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RESEARCH ARTICLE

Dental Pulp Stem Cells (DPSCs): An Overview

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Abstract

Dental pulp stem cells (DPSCs) are a promising source of cells for numerous and varied regenerative medicine applications. Their natural function in the production of odontoblasts to create reparative dentin support applications in dentistry in the regeneration of tooth structures. 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. It is clear that a deep understanding of the mechanisms of stem cells, including their aging, self-renewal, micro environmental homeostasis, and differentiation correlated with cell activity, the energy for which is provided from mitochondria, should provide new approaches for DPSC research and therapeutics.

Keywords: Dental Pulp, Differentiation, Isolation, Stem cells

1 | INTRODUCTION

Stem cells are undifferentiated biological cells that can differerntiate into specialized cells and can divide (through mitosis) to produce extra stem cells. (1) It is the ancestor at the top of the family tree of related cell types. One blood stem cell gives rise to red cells, white cells and platelets. (2) Stem cells are the master cells of the body that meet the two conditions of self-replication and the ability to differentiate into at least two different type of cells. (3) Therapeutic application of stem cells has created an increasing interest in the study of undifferentiated cell types that constitute the ability to proliferate and differentiate into various tissues. (4)

In dentistry, interest in tissue engineering researches on different types of dental stem cells done in vivo and in vitro, increased rapidly among researchers and institutes. (5) Various types of tooth derived stem cells have been utilized in the field of regeneration medicine. (6)

1.1 | The different types of Dental Stem Cells are:

Dental Pulp Stem Cells (DPSCs), Stem Cells from Human Exfoliated Deciduous Teeth (SHEDs), Periodontal Ligament Stem Cells (PDLSCs), Stem Cells from Apical Papilla (SCAP), Dental Follicle Progenitor Cells (DFPCs). (1)

In this review article, we will discuss in detail about the Dental Pulp Stem Cells (DPSCs).

2 | DISCUSSION

Mineralized tissue that has a great deal of similarity to bone is dentin. Although dentin does not turn over throughout life, as bone does, limited dentinal repair in the postnatal organism does occur. It was postulated that the ability for limited repair is maintained by a precursor population, associated with pulp tissue, that has the ability to mature into odontoblasts. Clonogenic and highly proliferative cells have been derived from enzymatically disaggregated adult human dental pulp, which have been termed dental pulp stem cells (DPSCs), that form sporadic, but densely calcified nodules in vitro. (3) When DPSCs were transplanted with hydroxyapetite/tricalcium phosphate into immuno compromised mice they generated a dentin-like structure with collagen fibers running perpendicular to the mineralizing surface as is found in vivo, and contained the dentin-enriched protein, dentin sialo-phosphoprotein. The newly formed dentin was lined with human odontoblast like cells that extended long cellular processes into the mineralized matrix, and surrounded an interstitial tissue i.e. reminiscent of pulp in vivo with respect to the organization of the vasculature and connective tissue. In contrast to BMSCs, DPSCs did not support the establishment of a hematopoietic marrow or adipocytes, elements that are also absent in dental pulp tissue in vivo. (3, 7)

DPSCs can be differentiated by modulation with growth factors, transcriptional factors, extracellular matrix proteins and receptor molecules into different cell types include odontoblast, osteoblast, chondrocyte, cardiomyocytes, neuron cells, adipocyte, corneal epithelial cell, melanoma cell and insulin secreting Beta cells. DPSC remain quiescent within the dental pulps, but respond quickly after injury. DPSCs have high proliferative capacity and immediately differentiate into odontoblasts, osteoblasts and chondrocytes to produce dentin, bone and cartilage tissues respectively for this repair process. (8)

By immunophenotyping, the DPSCs are virtually identical to BMSCs, yet each population produces quiet different mineralized matrices. To identify possible differences between these two populations, their gene expression profiles were characterized using a commercially available microarray. Human DPSCs and BMSCs were found to have a similar level of gene expression for more than 4,000 known genes represented on the filter. A few differentially expressed genes including collagen type VIII alpha

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1, insulin-like growth factor 2, discordin domain tyrosine kinase 2, NAD(P)H menadione oxidoreductase, homolog 2 of Drosophila large disk, and cyclin dependent kinase 6 were highly expressed in DPSCs, while insulin-like growth factor binding protein 7 and collagen type I alpha 2 were more highly expressed in BMSCs. This characterize the functional roles of the differentially expressed genes in the development of dentin and bone. (7, 9)

2.1 | Isolation of DPSCs

Based on many experiments and investigations, isolation of stem cells from human dental pulp could be done by several methods. Some methods are discussed below:

(1) Raoof et al 2014 described 3 different methods of DPSCs from dental pulp tissue.

(a) Dental pulp tissue is digested with collagenase or dispase enzyme and isolated trypsinised cells are plateed in culture dishes;

(b) Explanted undigested dental pulp small tissue pieces directly to petridishes;

(c) Dental pulp tissues are initially trypsinised and then small tissue pieces are explanted to petridishes for their outgrowth.

These cultures have grown in Minimum Essential Medium (MEM) supplemented with 20% Fetal Bovine Serum (FBS) at 37°C with 5% CO_2 and 90% humidity in CO_2 incubator.

Among three methods, third method gives better cell outgrowth with achieving confluency at 60% within 2 days of culture. (8, 10)

(2) Lindemann et al 2014 isolated dental pulp cells from 7 days old non-cryopreserved and cryopreserved human deciduous teeth and culture them simultaneously.

No change in differentiating and immuno phenotype properties of both these cells were observed. But there is a change in the morphology, proliferative capacity of cryopreserved cells than non-cryopreserved cells. (8, 11)

(3) Lin et al 2014 isolated DPSCs from extracted teeth are frozen and then stored at -196°C for 24 hours. During freezing, the cells are suspended in freezing media containing 10% dimethylsulfoxide (DMSO).

Survival rated of revived DPSCs increase by 2 to 2.5 folds when the freezing medium is DMSO free. (8)

DPSCs isolated without laser piercing have significant loss of cell viability and proliferation rate as compared to teeth cryopreserved by laser piercing. (8)

2.2 | Stem Cell Handling and Cryopreservation

Extracted permanent and deciduous teeth can be preserved for future use with cryopreservation. The cells rapidly cooled to subzero temperatures as low as -196°, stopping any cellular or biochemical activity. Rapid freezing prevents ice from forming around or inside the cells and to prevent dehydration, as these would cause damage and death. (12) Stem cells derived from dental pulp of extracted third molars retain the ability to differentiate into multiple cell types following thawing after cryopreservation. After two years of cryopreservation, stem cells have been able to differentiate and proliferate. (13)

2.3 | Differentiation of DPSC

DPSC represent rapidly proliferating cell population that readily differentiates into osteoblastic, neural, myocytic and hepatocytic lineages. This suggests that DPSC may have a more broad therapeutic application than lineage-restricted adult stem cell population. DPSCs differentiate into different kinds of cells and tissues and their multi-potency has been compared to those of Bone Marrow Stem Cells (BMSCs). Proliferation, availability and cell number of DPSCs are greater than BMSC. (14)

2.4 | Differentiation Markers

Leukemia Inhibitory Factor (LIF) is involved in the induction of hematopoietic differentiation in normal and myeloid leukemia cells. It plays a role in immune tolerance at the maternal fetal interface. LIF has the ability to induce the terminal differentiation of myeloid leukemic cells. The other differentiating marker is a Keratin 18. It is a Keratin Associated-

Protein (KAP) which forms a matrix of keratin intermediate filaments of cell cytoskeleton structure. Keratin 8, Keratin 18 and Keratin 19 are used as a marker for epithelial cells and differentiate from hematopoietic cells. (15)

2.5 | Clinical Application of DPSC

Stem cell based therapies are being investigated for the treatment of many conditions including neurodegenerative conditions such as Parkinson's disease and Multiple sclerosis, liver disease, diabetes, cardiovascular disease, autoimmune diseases, musculoskeletal disorders and for nerve regeneration following brain or spinal cord injury. Currently, patients are being treated using stem cells for bone fractures, cancer and spinal fusion surgery. The diseases and conditions currently being treated using DPSCs include Acute and chronic leukemias, Myeloproliferative disorders, Myelodysplastic syndromes, Lymphoproliferative disorders, Inherited Erythrocyte Abnormalities, Liposomal storage diseases, Histiocytic disorders, Phagocytic disorders, Congenital immune system disorders, Plasma cell disorders and malignancies. (13)

The applications related to oral health care included regeneration of an immature tooth with extensive coronal and pulp damage, regeneration of resorbed roots, cervical or apical dentin, whole tooth regeneration, repair and replacement of bone in craniofacial defects can facilitate restoring the physiologic structural integrity. (13)

The regeneration of orodental tissues is dependent on four basic components. The appropriate signals, cells, blood supply and scaffold that are needed to target the tissue at the site of defect. All these components are essential for reconstruction and healing of lost tissues. The cells provide the machinery for new tissue growth and differentiation, whereas the growth factors modulate the cellular activity and stimulate the cells to differentiate as well as produce tissue matrix. The new vascular tissues provide the nutritional base for tissue growth and the scaffolds guide and create a template structure in three- dimension to facilitate the tissue regeneration process. (16)

Suitable stem cells for tissue engineering should be able to differentiate into the target tissue/organ and should be easily collected and prepared to provide a further benefit to ensure the safety of the patient. DPSCs hold great clinical procedure due to their differentiation capacity and easy accessibility. (8)

1. De novo pulp regeneration: When the entire pulp tissue is lost, regeneration requires the de novo creation of pulp. To create functional pulp for clinical application, three issues must be considered: first, regenerated pulp tissue must be vascularised; second, newly differentiated odontoblasts should form on the existing dentinal wall of the root canal space; finally, new dentin must be produced by differentiated odontoblasts on the existing dentin. (17)

2. Tooth reconstruction: It may be possible to generate a method to biologically replace lost teeth with the help of stem cells. A functional biological replacement tooth must include generation of a root and periodontal ligament with nerve and blood supplies. The crown, is less important since replacement of crowns with synthetic functioning is possible. (18)

3. Neurology: Dental pulp cells have been proposed as a treatment for peripheral nerve injury. The dental pulp cells form blood vessels and myelinating tissue and contributed to the promotion of normal nerve regeneration. A degradable poly (dl-lactide-co-glycolide) (PGLA) tube was developed to avoid the need for a potential second operation to remove the silicon tube. (19)

4. Angiogenesis and Vasculogenesis: Stem cells and endothelial progenitor cells (EPCs) can be utilized to stimulate vasculogenesis as a potential treatment for ischaemic disease. Therapeutic benefits of injection of bone marrow or adipose derived MSCs after myocardial infarction (MI) and other heart diseases have also been reported. DPSCs and sub-fractions of DPSCs also serve as mode of treatment for myocardial infarction and ischaemia. (20)

5. Endocrinology: DPSCs have been differentiated to produce Hepatocyte like cells (HLCs) with acquired hepatocyte functions, such as glycogen storage and urea production. Differentiated cells possessed a polygonal shape and normal karyotype and expressed hepatic metabolic function genes and liver-specific genes. Glycogen storage and urea production results indicated that the differentiated DPSCs were functionally close to normal hepatocyte -

like cells (HLCs). The use of cryopreserved tissue to generate HLCs provides a promising alternative for the treatment of liver diseases. (21)

2.6 | Tooth Stem Cell Banking

Although tooth banking is currently not very popular the trend is gaining acceptance mainly in the developed countries. BioEden (Austin, Texas, USA), has international laboratories in UK (serving Europe) and Thailand (serving South East Asia) with global expansion plans. Stem cell banking companies like Store-A-Tooth (Provia Laboratories, Littleton, Massachusetts, USA) and StemSave (Stemsave Inc, New York, USA) are also expanding their horizon internationally. In Japan, the first tooth bank was established in Hiroshima University and the company was named as "Three Brackets" (Suri Buraketto) in 2005. Nagoya University (Kyodo, Japan) also came up with a tooth bank in 2007. Taipei Medical University in collaboration with Hiroshima University opened the nation's first tooth bank in September, 2008. The Norwegian Tooth Bank (a collaborative project between the Norwegian Institute of Public Health and the University of Bergen) set up in 2008 is collecting exfoliated primary teeth from 1,00,000 children in Norway. Not last but the least, Stemade introduced the concept of dental stem cells banking in India recently by launching its operations in Mumbai and Delhi. (22)

2.7 | Future Prospectives

Researchers have observed promising results in several preclinical animal studies and numerous clinical trials are now on-going globally to further validate these findings. The Obama administration has made stem cell research one of the pillars of his health program. The U.S. Army is investing over $250 million in stem cell research to treat injured soldiers in a project called Armed Forces Institute for Regenerative Medicine. It is likely that the next stem cell advance is the availability of regenerative dental kits, which will enable the dentists the ability to deliver stem cell therapies locally as part of routine dental practice. An innovative method that holds promising future is to generate induced stem cells from harvested human dental stem cells. This approach reprograms dental stem cells into an embryonic state, thus expanding their potential to differentiate into a much wider range of tissue types. Researchers have so far succeeded in making specific dental tissues or tooth like structures although in animal studies but future advances in dental stem cell research will be the regeneration of functional tooth in humans. (22, 23)

3 | CONCLUSION

DPSCs are useful in the treatment of various diseases. They have great potential and is a very powerful tool in regenerative medicine. They can be obtained safely and easily without significant morbidity or ethical concerns; however, the challenge of understanding the mechanisms underlying the therapeutic effects of DPSCs requires more research. The future treatment modality will be regenerative based; however, further studies are needed to test the various other applications of DPSCs with long-term follow-up.

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