCs, which are, Nanog, CD73, CD105, CD106, CD10, CD59, CD 117, CD150, CD13, CD24, CD166, CD29, CD44, CD146, Oct4, CD90, and $\beta 2$ integrin and are negative to HLA-DR, CD14, CD45 and CD34.⁸ It also shows two very early mesenchymal stem cells markers i-e STRO-1, CD146 as well Msx-1, CD31 and vimentin⁹. Its primitive nature is established by existence of pluripotent embryonic stem cell marker Sox-2. Even in one study embryonic markers for stem cells were still expressed in DPSC from 58 year old patient DPSCs¹⁰. TGF β 1, and bone morphogenic proteins BMP-2, are engaged in proliferation and differentiation of these cells¹. Data collected from different studies have proved that DPSCs have the capacity to differentiate into adipogenic, chondrogenic, myogenic, and neuron-glial cell lines¹⁰.

DPSC, when compared to bone marrow stromal cells, which is the known source of osteoblast, display similar expressions for markers, related with endothelium, smooth muscle, bone and fibroblast. DPSC also shows ectodermal markers, i-e ncam-1, β

3 tubulin and nestin, mesodermal markers hand1, bmp4 and gapdh but does not show endodermal marker¹¹.

Methods

An electronic, customized search of scientific articles was conducted using the PubMed/ MEDLINE and Google Scholar databases. We systematically searched and reviewed articles until July 2020. The inclusion criteria were applied, and the articles that described the use of DPSC for various tissue regeneration were selected for complete evaluation. The articles were classified according to successful differentiation into various cell types with promising results. After the application of the eligibility criteria, a total of thirty two studies were chosen and fully analyzed. Key word used to search the required topic was 'mesenchymal stem cells', 'dental stem cells', 'human dental pulp stem cells', 'differentiation potential of DPSCs', 'tissue regeneration', 'uses', 'immunosuppressive property of DPSCs', 'cryopreservation', and 'banking of stem cells'. Suitable full-text articles were selected and data was extracted.

Teeth are an amusing source of MSCs and can act as a very important reservoir. DPSCs can be used for engineering of various tissues, under influence of differentiation specific supplements.¹²

Induced pluripotent stem cells (iPSCs) are created from human somatic cells under influence of Oct4, c-Myc, Klf4, Nanog, and Sox2 or Lin28¹³. These cells resemble human ESC, but unlike ESC, these cells are obtained from donor itself and in this way increasing chances of derivation of tissues that is histocompatible. (iPSCs) derivation from adult somatic cells have been extensively studied. Shinya Yamanaka and John Gurdon have won Noble prize award in year 2012 for their exceptional work regarding iPSCs. Cell types which have been reprogrammed for this purpose are Human dermal fibroblasts, mesenchymal stem cells (MSCs), neonatal foreskin fibroblast, and amniotic fluid-derived cells. Tooth source for iPSCs includes dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous teeth (SHED), and stem cells from apical papilla (SCAP). Kerkis and Caplan described that the derivation of iPSCs from DPSCs are significantly more efficient compared with that of human fibroblasts¹⁴.

It has been proved that one of the source of origin of DPSCs is Neural crest cells, so a pronounced deal of exploration has been carried on these cells for the fabrication of neural lineage. Even earlier to exposure with neural differentiation medium, DPSCs express the neural stem cell marker, nestin¹⁵. Under influence of FGF2, FGF8, glial cell-derived neurotrophic factor, and Shh homolog, DPSC displays affirmative expression of neural cell markers such as glial fibrillary acidic protein (GFAP), β III-tubulin, Ca²⁺/calmodulin-dependent protein kinases II (Cam kinase II), and tyrosine hydroxylase, in a formed neurospheres in neural differentiation culture medium, demonstrating DPSC has the capability to be differentiated into glial cells and neurons¹⁶.

DPSC in the presence of (TGF) β 1, unaccompanied or with FGF, can be differentiated into odontoblast. In experiment conducted on rabbits, DPSCs cultivated on a 3D scaffold after transplantation, developed into a mineralized assembly comprising of osteodentin. This mineralized tissue possessed conventional cell organization and blood vessels. In another study, bioengineered molar tooth germ in 3D was constructed in area of lost tooth by introducing a mixture of epithelial cell derived from molar tooth germ, DPSC, and collagen gel. These findings may perhaps recommend the imaginable possible use of DPSCs in constructing functional tooth structure¹⁷.

Undifferentiated stem cells plays important role in keeping the vitality of dental pulp. When DPSCs are introduced into pulp cavity, unaided or in mixture with BMP2, DMP1, and collagen scaffold, these cells encouraged reestablishment and renovation of dentine pulp like complex¹⁸. Vascularity of dentine pulp can be rejuvenated denovo in emptied root canal space by consuming SHED or DPSCs supported by VEGF. Latest studies have exposed that SCAP have the ability to undergo odontogenic differentiation expressing DSP, BSP, and ALP and when these cells were transplanted in vivo into 5-6 mm-long root canals, it resulted in production of vascularized pulp-like tissue¹⁹.

Periodontal ligament stem cells (PDLSCs) are found to express MSCs markers CD146/MUC18, CD105, CD166, and STRO-1 and tendon specific markers. A recent study suggested that PDLSC are an exceptional population, when transplanted into immunodeficient mice with either DPSCs or SCAP and

HA/TCP, these cells were able to produce a PDL/cementum-like assemblage and can contribute to PDL tissue repair²⁰.

Corneal blindness affects millions of individual and is presently cured by cadaveric tissue graft. DPSCs, after inducing differentiation in vitro under proper cultural conditions, expresses molecules characteristics of, keratocan, keratocytes, and keratin sulphate proteoglycans²¹. After injecting *in vivo* into mouse corneal stroma, DPSCs produces corneal stromal extracellular matrix, comprising of keratocan and human type 1 collagen. These cells did not induce immunological rejections after transplantation. There is a prospective for clinical use of DPSCs in tissue engineering treatments for corneal stromal blindness. In vivo, iDPSCs were capable of regenerating damaged corneal tissues in animal models. Two studies shows that SHED expresses markers similar to, corneal limbal stem cells²².

Myogenic potential of human DPSC has been investigated, by evaluating their differentiation into smooth and skeletal muscle cell phenotype in myogenic differentiation medium. For skeletal muscles, gene expression of human MyoD is spotted in the co-cultures, demonstrating that under influence of proper differentiating media, muscle-specific genes in DPSCs can be turned on. For differentiation into smooth muscle, cultures undergoing TGF- β treatment shows a high expression of smooth-muscle actin²³.

It has already been established by Stevens *et al*, the spontaneous differentiation of DPSCs into mature non-mesenchymal melanocytes with presence of melanocyte specific markers and melanosomes up to the final stage of maturation, provided that conditions are optimal for differentiation in a specific melanocyte differentiating medium²⁴.

Human dental pulp stromal cells (HDPSCs) are acknowledged to be extremely proliferative, multipotent, and has ability to fabricate a dentine-like regenerative tissue, or to produce mineralized nodules in monolayer cultures analogous to bone^{25,26}. Numerous researches have reported invitro osteoblastic/cementoblastic differentiation of DPSCs in osteogenic medium containing, ascorbic acid, b-glycerophosphate, and dexamethasone. High activity of alkaline phosphatase, osteocalcin (OCN), RUNX2,

type I collagen (COL I), bone sialoprotein (BSP) and osterix (OSX) was evident in these cultures. Various scaffolds are being introduced as a promising means for improving bone regeneration. Use of Silicate bioactive glasses, glass ceramics and composite scaffolds in bone tissue engineering is expanding. Recently DPSC was combined with PRP (Platelet rich Plasma) scaffolding and was compared with the osteogenic potential of other cell sources, including BMSCs and PSCs.²⁷

Alopecia can be treated by use of DPSCs. Human unfractionated DPSCs relocated into surgically deactivated hair follicles work together with follicle epithelium to redevelop new end bulbs and produce several differentiated hair fibers²⁸.

DPSCs cultured on nanopatterned PEG-GelMA-HA scaffolds exhibited a substantial inclination towards cartilage formation. Gene expression exploration of the DPSCs recognized the existence of chondrogenic marker such as alkaline phosphatase, Sox9, Procollagen type II, Aggrecan and Procollagen type X²⁹.

Several studies, indicating hDPSCs can be differentiated to adipocytes under appropriate environment and buildup of lipids in newly formed adipocytes can be evaluated by oil red O staining³⁰.

Discussion:

Two elementary methods for the isolation of DPSCs from tooth have been defined: the explant method and the enzymatic digestion of pulp tissue method. A chief advantage of DPSCs is that they can be isolated in a routine dental procedures such as the extraction of deciduous teeth or impacted wisdom teeth. The use of DPSCs can be very economical, less invasive, convenient, and safer with very low morbidity and no ethical issues. These cells can easily be cultivated and expanded for autologous as well as allogenic medical use. Further these cells are immunoprivilege, also when grafted into allogenic tissues exhibit anti-inflammatory abilities. Cell-to-cell contact and secreted soluble factors such as HLA-G, 3-dioxygenase (IDO), indoleamine 2, prostaglandin E2, nitric oxide (NO), TGF- β , interferon (IFN)- γ , and interleukin (IL)-1 β , all are responsible for the immunosuppressive property of DPSCs³¹. These cells can be used for the management of autoimmune

diseases such as rheumatoid arthritis, promoting transplant tolerance and patient survival. It's highly proficient interactivity with biomaterials makes it ultimate choice for tissue engineering as these cells can easily be differentiated into odontoblasts, chondrocytes, adipocytes, endothelial, neuronal and melanocytes in optimal environment.

Cells, tissues and organs can be stored at temperatures below -80°C , and generally as cold as -196°C to preserve their viability through a procedure termed as cryopreservation³. DPSCs can easily be cryopreserved, utilizing Papaccio method or Kamath method, as both are found to be extremely productive as documented by Munévar JC³² and defrosting is done by liquid nitrogen. DPSCs cells after cryopreservation showed high vitality and proliferation potential as shown in various studies. In this way we can make an extracted tooth useful by storing it for future therapies for various tissue regenerations.

Conclusion

A suitable combination of growth factors and scaffolds can improve the differentiation capability of DPSCs. Presence of DPSCs in all teeth whether deciduous or permanent, makes them readily reachable source throughout one's life period. This has headed to the creation of numerous dental cell banks around the world to preserve the extracted healthy tooth for future use. Many famous banks are already working on this matter. Store-a-Tooth Stem Cell Bank, Bio bank, UK Stem Cell Bank, and UK Bio bank are few examples. DPSC therapy is promising but, its practice in broad clinical trials is not yet optimized in all countries. Still, more research is needed to illuminate the source and number of passages of DPSCs that are best suited to induce tissue regeneration.

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