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Dental Pulp Stem Cells: A Promising Tool for Tissue Regeneration

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Abstract *: Stem cells are the "master " cells of our body that act as building block to regenerate and turn into cells that form tissues, organs and systems in the human body. Several "loci '' or "niches" within the adult human body are colonised by a significant number of stem cells. However, access to these potential collection sites often is a limiting point. Growing evidence demonstrates that stem cells are primarily found in niches and that certain tissues contain more stem cells than others. Among these tissues, the dental tissues are considered a rich source of mesenchymal stem cells that are suitable for tissue engineering applications. It is known that these stem cells have the potential to differentiate into several cell types, including odontoblasts, neural progenitors, osteoblasts, chondrocytes, and adipocytes. The interaction with biomaterials is a further point that needs to be considered for the therapeutic use of stem cells. Dental pulp stem cells (DPSCs) have been demonstrated to answer all of these issues: access to the collection site of these cells is easy and produces very low morbidity; extraction of stem cells from pulp tissue is highly efficient; they have an extensive differentiation ability; and the demonstrated interactivity with biomaterials makes them ideal for tissue reconstruction. Mesenchymal stem cells were demonstrated in dental tissues, including dental pulp,periodontal ligament, dental papilla, and dental follicle. These stem cells can be isolated and grown under defined tissue culture conditions, and are potential cells for use in tissue engineering, including, dental tissue, nerves and bone regeneration. More recently, another source of stem cell has been successfully generated from human somatic cells into a pluripotent stage, the induced pluripotent stem cells (iPS cells), allowing creation of patient and disease-specific stem cells. Collectively, the multipotency, high proliferation rates, and accessibility make the dental stem cell an attractive source of mesenchymal stem cells for tissue regeneration. This review describes about different sources of stem cells eg human embryonic stem cells (ES) cells , somatic or adult stem cells , stem cells of Dental tissue origin and induced pluripotent stem cells and their characteristic and new findings in the field of dental stem cell research and on their potential use in the tissue regeneration.*
Keywords: *stem cells, Dental pulp stem cells , embryonic stem cell , regeneration*

I. Introduction

Stem cell possess a remarkable potential to proliferate and develop into many different cell types to form the desired tissue, these cells hold great promise for regenerative therapy. The most commonly used and studied cells are Embryonic stem cell (ES), Somatic or Adult stem cells and Induced Pluripotent stem cells. Embryonic stem cells (ES) are derived from the inner cell mass of early embryos, called blastocysts. ES cell were 1st isolated from mouse embryos in 1981.[123] The success of this work led to the derivation of human ES cell from in vitro fertilised human blastocysts in 1998 [4] Stem cells are cells that have the ability to renew themselves through mitosis and can differentiate into several specialized cells. The embryonic stem cells (ESC) are pluripotent and have the ability to become almost any kind of cell of the body [4] The local microenvironment represents an important compartment in maintaining the stem cells status. The microenvironment regulates the balance between self-renewal and differentiation. This intercellular communication has been characterized between embryonal carcinoma cells and stromal cells, and indicates changes in the expression on both cellular compartments [5] Scientists can induce these cells to replicate themselves in an undifferentiated state. However, the use of ESC is controversial and associated with ethical and legal issues, thus conditioning their application for the development of new therapies [4] Another source of stem cells is the umbilical cord. Blood from the umbilical cord contains stem cells that are genetically identical to those of the newborn baby. These cells are multipotent, and are able to differentiate into certain cell types. Umbilical cord stem cells can be stored cryogenically after birth for use in a future medical therapy [6]. Somatic or Adult stem cells :In general , regenerative capacity of adult tissues depends on tissue specific stem cell populations that maintain stable numbers by self renewal and possess the ability to differentiate into distinct cell lineages. Regeneration and renewal in adult mammals has been studied in several organs , including blood, mammary glands , gut, brain, skin , muscle and hair. These tissue contain adult stem cells such as hematopoietic, endothelial ,mammary ,intestinal, neural , skin, muscle, hair follicle stem cells [7]. Similarly teeth and supporting structures contain multiple lineage of stem cells including :

Mesenchymal stem cells isolated from the dental pulp of permanent teeth , termed Dental Pulp Stem Cells (DPSC) [10] and from the dental pulp of exfoliated deciduous teeth termed stem cells from Human Exfoliated Deciduous teeth (SHED). [10, 14, 15]
Mesenchymal stem cells isolated from the periodontal ligament (PDLSC) [18]
Mesenchymal stem cells isolated from the apical end of developing tooth roots, termed Stem cells from the Apical Papilla (SCAP) [16]

Adult stem cells normally generate cell types of the tissue in which they reside. However, studies have shown that stem cells from one tissue could generate cell types of a completely different tissue. [4]Unlike ESC, adult stem cells have the potential to be used for treatment of regenerative disease, cardiac ischemia, and bone or tooth loss. Future applications for stem cells include the treatment of Parkinson's disease and cancer .[7] The use of adult stem cells in research and medical applications is less controversial because they can be harvested without destroying an embryo. Adult or Postnatal stem cells have been found in almost all body tissues, including dental tissues. Dental stem cells have been identified as candidates for tissue engineering . [10] Because of their multipotent differentiation ability, they provide an alternative for use in regenerative medicine since they can be used for not only to dental tissue regeneration, but also to facilitate repair of non-dental tissues such as bone and nerves [10,14, 24, 25] A new source of stem cell has been generated from human somatic cells into a pluripotent stage, the induced pluripotent stem cells (iPS cells) [12, 13] iPS cells resemble human ESC and can differentiate into advanced derivates of all three primary germ layers. Unlike ESC, iPS cell technology can derive patient-specific stem cells allowing derivation of tissue-matched differentiation donor cells for basic research, disease modelling, and regenerative medicine . [13]This review discusses the application of stem cell-in tissue regeneration, addressing sources of stem cells identified in dental tissues; and new findings in the field of dental stem cell research and their potential use in the dental tissue engineering.

It is considered that dental pulp stem cells are undifferentiated mesenchymal cells present in dental tissues and characterized by their unlimited self-renewal, colony forming capacity, and multipotent differentiation(1). During the characterization of these newly identified dental stem cells, certain aspects of their properties have been compared with those of bone-marrow-derived stromal stem cells (BMMSC). Dental stem cells display multi differentiation potential, with the capacity to give rise to distinct cell lineages, osteogenic, adipogenic, and neurogenic. Therefore, these cells have been used for tissue-engineering studies to assess their potential in preclinical applications . [7]

II. Dental Pulp Stem Cells

The first stem cells isolated from adult human dental pulp were termed dental pulp stem cells (DPSC). They were isolated from permanent third molars and exhibited high proliferation and colony formation that produced calcified nodules [14] DPSC cultures from impacted third molars at the stage of root development were able to differentiate into odontoblast like cells with a very active migratory and mineralization potential, leading to organized three-dimensional dentin like structures in vitro . [21] There are different cell densities of the colonies in DPSC, suggesting that each cell clone may have different grown rate .[14] Different cell morphologies and sizes can be observed in the same colony. The differentiation of DPSC to a specific cell lineage is mainly determined by the components of local microenvironment, such as, growth factors, receptor molecules, signaling molecules, transcription factors and extracellular matrix protein. DPSC can be reprogrammed into multiple cell lineages such as, odontoblast, osteoblast, chondrocyte, myocyte, neurocyte, adipocyte, corneal epithelial cell, melanoma cell, and even induced pluripotent stem cells (iPS cells) [22] Almushayt et al. [23] demonstrated that dentin matrix protein 1 (DMP1), a non-collagen extracellular matrix protein extract from dentin, can significantly promote the odontoblastic differentiation of DPSC and formation of reparative dentin over the exposed pulp tissue. Additionally, DPSC can be induced into odontoblast lineage when treated with transforming growth factor β1 (TGFβ1) alone or in combination with fibroblast growth factor (FGF2) [22] Histologically, dentin lies outside of dental pulp, and they intimately link to each other. Functionally, dental pulp cells can regenerate dentin and provide it with oxygen, nutrition and innervation, whereas the hard dentin can protect soft dental pulp tissue. Together, they maintain the integrity of tooth shape and function. Any physiological or pathological reaction occurring at one part, such as trauma, caries, and cavity preparation, will affect the other. Both of them act as a dentin-pulp complex and simultaneously participate in various biological activities of the tooth. Several studies have shown that DPSC play a vital role in the dentin-pulp tissue regeneration [14] In vivo transplantation into immunocompromised mice DPSC demonstrated the ability to generate functional dental tissue in the form of dentin/pulp-like complexes. [24] Transplanted ex vivo expanded DPSC mixed with hydroxyapatite/ tricalcium phosphate form ectopic dentin/pulp-like complexes in immuno compromised mice. These polls of heterogeneous DPSC form vascularised pulp like tissue and are surrounded by a layer of odontoblast-like cells expressing factors that produce dentin containing tubules similar those found in natural dentin. [24, 25] Huang et al. [26] reported that dentin-pulp-like complex with well-established vascularity can be regenerated de novo in emptied root canal space by DPSC. These studies provide

a novel advance for future pulp tissue preservation and a new alternative for the biological treatment for endodontic diseases. In addition, DPSC can express neural markers and differentiate into functionally active neurons, suggesting their potential as cellular therapy for neuronal disorders [11]

STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

Stem cells may be also isolated from the pulp of human exfoliated deciduous teeth (SHED). These cells have the capacity of inducing bone formation, generate dentin and differentiate into other non dental mesenchymal cell derivatives in vitro. SHED exhibit higher proliferation rates, increased population doublings, in addition to osteoinductive capacity in vivo and an ability to form sphere-like clusters. However, unlike DPSCs, they are unable to regenerate complete dentin/pulp-like complexes in vivo. [15] With the osteoinductive potential, SHED can repair critical sized calvarial defects in mice with substantial bone formation. [28] Given their ability to produce and secrete neurotrophic factors, dental stem cells may also be beneficial for the treatment of neuro degenerative diseases and the repair of motor neurons following injury. Indeed, dental stem cells from deciduous teeth have been induced to express neural markers such as nestin . [29] The expression of neural markers in dental stem cells stimulates the imagination for their potential use in neural regeneration such as in the treatment of Parkinson's disease. The potential of dental stem cells in non-dental regeneration continues to be further explored by researchers.

STEM CELLS FROM APICAL PAPILLA

The physical and histological characteristics of the dental papilla located at the apex of developing human permanent teeth has been recently been described and this tissue has been termed apical papilla. This tissue is loosely attached to the apex of the developing root and can be easily detached. A population of stem cells isolated from human teeth was found at the tooth root apex. These cells are called stem cells from apical papilla (SCAP) and have been demonstrated to differentiate exhibit higher rates of proliferation in vitro than do DPSC. There is an apical cell-rich zone lying between the apical papilla and the pulp. Importantly, stem /progenitor cells were located in both dental pulp and the apical papilla, but they have somewhat different characteristics. [16]

PERIODONTAL LIGAMENT STEM CELLS

Periodontal ligament (PDL) is a space interlying the cementum and alveolar bone, a replacement of the follicle region surrounding the developing tooth in cap and bud stages of development. Fibers inserted into the cementum layer may be of follicle origin (termed Sharpey's fibers) or cementoblast origin (in cellular intrinsic fiber cementum). The PDL matures during tooth eruption, preparing to support the functional tooth for the occlusal forces. In the mature PDL, major collagen bundles (principal fibers) occupy the entire PDL, embedding in both cementum and alveolar bone. Fibers are arranged in specific orientations to maximize absorption of the forces to be placed on the tooth during mastication. The PDL has long been recognized to contain a population of progenitor cells and recently, studies identified a population of stem cells from human PDL capable of differentiating along mesenchymal cell lineages to produce cementoblast-like cells, adipocytes and connective tissue rich in collagen I [18] PDL stem cells (PDLSC) display cell surface marker characteristics and differentiation potential similar to bone marrow stromal stem cells and DPSC.[18] After PDLSC were transplanted into immunocompromised mice, cementum/PDL-like structures were formed. Human PDLSC expanded ex vivo and seeded in three dimensional scaffolds (fibrin sponge, bovine-derived substitutes) were shown to generate bone [30] These cells have also been shown to retain stem cell properties and tissue regeneration capacity. These findings suggest that this population of cells might be used to create a biological root that could be used in a similar way as a metal implant, by capping with an artificial dental crown.

DENTAL FOLLICLE PRECURSOR CELLS

The dental follicle is a loose connective tissue that surrounds the developing tooth. The dental follicle has long been considered a multipotent tissue, based on its ability to generate cementum, bone and PDL from the ectomesenchyme-derived fibrous tissue. Dental follicle precursor cells (DFPC) can be isolated and grown under defined tissue culture conditions, and recent characterization of these stem cells has increased their potential for use in tissue engineering applications, including periodontal and bone regeneration . [16,31] DFPC form the PDL by differentiating into PDL fibroblasts that secrete collagen and interact with fibers on the surfaces of adjacent bone and cementum. Dental follicle progenitor cells isolated from human third molars are characterized by their rapid attachment in culture, and ability to form compact calcified nodules in vitro. [31] DFPC, in common with SCAP, represent cells from a developing tissue and might thus exhibit a greater plasticity than other dental stem cells. However, in the same way as for SCAP, further research needs to be carried out on the properties and potential uses of these cells.

DENTAL PULP STEM CELLS USED FOR TISSUE REGENERATION

There are several areas of research for which dental stem cells are presently considered to offer potential for tissue regeneration. These include the obvious uses of cells to repair damaged tooth tissues such as dentin, PDL and dental pulp. [7,26] Even the use of dental stem cells as sources of cells to facilitate repair of additional tissues as bone and nerves. [7,11,21]

Regeneration of Periodontium :- Periodontitis is the most common cause for tooth loss in adults due to irreversible waste of connective tissue attachment and the supporting alveolar bone. The challenge for cell-based replacement of a functional periodontium is therefore to form new ligament and bone, and to ensure that the appropriate connections are made between these tissues, as well as between the bone and tooth root. In recent years, guided tissue regeneration has become the gold-standard surgery for periodontal tissue regeneration. This procedure involves draping a biocompatible membrane over the periodontal defect from the root surface to the adjacent alveolar bone, often in combination with a bone graft . [38] The barrier membrane prevents unwanted epithelium and gingival connective tissue from entering the healing site, while promoting repopulation of the defect site by cells migrating in from the PDL . [30] The rather limited success of this approach has led scientists to develop methods to improve this therapy, through the addition of exogenous growth factors and via stem cell therapy [38]. One goal of current research is to use different populations of dental stem cells to replicate the key events in periodontal development both temporally and spatially, so that healing can occur in a sequential manner to regenerate the periodontium [16] Commonly used growth factors for PDL regeneration therapies include bone morphogenetic proteins, platelet derived growth factor, Emdogain and recombinant amelogenin protein. The resultant improved regenerative capability could be related to increased recruitment of progenitor MSC, which subsequently differentiate to form PDL tissue. Recently, PDLSC transfected with expression vectors for platelet-derived growth factor and bone morphogenetic protein were investigated in periodontal tissue engineering models. [39]These studies revealed the regeneration of normal periodontal tissues, containing organized cementum, alveolar bone and the PDL attachment apparatus. Successful therapies for PDL tissue regeneration will not only facilitate the treatment of periodontal diseases, but may also be used to improve current dental implant therapies. Numerous attempts to reconstruct periodontal tissues around dental implants revealed the challenge of avoiding fibrous tissue encapsulation and the formation of functional cementum on the implant surface. [41]

Regeneration of Dentin/Pulplike complex:- Efforts to induce tissue regeneration in the pulp space have been a long search. In 1962, Ostby [32] proposed inducing haemorrhage and blood clot formation in the canal space of mature teeth in the hope of guiding the tissue repair in the canal. However, the connective tissue that grew into the canal space was limited and the origin of this tissue remains unproved.

Regenerative Endodontics represents a new treatment modality that focuses on reestablishment of pulp vitality and continued root development. This clinical procedure relies on the intracanal delivery of a blood clot (scaffold), growth factors (possibly from platelets and dentin), and stem cells. [33] In a recent study, it was demonstrated that mesenchymal stem cells are delivered into root canal spaces during regenerative endodontic procedures in immature teeth with open apices [33]These findings provide the biological basis for the participation of stem cells in the continued root development and regenerative response that follow this clinically performed procedure. As DPSC have the potent dentinogenic ability,they could be used for the vital pulp therapy. When DPSC are transplanted alone or in combination with BMP2 in the pulp cavity, these stem cells can significantly promote the repair and reconstruction of dentin-pulplike complex [32] Prescott et al. [35] placed the triad of DPSC, a collagen scaffold, and DMP1 in the simulated perforation sites in dentin slices, and then transplanted the recombination subcutaneously into the nude mice. After 6 weeks of incubation, well-organized pulplike tissue could be detected in the perforation site. Cordeiro et al. [36] demonstrated that SHED/scaffold recombination prepared within human tooth slices also have the potential to form dental pulp-like structures. Huang et al. [26] reported that dentin-pulp-like complex with well-established vascularity can be regenerated de novo in emptied root canal space by either DPSC or SHED. [26] One of the most challenging aspects of developing a regenerative endodontic therapy is to understand how the various procedures involved can be optimized and integrated to produce the outcome of a regenerated pulp-dentin complex. The future development of regenerative endodontic procedures will require a comprehensive research program directed at each of these components and their application in the clinical practice.

Bone Regeneration : Kenji Ito et al [43] have studied osteogenic potential of effective bone engineering using DPSCs , bone marrow cells & periosteal cells for osseointegration of dental implants. Study concluded that DPSCs showed the highest osteogenic potential and may be useful cell source for tissue engineered bone around dental implants. DPSCs showed differentiation profiles similar to those showed during bone differentiation and this event make them very interesting as a model to study osteogenesis and the relationship with scaffolds. [44]

III. Summary: -

Stem cells are a promising tool for tissue regeneration, due to their particular characteristics of proliferation, differentiation and plasticity. Human dental pulp stem cells (hDPSCs) derived from adult pulp tissue maintained the characteristic of stem cells including self renewal & multipotency. DPSC can give rise to variety of cells and tissue other than dentin, such as adipocytes, neural progenitor cells and myotubes. DPSCs have gained great importance for use in the regeneration of dental tissues, particularly those in the dentin pulp complex. In dentistry stem cell biology and tissue engineering are of great interest since they provide an innovative for regeneration of clinical material and tissue regeneration.

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