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Review Article

Therapeutic potential of dental pulp stem cells in regenerative medicine: An overview

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ABSTRACT

The purpose of this review is to gain an overview of the applications of the dental pulp stem cells (DPSCs) in the treatment of various medical diseases. Stem cells have the capacity to differentiate and regenerate into various tissues. DPSCs are the adult stem cells that reside in the cell rich zone of the dental pulp. These are the multipotent cells that can be explained by their embryonic origin from the neural crest. Owing to this multipotency, these DPSCs can be used in both dental and medical applications. A review of literature has been performed using electronic and hand-searching methods for the medical applications of DPSCs. On the basis of the available information, DPSCs appear to be a promising alternative for the regeneration of tissues and treatment of various diseases, although, long-term clinical trials and studies are needed to confirm their efficacy.

Key Words: Alzheimer's disease, dental pulp stem cells, myocardial infarction, neurogenic differentiation, tissue differentiation

INTRODUCTION

Stem cells are defined as clonogenic, self-renewing, progenitor cells that can generate one or more specialized cell types and are one of the key elements of the tissue engineering triad, the other two being the growth factors/signals and scaffold or extracellular matrix.[1] Stem cells have the potential to renew themselves for long periods through cell division, and under certain physiological or experimental conditions, they can be induced to become cells with special functions.[2] They are broadly classified into *embryonic*, located within the inner cell mass of the blastocyst stage of the development, and *adult* stem cells.[3] Adult stem cells have been isolated from various tissues and fluids such as bone marrow, peripheral blood, Wharton jelly, placenta, amniotic fluid and membrane, skeletal muscle, central nervous system, olfactory bulb, retina, and liver. From these sources progenitors of mesenchymal-epithelial, neural, endothelial, hematopoietic, neural, epithelial, and trophoblastic lineages have been identified.[4,5] Mesenchymal stem cells are most widely used in tissue engineering, as they can be obtained from a wide variety of sources, they have the ability to self-renew, and also have multilineage potential following adequate induction.[6]

In recent times, the mesenchymal stem cell populations having high proliferative capacity and multilineage differentiation have been isolated from dental tissues. These are dental pulp stem cells (DPSCs),[7] stem cells from human exfoliated deciduous teeth (SHEDs),[8] periodontal ligament stem cells (PDLSCs),[9] dental follicle progenitor stem cells (DFPCs),[10] and stem cells from apical papilla

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(SCAPs).[11] DPSCs and SHEDs originate from the cranial neural crest and express early markers for both mesenchymal and neuroectodermal stem cells.[7,8] This explains their multipotency and pluripotency. Sharpe and Young[12] were the pioneers in use of stem cells in dental tissue engineering. Various studies have shown that these cells have the unique features of stem/progenitor cells, having the capacity to differentiate the dentin forming odontoblasts.[13-15]

The roots of the third molar are often incomplete at the age of 18 years, therefore, these teeth contain a conspicuous pool of undifferentiated cells, resident within the 'cell-rich zone' of the dental germ pulp.[16] In an *in vitro* model, Hwang *et al*.[17] derived DPSCs from supernumerary mesiodens and it has been seen that DPSCs derived at the stage of crown development are more proliferative than at later stages.[18] Apart from this, the cells obtained from the loosely attached tissue at the root apex (SCAP) and periodontal ligament (PDLSC) have been used for bio-root engineering.[19,20] More recently, stem cells obtained from the dental tissues have been shown to develop into fat, bone cartilage, and neural cells.[21,22]

Besides being therapeutic in dentin regeneration, regeneration of periodontal tissues, and skeletoarticular tissues of the craniofacial region, DPSCs were also reported to be used in the treatment of neurotrauma, autoimmune diseases, myocardial infarction, muscular dystrophy, and connective tissue damages.[23] Simplicity of their procurement and easy availability make them a promising tool in regenerative medicine. Hence, this endeavor was undertaken to highlight and evaluate various medical applications of DPSCs.

MATERIALS AND METHODS

The related dental literature was searched for using the Medline / PubMed database and Google scholar, with an emphasis on peer-reviewed dental journals, until April 2012. The medical subject heading (MeSH) terms used were 'dental pulp stem cells', 'regeneration', 'medical applications,' and 'tissue engineering'. Pertinent articles on the topics and abstracts of relevant articles were scrutinized thoroughly, and finally articles pertaining to the clinical application were included. Relevant literature in common textbooks on tissue engineering and regenerative medicine, bibliographies of articles and review articles, together with appropriate peer-reviewed print journals were also analyzed for additional information.

THERAPEUTIC APPLICATIONS

The dental pulp stem cells are multipotent in nature, thus many cytotypes can be obtained from them. These can be easily obtained from exfoliated human teeth or after extraction of wisdom teeth and their collection can be made with very less tissue sacrifice. Stem cell therapy is being used already for various degenerative diseases such as Alzheimer's disease, myocardial infarction, diabetes mellitus, bone defects, spinal cord injuries, and so on.

Cardiomyocyte differentiation of the dental pulp stem cells

Despite recent advances in its prevention and treatment, myocardial infarction (MI) remains one of the major causes of mortality. The treatment options based on stem cell therapy can provide a promising alternative to the conventional therapies for this life-threatening condition. In this regard, cardiomyocyte differentiation of DPSCs has been studied by different researchers.[24,25]

The capacity of the stem cells derived from the bone marrow stem cells (BMSCs), adipose tissue cells (ATSCs), and DPSCs, to differentiate cells with a cardiac phenotype, was evaluated by Arminan *et al*.[24] The result showed that tissue-specific mesenchymal stem cells (MSCs) could change into cardiomyocytes and support the potential use of MSCs in stem cell-based cardiac therapies. The expression of cardiac specific markers like Troponin-1, beta-myosin heavy chain, atrial natriuretic peptide, and alpha sarcomeric actinin was detected in BMSCs, ATSCs, and DPSCs. The therapeutic potential of DPSCs in the repair of myocardial infarction was also evaluated by Gandia *et al*.,[25] who concluded that human DPSCs secrete multiple proangiogenic apoptotic factors. The cardiac function improved in the cell-treated animals at four weeks, as shown by the percentage of change in the anterior wall thickening, left ventricular fractional area change, reduction in the infarct size, and increased angiogenesis.[25] The angiogenesis was also increased relative to the control-treated animals.

Differentiation into muscular tissue

Various studies evaluated the myogenic potential of DPSCs. Kerkis *et al*.[26] used human DPSCs for the treatment of muscular dystrophy in golden retriever dogs, transplanted by arterial or muscular injections. The samples from the biopsies were checked by immunochemistry (dystrophin markers) and the

researchers showed that DPSC presented significant engraftment in dog muscles.

Their potential to differentiate into dystrophin-producing, multinucleated muscle cells can be utilized in disorders such as muscular dystrophy, wherein, the body is unable to produce dystrophin. Utilization of myogenic progenitor cells derived from dental pulp produced more dystrophin as compared to the heterogeneously present stem cells.[27] Thus, DPSCs can prove to be a potential alternative for stem cell therapy in muscular dystrophy patients.

Corneal reconstruction with dental pulp stem cells

Attempts have been made for corneal reconstruction with the help of human DPSCs. In an animal model by Gomes *et al.*,[28]a tissue-engineered DPSC sheet was transplanted on the corneal bed and then covered with de-epithelialized human amniotic membrane. After three months, it was confirmed by histological analysis that healthy uniform corneal epithelium was formed. It was concluded that a tissue-engineered DPSC sheet was successful in the reconstruction of corneal epithelium.

Vasculogenic differentiation of dental pulp stem cells (treatment of ischemia)

A highly vasculogenic subpopulation of DPSCs, similar to endothelial progenitor cells, has been isolated from the dental pulp by Iohora *et al.*[29] These cells showed high proliferation and migration activities, multilineage differentiation, and vasculogenic potential. It resulted in successful engraftment and an increase in the blood flow, including a high density of capillary formation in a rat model with hind limb ischemia. The transplanted cells were in the proximity of the newly formed vasculature and expressed several pro-angiogenic factors such as the vascular endothelial growth factor – A (VEGF-A).

Hepatocyte differentiation of dental pulp stem cells

DPSCs could be differentiated into cells with morphological, phenotypic and functional characteristics of hepatocytes. Ishkitieve *et al.*[30] isolated stem cells from the dental pulp and established that DPSCs have the capacity to differentiate into a hepatic lineage.

Neural differentiation of dental pulp stem cells

Apel *et al.*[31] investigated the neuroprotective effect of DPSCs in the *in vitro* models of Alzheimer's and Parkinson's disease. They isolated the DPSCs from adult rat incisors and these were added to the neuron cultures two days prior to the neurotoxin treatment. It was seen that DPSCs expressed a neuronal phenotype and produced neurotrophic factors like NGF (nerve growth factor), GDNF (Glial cell-derived neurotrophic factor), BDNF (Brain-derived neurotrophic factor), and BMP2. Also, DPSCs protected the primary neurons and helped in cell viability.

It has been demonstrated that DPSCs are capable of stimulating long-term regeneration of nerves in the damaged spinal cord.[32] In an experiment, the DPSCs were transplanted into rats with completely severed spinal cords. It was demonstrated that DPSCs promoted the regeneration of transected axons by directly inhibiting multiple axon growth inhibitors and by preventing the apoptosis of neurons, astrocytes, and oligodendrocytes. The DPSCs also differentiated into mature oligodendrocytes to replace cells that were lost. It was found that SHEDs and DPSCs expressed several neural lineage markers. When compared to BMSCs, DPSC-implanted rats showed improved recovery soon after the operation, during the acute phase of spinal cord injury. de Almeida *et al.*[33] also supported this fact in their study, wherein, they investigated the effects of human dental pulp stem cells in a mouse model with compressive spinal cord injury. They concluded that the group with human DPSCs showed better white matter preservation, higher trophic factor expression, and better tissue organization, with a presence of many axons being myelinated by both Schwann cells and oligodendrocytes.

Kiraly *et al.*[34] transplanted DPSCs, which were predifferentiated and labeled with a vital cell dye, vibrant D, into the cerebrospinal fluid of three-day old Wister rats. It was concluded that DPSC-derived cells integrated into the host brain and showed neuronal properties like expression of neuron-specific markers and voltage-dependent sodium and potassium channels.

Dental pulp stem cells have proved to provide relief to patients with Parkinson's disease, which is a neurodegenerative disorder. In a study done by Nosrat *et al.*,[35] researchers have cultured dental pulp stem cells, which produce neuronal precursor cells and cells that produce beneficial neurotrophic factors for the treatment of the disease. It has also been suggested that tooth cells provide neurotrophic support to dying nerve cells and replace dead cells.[35] The protective effect may be attributed to the BDNF and GNF released by the DPSCs, and they are also

capable of differentiating into dopaminergic neuron-like cells.[35-37]

The comparison between human DPSC and BMSC cells, for better neural epithelial stem cell properties, was done by Karaoz *et al*.[38] The DPSCs from the third molar were evaluated for their proliferation capacity and gene-expression profiles, phenotypic, ultrastructural, and differentiation characteristics, where HDPSCs were seen to be more metabolically active cells. They expressed cytokeratin (CK 18-19), which is involved in both odontoblast differentiation and dentin repair. Additionally, the immortalized tooth germ cells retained their properties of differentiation. Results of the various studies suggested that the immortalized DPSCs might slow their senescence, and biomaterials coated with DPSCs could also be used for neural stem cell differentiation.[39,40]

Osteogenic differentiation of dental pulp stem cells

Mesenchymal stem cells are used more widely in surgical repair/regeneration,[41] as they originate from the neural crest and migrate, differentiate, and participate in morphogenesis, to give rise to structures of the craniofacial region, including muscle, ligament, cartilage, bone, periodontal membrane, and teeth.[42] The DPSCs are ectomesenchymal in origin and contain osteogenic markers thatrespond to the inductors of osteogenic and odontogenic differentiation.[43] Various studies have shown the differentiation of DPSCs into functional osteoblasts *in vitro* and they have also been seen to produce extracellular and mineralized matrix in abundance. According to the researchers, the stromal pulp stem cells differentiate into osteoblasts, which synthesize the three-dimensional woven bone tissue chips *in vitro*.[44,45]

Graziano *et al.*,[46] in their study, collected dental pulp cells and subjected them to magnetic-activated cell sorting, and the CD34 (+) stem cell population capable of differentiating into pre-osoteoblasts were selected, adhered to a polylactic scaffold, and transplanted to immunocompromised rats. The recovered transplants showed nodules of bone similar to the dimension of the original scaffold. The investigators detected bone-specific proteins within the nodules, by immunofluorescence, as also characteristic features of the bone as revealed by X-ray diffraction patterns.

D'Aquino *et al*.[47] evaluated bone regeneration by DPSCs both clinically and radiographically, using a collagen scaffold. Their results showed that within three months of colonization on the scaffold, complete radiographic bone regeneration could be observed. de Mendonça Costa *et al*.[48] evaluated the capacity of human DPSCs to reconstruct large cranial defects in non-immunosuppressed rats and found that a more mature bone was formed in the cranial defects, supplied with collagen membrane and HDPSCs. Chadipiralla *et al.*[49] studied the osteogenic differentiation of stem cells derived from human periodontal ligaments and the pulp of human exfoliated deciduous teeth and suggested that PDLSC is a better osteogenic stem cell source.

Type 1 diabetes

Diabetes is a chronic degenerative disease. One of the treatments for diabetes includes transplantation of pancreatic islet cells. Embryonic and adult stem cells have been used for the production of insulin producing cells derived from amniotic fluid, bone marrow, and adipose tissue.[50,51] Chen *et al*.[52] demonstrated that insulin-producing cells (IPCs) can be derived from monoclonal and polyclonal DPSCs. Furthermore, when subjected to the same IPC-producing protocol, they demonstrate that the insulin yield of polyclonal and clonal DPSCs is higher than that of BMSCs.[52] DPSCs have the capacity to differentiate into islet-like aggregates, also shown by Govindsamy *et al*.[53]

Infertility

The potential of DPSCs can also be used in the treatment of infertility. Leake and Templeton[54] isolated HDPSCs and injected them into the testes of live male mice. The mice were killed at various intervals after the injection and their testes were examined to see whether the stem cells survived. It was found that stem cells settled in the testes and also differentiated into cells that were producing viable sperm.

PROCUREMENT OF DENTAL PULP STEM CELLS

Extraction, preservation, and isolation of DPSCs from the teeth planned for orthodontic extraction, from exfoliated deciduous teeth or extracted impacted teeth can be beneficial for future regenerative medicine. It is possible to bank DPSCs, as they can be successfully cryopreserved with good viability and function upon thawing, as shown in various studies.[55,56]

Perry *et al*.,[57] in an experimental study for the collection and cryopreservation of DPSCs for clinical use as well as banking, concluded that approximately

80% of the extracted human third molars yielded DPSC culture within 24 hours of extraction and at least 12 hours post extraction, if stored at 4ºC. Moreover, DPSCs can be stored at - 85ºC to - 196ºC for at least six months without loss of functionality.[58] The DPSCs can be cultured by the enzyme digestion method, which uses the collagenase type 1 enzyme, as also the explant method, which uses the pulp tissue cut into specific sizes and incubated with essential medium. After culturing, the various cells obtained are differentiated into the desired cell lines depending on the contents of the culture medium.[58,59]

CONCLUSION

Dental pulp is made of ectomesenchymal components containing neural crest-derived cells that display plasticity and multipotential capabilities.[9] Their easy availability, preservation for longer periods, lack of ethical/legal issues in its extraction, availability from the homogenous source, and multipotency, are some of the advantages that make it a lucrative option over other types of stem cells.[59,60] DPSCs are readily available from exfoliating/extracted teeth that are otherwise discarded as medical waste and its rapid proliferation provides a potential for expansion.[52]

D'aquino *et al*.[16] advocated that 'dental pulp is a remarkable site of stem cells; collecting stem cells from dental pulp is a non-invasive practice that can be performed in the adult during life and in the young after surgical extraction of wisdom teeth, a common surgical practice. Tissue sacrifice is very low when collecting dental pulp stem cells. Several cytotypes can be obtained from dental pulp stem cells owing to their multipotency. Transplantation of new-formed bone tissue obtained from dental pulp stem cells leads to the formation of vascularized adult bone and integration between the graft and the surrounding host blood supply. Dental pulp stem cells can be cryopreserved and stored for long periods. Dental pulp is ideal for tissue engineering, for clinical use in several pathologies requiring bone tissue growth, and repair and tooth extraction is a clinical/therapeutical need. They further proposed that 'if bone marrow is the site of first choice for hematopoietic stem cell collection, dental pulp must be considered as one of the major sites for mesenchymal cell collection'.

Thus, it can be concluded that stem cells of dental origin are easily procurable and have the proven capacity to differentiate into distinct cell lines. This lesser used source of stem cells can have a significant role in not only regenerative endodontics, but also in the treatment of many degenerative diseases. However, future long-term studies are required to evaluate the specific dental stem cell sub-populations for specific clinical situations.

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