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Imperative Role of Dental Pulp Stem Cells in Regenerative Therapies: A Systematic Review

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Abstract

Stem cells are primitive cells that can differentiate and regenerate organs in different parts of the body such as heart, bones, muscles and nervous system. This has been a field of great clinical interest with immense possibilities of using the stem cells in regeneration of human organ those are damaged due to disease, developmental defects and accident. The knowledge of stem cell technology is increasing quickly in all medical specialties and in dental field too. Stem cells of dental origin appears to hold the key to various cell-based therapies in regenerative medicine, but most avenues are in experimental stages and many procedures are undergoing standardization and validation. Long-term preservation of SHED cells or DPSC is becoming a popular consideration, similar to the banking of umbilical cord blood. Dental pulp stem cells (DPSCs) are the adult multipotent cells that reside in the cell rich zone of the dental pulp. The multipotent nature of these DPSCs may be utilized in both dental and medical applications. A systematic review of the literature was performed using various internet based search engines (PubMed, Medline Plus, Cochrane, Medknow, Ebsco, Science Direct, Hinari, WebMD, IndMed, Embase) using keywords like "dental pulp stem cells", "regeneration", "medical applications", "tissue engineering". DPSCs appears to be a promising innovation for the re-growth of tissues however, long term clinical studies need to be carried out that could establish some authentic guidelines in this perspective.

Keywords: Dental pulp stem cells, myocardial infarction, regenerative therapy, tissue engineering

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Introduction

The term stem cell was proposed for scientific use by Russian histologist Alexander Maksimov in 1909. He was the first to suggest the existence of hematopoietic stem cells (HSC) with the morphological appearance of a lymphocyte, capable of migrating throughout the blood to micro ecological niches that would allow them to proliferate and differentiate.[1] Tissue engineering as a scientific discipline has shown promising results in the field of dentistry also. Tissue engineering approaches can aid in either the replacement of damaged tooth structures and/or in the repair/regeneration of pulp-dentin complex (regenerative endodontics).

The science of tissue engineering and regenerative medicine has seen tremendous development, especially in the field of stem cell research. Tissue engineering approach requires the three main key elements (triad): Stem cells, scaffold (or matrix) and growth factors (morphogens).[2] These key elements can be used in three principal therapeutic strategies to obtain the desired result. Today stem cell biology is one of the most fascinating areas of science which brings in the hope for improved outcomes by replacing damaged or absent tissues with healthy regenerated tissue.[3] Dental pulp stem cells (DPSCs) can be found within the "cell rich zone" of dental pulp. Their embryonic origin, from neural crests, explains their multipotency.[4] The term stem cell was projected by Alexander Maksimov a Russian histologist, during 1908 in congress of hematologic society at Berlin.[5] Stem cells have the potential to renew themselves for long periods through cell division and under certain physiologic or experimental conditions, they can be induced to become cells with special functions.[6] Several studies have been carried out to verify whether stem cells could become a source of stable differentiated cells. These studies have confirmed their capacity to induce tissue formation during the embryonic development and proliferation along with differentiation to generate all other tissues.[7-10]

By definition the pluripotency of biological compounds describes the ability of certain substances to produce several distinct biological responses whereas multipotency means the ability to differentiate to a limited number of cell fates or into closely related family of cells. Recent advances in the tissue engineering have drawn scientists to test the possibility of tooth engineering and regeneration. However, these biotechnologies are in its initial phase, it is expected to be used to restore missing teeth and replace artificial dental implants.

Researchers have observed that these stem cells act differently than other adult stem cells. These dentally-derived mesenchymal stem cells are capable of extensive proliferation and differentiation, which makes them an important resource of stem cells for regeneration and repair of a multitude of diseased and injured organs and tissues.[10-11] Because of their ability to produce and secrete neurotrophic factors, these stem cells may also be beneficial for the treatment of neurodegenerative diseases and the repair of motoneurons following the injury. Research works on dental mesenchymal stem cells is expanding at an unprecedented rate. More than 1,000 research studies from institutions around the world have been published since the year 2000 that make reference to the dental stem cells. In the year 2007 alone, over 1,000 research articles were published on Dental Stem Cells.[12] Additionally, over 60 clinical investigations with animals and human volunteers have been published seeking to identify the potential new medical treatments from adult stem cells.[10] Stem cell-based therapies are being investigated for the treatment of many conditions including: Neurodegenerative conditions, liver disease, diabetes, cardiovascular disease, autoimmune diseases, musculoskeletal disorders, and for nerve regeneration following the brain or spinal cord injury.

Riccardo and co workers postulated two school of thoughts; one argues that these cells produce a "dentin-like tissue",[7] whereas the other research group[11] has demonstrated that these cells are capable of producing bone, both *in vitro* and *in vivo*. Beyond natural capacity of response to the injury, dental pulp stem cells are attractive for their potential to differentiate, *in vitro*, into several cell types including odontoblasts, neural progenitors, chondrocytes, endotheliocytes, adipocytes, smooth muscle cells and osteoblasts.[12,13] The potential application of dental pulp stem cells and tissue engineering in medicine and dentistry in particularly are discussed in the present review.

At present, the mesenchymal stem cell populations having the high proliferative capacity and multi-lineage differentiation have been isolated from the dental tissues.[14,15] These are dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), dental follicle progenitor stem cells (DFPCs), and stem cells from apical papilla (SCAPs). DPSCs and SHEDs originate from the cranial neural crest and express early markers for both mesenchymal and neuroectodermal stem cells.[16-18] This explains their multipotency and pluripotency. Sharpe and Young were pioneered the use of stem cells in the dental tissue engineering.[19,20] Various studies have shown that these cells have unique features of stem/progenitor cells having the capacity to differentiate into dentin forming odontoblasts.[21,22] The roots of the third molar are often incomplete at the age of eighteen, therefore these teeth contains a conspicuous pool of undifferentiated cells, resident within the "cell rich zone" of the dental germ pulp.[23-24] In an *in vitro* model, Hwang *et al.* 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.[25] Apart from these, the cells obtained from loosely attached tissue at the root apex (SCAP) and periodontal ligament (PDLSC) have been used for bio-root engineering.[26-28] More recently, stem cells obtained from the dental tissues have been shown to develop into fat, bone cartilage and neural cells.[29,30]

In addition to their therapeutic use in dentin regeneration, regeneration of periodontal tissues and skeletal articular tissues of craniofacial region, DPSCs were also reported to be used in the treatment of neurotrauma, autoimmune diseases, myocardial infarction, muscular dystrophy and connective tissue damages.[31] This review article is an attempt to highlight main strategies as related to the use of dental pulp stem cells, their characterization, storage, tissue engineering strategies and useful clinical applications in the field of modern dentistry.

Literature search philosophy

Various internet based popular search engines (Google, Google Scholar, Yahoo), scholarly search bibliographic databases (PubMed, PubMed Central, Medline Plus, Cochrane, Medknow, Ebsco, Science Direct, Hinari, WebMD, IndMed, Embase) and textbook (Sibel Yildirim. Dental Pulp Stem Cells, 2013, Springer New York) were searched until April 2013 using MeSH (Medical Subject Headings; PubMed) based keywords such as "dental pulp stem cells", "regeneration", "medical applications", "tissue engineering". The search was limited to reviews, systematic researches, meta-analyses and clinical guides in the medical and dental journals published over the last 25 years in English.

The literature search was ended up with the identification of 157 relevant articles. Only those articles were selected that gave information on the use of these dental stem cells in various fields of regenerative medicine. Throughout the process firstly the articles were judged based on their titles, then on the abstracts, and finally on the entire text. The articles containing no information on adult dental stem cells and regenerative medicine were excluded, as were doctoral theses, case reports and expert opinions. After examining the titles and abstracts, this number was finally reduced to 78 articles.

Banking of stem cells

There is an abundant source of adult stem cells in the human exfoliated deciduous teeth (SHED). Recent studies have shown that SHED has the ability to develop into more types of body tissues than other types of stem cells. Researchers have found the pulp of exfoliated deciduous teeth to contain chondrocytes, osteoblasts, adipocytes, and mesenchymal stem cells. All of these cell types hold enormous potential for the therapeutic treatment of: Neuronal degenerative disorders such as Alzheimer's, Parkinson's, and ALS (Amyotrophic Lateral Sclerosis or Lou Gehrig' Disease); chronic heart conditions such as congestive heart failure and chronic ischemic heart disease; periodontal disease and to grow replacement teeth and bone.[32-34]

Keeping this premise in mind, the concept of the tooth banking has popularized and various companies have set up tooth banks to tap the potential of this new and innovative approach for preserving SHED and stem cells from other dental sources.[33,35] Thus the ultimate key to successful stem cell therapy is to harvest cells and store them safely until accident or disease requires their usage. The tooth banking is not very popular, but the trend is catching up, mainly in the developed countries. Also, it is now been proven that the primary teeth are a better source of therapeutic stem cells for use in regenerative medicine than wisdom teeth, and orthodontically extracted teeth.[36,37]

Dental stem cells isolated from different parts of the teeth are
1. SHED.
2. Adult dental pulp stem cells (DPSC).
3. Stem cells from the apical part of the papilla (SCAP).
4. Stem cells from the dental follicle (DFSC).
5. Periodontal ligament stem cells (PDLSC).
6. Bone marrow derived mesenchymal stem cells (BMSC).

Advantages of SHED banking[37,38]

1. Provides an autologous transplant for life.
2. Simple and painless procedure.
3. SHED cells are complementary to stem cells from the cord blood.
4. Useful for close relatives of the donor.
5. Not subjected to the same ethical concerns as embryonic stem cells.

A new method to cryopreserve DPSC inside a whole tooth was proposed by Silvia and associates. They showed the possible avoidance of purification of the cells before cryopreservation and reducing the initial costs and workload of the tooth banking. They further demonstrated that DPSC isolated from laser pierced cryopreserved teeth show mesenchymal stem cells morphology, immune-phenotype, viability and proliferation rate similar to those of the cells isolated from fresh, non cryo-preserved teeth, whereas significant loss of the cell viability and proliferation rate was shown by the cells isolated from teeth cryopreserved without laser piercing.[39]

It is necessary to store stem cells that could act as a fundamental resource of biological materials for both basic and advanced research projects. There are various stem cell banks, storages that preserve and maintain ethically sourced stem cell obtained from different origins.[34] Recent past has evidenced global initiatives to address management and standardization processes for the stem cell research and banking. The "International Society for Stem Cell Research and the International Stem Cell Banking Initiative" is one of the pioneer of this program.[35] These banks also serve to freeze the dental stem cells of baby teeth when the anterior primary tooth is shedding (DPSC is preferred for storage). The tooth is extracted by the dentist and well preserved in a special kit provided separately from the stem cell bank. This assembly is then transferred to their special labs to harvest the dental stem cells and store them in their bank for each child confidentially until they are needed later for the child himself or a member of his family. In India, 'Life Cell International', was the first private sector stem cell banking services started in Bangalore (India) in the year 2009.

Initially, via individual and corporate approaches, charges for collecting and saving the stem cells were barely affordable (approximately 3000 USD); that is why it could not receive much promotion in India.[36,37]

Licensed tooth stem cell banks, Internationally and in India, used for cryopreservation and isolation are as follows[33]
1. In Japan, the first tooth bank was established in Hiroshima University and the company was named as 'Three Brackets' (Suri Buraketto).
2. BioEden (Austin, Texas), StemSave, and Store-a-Tooth (USA)
3. The Norwegian tooth bank.
4. In India, Stemade Biotech Pvt. Ltd. (Delhi, Chennai, Chandigarh, Pune, and Hyderabad).

Dental pulp stem cells

DSCs can be obtained from the pulp of the primary and permanent teeth, from the periodontal ligament, and from the other tooth structures. Periodontal ligament derived stem cells are able to generate periodontal ligament and cementum. Extracted third molars, exfoliating/extracted deciduous teeth and teeth extracted for orthodontic treatment, trauma or periodontal disease are all sources of dental stem cells from the dental pulp.[40] The dental pulp offers a source of stem cells (in post-natal phase) that is readily available, with minimally invasive process that results in minimal trauma. Exfoliating or extracted deciduous teeth offer extra advantages over the other teeth as a source of stem cells. Stem cells from deciduous teeth have been found to grow more rapidly than those from other sources, and it is believed that this is because they may be less mature than other stem cells found in the body. Additional advantages of sourcing the stem cells from exfoliating deciduous teeth are that the cells are readily available, provided they are stored until they may be needed later in life; the process does not require a patient to sacrifice a tooth to source the stem cells; and there is little or no trauma.[41]

Therapeutic applications

Dental pulp tissue engineering is a promising field that can potentially have a major impact on the oral health. Though, the actual resource of elements required for the stem cell propagation into odontoblasts and scaffold characteristics are still unclear. Depending on specific signals from their environment, DPSCs can either regenerate new stem cells or undergo a differentiation process. In the dental pulp, there are different progenitor cell subpopulations, which differ in terms of self renewal ability, proliferation rate and differentiation potential[14] DPSCs can differentiate to odontoblasts, osteoblasts, endotheliocytes, smooth muscle cells, adipocytes, chondrocytes and neurons. Carnevale *et al.* have recently found that human amniotic fluid stem cells and human DPSCs differentiate into insulin-producing cells, offering a nonpancreatic, low-invasive source of cells for islet regeneration.[42] Dental tissues usually have a complex structural composition that warrants both hardness and durability. However, this structure is highly susceptible to trauma and microbial attacks. Following any external insult but still in a reparable condition, regeneration of the tooth structure may avert or delay the loss of the whole tooth. Of all the dental structures, only enamel is incapable of regenerating its original structure, while the remaining tissues possess that capacity in varying degrees, dependent on the multiple factors. In 2003 Dr. Songtao Shi unexpectedly discovered deciduous tooth stem cells by using the deciduous teeth of his 6-year-old daughter.[43] He was luckily able to isolate, grow and preserve these stem cells' regenerative ability, and he named them as SHED (Stem cells from Human Exfoliated Deciduous teeth).

The dental pulp stem cells are multipotent in nature and 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 in medical sciences for various degenerative diseases other than those of dental origin such as Alzheimer's disease, myocardial infarction, diabetes mellitus, bone defects and spinal cord injuries etc.[39] Dentistry has long exploited the life-long regeneration potential of AS cells in human dental pulp which give rise to tertiary dentin, therapeutically employed for direct and indirect pulp capping after caries excavation near the pulp. The application of calcium hydroxide or calcium phosphate, among other substances, can induce pulpal progenitor cells to differentiate into odontoblasts. In the future, DPSCs could also be used to treat perforated furcations.[44]

Role of DPSCs in corneal reproduction

The role of DPSCs in successful reconstruction of cornea has also been explored in the recent past. In an animal study by Gomes *et al.*, a tissue engineered DPSC sheet was transplanted on the corneal bed and then covered with de-epithelialized human amniotic membrane.[45] Histological analysis at three months postoperative phase confirmed that healthy uniform corneal epithelium was formed. Moreover, it was concluded that tissue engineered DPSC sheet was successful for the reconstruction of corneal epithelium.

Role in myocardial infarction

Regardless of the recent advances in prevention and treatment of myocardial infarction (MI), it remains one of the major causes of mortality worldwide. In this perspective, cardiomyocytic differentiation of DPSCs has been studied by various researchers. The overall capability of the stem cells derived from the bone marrow stem cells (BMSCs), adipose tissue cells (ATSCs) and DPSCs to differentiate to cells with a cardiac phenotype was first estimated by Arninam and co-workers.[46] They postulated that the tissue specific mesenchymal stem cells (MSCs) can change into cardiomyocytes and support the potential use of MSCs in the stem cell based cardiac therapies. Furthermore, the therapeutic potential of DPSCs in repair of myocardial infarction was evaluated by Gandia *et al.* in 2008, who concluded that human DPSC secrete multiple pro-angiogenic apoptotic factors.[47]

Therapeutic role of DPSCs in Ischemia

Iohora *et al.* successfully isolated a highly vasculogenic DPSCs similar to endothelial progenitor cells from the dental pulp. Increased proliferative and migration activities including multi-lineage differentiation and vasculogenic potential (Innate capacity to differentiate into blood vessels and associated structures) have also been shown by these cells. Therapeutic action of DPSCs resulted in successful engraftment and an increase in the blood flow including high density of capillary formation in a rat model with hind limb ischemia.[48]

Segregation of DPSCs into muscular tissue

Literature has well evidenced various studies evaluating the myogenic potential of DPSCs. Yang *et al.* was among the initial researchers who actually used human DPSCs for the treatment of muscular dystrophy in golden retriever dogs transplanted by arterial or muscular injections. The samples from biopsies were checked by immunochemistry (dystrophin markers) and the researchers showed that DPSC presented significant engraftment in dog muscles. The therapeutic potential of DPSC to differentiate into dystrophin producing multinucleated muscle cells can be successfully utilized in diseases like muscular dystrophy in where body is incapable to produce dystrophin.[49]

Neural differentiation of DPSCs

Several studies have shown that DPSCs are competent of provoking long term regeneration of nerves in damaged spinal cord. Apel *et al.* potentially investigated the neuro-protective effect of DPSCs *in vitro* models of Alzheimer's and Parkinson's disease.[50] They also obtained DPSCs from adult rat incisors by systematic isolation and were added to the neuron cultures two days prior to the neurotoxin treatment. It was also revealed that DPSCs expressed a neuronal phenotype and produced the neurotropic factors like NGF (nerve growth factor), GDNF (Glial cell-derived neurotrophic factor), BDNF (Brain-derived neurotrophic factor) and BMP2. Also, DPSCs protected primary neurons and helped in the cell viability.[51,52] In an experiment the DPSCs were transplanted into rats with completely severed spinal cords. It was demonstrated that DPSCs promoted the regeneration of transacted axons by directly inhibiting multiple axon growth inhibitors and by prevention of apoptosis of neurons, astrocytes and oligodendrocytes. The DPSCs also differentiated into mature oligodendrocytes to replace cells that were lost. Later on Almeida *et al*. supported the fact on the basis of their study where they explored the outcomes of human dental pulp stem cells in a mouse model with compressive spinal cord injury.[53]

DPSCs differentiation to Hepatocyte

By definition the hepatocyte is a cell of the main tissue of the liver that makes up 70-80% of the liver's cytoplasmic mass. Ishkitieve and co-workers were among initial researchers who demonstrated that DPSCs may possibly be differentiated into cells with morphological, phenotypic and functional characteristics similar to hepatocytes.[54] They further segregated the stem cells from dental pulp and postulated the fact that DPSC's have the capacity to differentiate into hepatic family.[55]

Differentiation of DPSCs into bone cells

The DPSCs are basically ectomesenchymal in origin and contain osteogenic markers that respond to the inductors of osteogenic and odontogenic differentiation. Such mesenchymal stem cells are extensively used in surgical repair/regeneration, as they instigate from neural crest and migrate, differentiate, participate in morphogenesis to give rise to structures of craniofacial region including muscle, ligament, cartilage, bone, periodontal membrane and teeth.[56-58] Graziano *et al*. and Aquino *et al*. assessed bone regeneration by DPSCs both clinically and radiographically using a collagen scaffold.[59,60] Their results demonstarted that within three months of colonization on the scaffold, complete radiographic bone regeneration could be observed. Costa *et al*. and Chadipiralla *et al*. studied the osteogenic differentiation of the stem cells derived from human periodontal ligaments and pulp of human exfoliated deciduous teeth and suggested that PDLSC is a better osteogenic stem cell resource.[61-62]

Treatment of infertility employing DPSCs

The prospective of DPSCs can also be used in the treatment of infertility. Kerkis *et al*. in a study successfully segregated HDPSCs and injected them to the testis of live male mice. The mice were killed at various intervals after the injection and their testis were examined to see whether stem cells survived. It was postulated that the stem cells settled in testes and also differentiated into cells that were producing viable sperm.[63]

DPSCs considerations for Type 1 diabetes

Diabetes is one of the most common chronic degenerative endocrinal diseases related to the pancreatic islet cells that may possibly be successfully managed by 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.[64,65] Chen *et al*. showed that insulin producing cells (IPCs) can be derived from monoclonal and polyclonal DPSCs. Furthermore, when subjected to same IPCs producing protocol, they demonstrated that the insulin yield of polyclonal and clonal DPSCs was higher than that of BMSCs.[65] DPSCs have the capacity to differentiate into islet-like aggregates also shown by Govindsamy *et al*.[66]

According to Laino and co workers, the extraction, preservation and isolation of DPSCs from the teeth intended for orthodontic extraction, from exfoliated deciduous teeth or extracted impacted teeth can be advantageous for upcoming regenerative medicine. DPSC are readily available from exfoliating/extracted teeth that are otherwise discarded as medical waste and its rapid proliferation provide a potential for expansion.[67,68] Moreover, it is also possible to bank DPSCs as it can be effectively cryo-preserved with excellent viability and function upon thawing as illustrated in different studies.[69,70]

Discussion and Conclusive Remarks

The dental pulp is primarily made of ecto-mesenchymal components, containing neural crest-derived cells, which display plasticity and multipotential capabilities. According to Saber and Huang, their easy availability, evident preservation for longer periods, relative lack of ethical/legal issues in its removal, frequent accessibility from the homogenous resources, and multi-potency are some of the crucial advantages which makes it a worthwhile option over other types of stem cells described in the literature.[71-76] D'aquino *et al*.[7] has rightly postulated 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 need". They further anticipated that, "If bone marrow is the site of first choice for hematopoietic stem cell collection, dental pulp must be considered one of the major sites for mesenchymal cell collection".

Thus the dental pulp is a remarkable site of stem cells and 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. Dental pulp is ideal for tissue engineering and for clinical use in several pathologies requiring bone tissue growth and repair.[77,78] Conversely, the stem cells of dental origin have proven capacity to differentiate into distinct cell lines. Regenerative endodontic procedures can be defined as biologically based procedures designed to replace the damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex. DPSCs can differentiate to odontoblasts that generate mineralized dentin with highly organized tubular structures however; has differentiation has not been reported in non vital cases. Histological analyses revealed a well-defined layer of odontoblast-like cells, with characteristic processes extending into tubular structures within the regenerated dentin, and a highly vascularized pulp tissue center. The orientation of the collagen fibers within the dentin was perpendicular to the odontoblasts-like cell layer, similar to the naturally formed dentin. Undoubtedly, this significantly less used reserve of stem cells may possibly have a key role in not only regenerative endodontics, but also for management of many degenerative diseases.

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