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Dental Stem Cells and their Applications in Regenerative Medicine-A Review

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ABSTRACT

Among the types of adult stem cells, those derived from tooth structures have been receiving the attention of researchers over the past decade and inspiring hope for practical applications in the future. Dental stem cells are a valuable source of stem cells and are found in teeth with healthy pulp Till date five different human dental stem cells have been isolated and characterized: Dental Pulp Stem Cells (DPSCs), Stem Cells From Exfoliated Deciduous Teeth (SHED), Periodontal Ligament Stem Cells (PDLSCs), Stem Cells From Apical Papilla (SCAP) and Dental Follicle Progenitor Cells (DFPCs). The discovery of dental stem cells and recent advances in cellular and molecular biology have led to the development of novel therapeutic strategies that aim at the regeneration of oral tissues that were injured by disease or trauma.

Dental Stem cells research has given newer conceptual approach to therapy of various diseases named “regenerative dentistry” and it will have its place in the clinical practice of dentistry in the future. This review, discusses the types of dental stem cells, their banking and their possible application in treatment of diseases in future.

Key words: Banking of dental stem cells, Regenerative Medicine, Scaffolds of Dental Stem Cell.

INTRODUCTION

The origin of the term ‘‘stem cell’’ can be traced back to the late 19th century. The term stem cell appears in the scientific literature as early as 1868 in the works of the eminent German biologist Ernst Haeckel. He used the term ‘‘Stammzelle’’ (German for stem cell) in two senses: as the unicellular ancestor of all multicellular organisms and as the fertilized egg that gives rise to all cells of the organism[1].

It was a Russian histologist named *Alexander* Maksimov who in 1908, first put forward the existence of the stem cell (and coming up with the term himself) as part of his theory of haematopoiesis. According to him, all cellular blood components were derived from haematopoietic stem cells[2]. In parallel, Artur Pappenheim, Ernst Neumann, and others used it to describe a proposed progenitor of the blood system[1].

It was in 1963 when two scientists from Canada, James E. Till and Ernest A. McCulloch, demonstrated the existence of self-renewing cells found in the bone marrow of mice and hence are credited for discovery of stem cells. They defined stem cells as clonogenic cells capable of both self-renewal and multilineage differentiation. Their findings have since paved the way for later scientists to make their own discoveries in this area of research[3]. From

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then till now Stem cells have been isolated from many tissues and organs, including dental tissue.

Properties of Stem Cells

Stem cells exhibit common inherent properties as Self-renewal capacity and potency.

a) Self-renewal means the ability to go through numerous cycles of cell division while maintaining the undifferentiated state[4].

b) Potency means genetically 'consistent' cell differentiation potential of stem cell[5].

Types of Stem Cells

In general stem cells can be divided into two types:

1. Embryonic stem cells
2. Non-Embryonic stem cells

a) Adult stem Cells
   I. Hematopoietic stem cells
   II. Mesenchymal stem cells

b) Induced pluripotent stem cells[7]

Embryonic stem cells

Human embryonic stem cells (ES cells) are primitive (undifferentiated) cells that can self-renew or differentiate into all cell types found in adult human body. They are found in the inner cell mass of the human blastocyst, an early stage of the developing embryo lasting from the 4th to 7th day after fertilization[5].

The possible sources of stem cells are embryos were created via:

1) In vitro Fertilization (IVF) (by Lanzendorf et al., 2001), embryos or fetuses obtained through elective abortion and

2) Embryos created via somatic cell nuclear transfer (SCNT) or cloning[7].

Adult stem cells

Stem cells that are found in developed tissue, regardless of the age of the organism at the time are referred to as adult stem cells[5]. They are derived from various sources as

- Bone Marrow & circulating blood
- Umbilical cord
- Placenta
- Perivascular area of small vessels
- Adipose tissue
- Heart
- Skeletal muscle
- Synovial tissue
- Pancreas
- Sources from dental tissues [6].

Induced pluripotent stem Cells

Series of landmark experiments have shown that the pluripotency of ESCs depends on the expression of four transcription factors:- Oct3/4, Sox2, c-myc, and Klf4 while the homeobox protein Nanog acts to prevent differentiation. Many adult cells like human fibroblasts, mature B lymphocytes, gingival fibroblasts etc. have been reprogrammed into pluripotent cells by the transduction of these four genes. The reprogrammed cells known as iPS cells, are able to generate cells from endodermal, mesodermal and ectodermal origins. Thus iPS cells may become a source of cells for patient specific stem cell therapy[8].

Concept of Dental Stem Cells

Tooth development results from sequential and reciprocal interactions between the oral epithelium and the underlying ectomesenchyme[9]. Hence, two different populations of stem cells have to be considered: epithelial stem cells (EpSC), (precursor of ameloblasts) and mesenchymal stem cells (MSC) (precursors of odontoblasts, cementoblasts, osteoblasts and fibroblasts of the periodontal ligament). Although significant progress has been made with MSC, there is no information available for dental EpSC in humans because dental epithelial cells such as ameloblasts and ameloblasts precursors are eliminated soon after tooth eruption[10].

Dental Mesenchymal stem cells have mesenchymal stem cell-like (MSC) qualities, including the capacity for self-renewal and multilineage differentiation potential[11]. The presence of stem cells in periodontal ligament was first proposed approximately by Melcher (1985), who queried whether the three cell populations of the periodontium (cementoblasts, osteoblasts and periodontal ligament fibroblasts) were derived from a single population of ancestral cells or stem cells[12]. Till date five different human dental stem/progenitor cells have been isolated and characterized:

1. Dental Pulp Stem Cells (DPSCs) [Gronthos et al (2000)]
2. Stem Cells From Exfoliated Deciduous Teeth (SHED) [Miura & Gronthos et al (2003)]
3. Periodontal Ligament Stem Cells (PDLSCs) [Seo et al (2004)]
4. Stem Cells From Apical Papilla (SCAP) [Sonoyama et al (2006)]
5. Dental Follicle Progenitor Cells (DFPCs)[13] [Yao S etal (2008)]

Possible application of these cells in various fields of medicine makes them a powerful tool for future research in therapeutics and tooth tissue engineering[14].

Differentiation potential of Dental Stem Cells (DSCs)

DPSCs, SHED and PDLSCs have a high proliferative potential[15, 16]. DPSCs can proliferate into Odontogenic, Osteogenic, Chondrogenic, Adipogenic, Neurogenic and Myogenic tissues [17,18]. SHED were identified to be a population capable of differentiating into a variety of cell types including neural cells, adipocytes, and odontoblasts[16]. PDLSCs could differentiate into Osteogenic, Odontogenic, cementogenic[12] and neurogenic tissue types[19,20]. SCAP and DFPCs have a multi differentiation potential [21, 22]. SCAP can differentiate into dentinogenic, osteogenic and adipogenic tissue[21]. DFSCs can differentiate toward a cementoblast, osteoblast or periodontal ligament lineage[22]. DPSCs, SHED, PDLSCs show immunosuppressive characteristics just as mesenchymal stem cells of other body tissues henceforth their possibilities of immunorejection are lowered[15, 23].

Isolation of DSCs

The tissue is isolated from crown and/or root and then enzymatically digested in a solution of collagenase and dispase. Cell suspensions obtained are seeded into Dulbecco's modified Eagle's medium (DMEM) which is a culture medium supplemented by various nutritional and growth factors and is incubated at 37°C in 5% CO_2[16,24].

Advantages of Dental stem cells

a) Tooth derived stem cells are readily accessible and provide an easy and minimally invasive way to obtain them.
b) Banking one's own tooth derived stem cells is a reasonable and simple alternative to harvesting stem cells [25].
c) They show good interaction with scaffolds[4].
d) They have a high proliferative and multi differentiation potential [15,16,21,22].

Banking of Dental stem cells

Once the tissue samples are obtained they are placed in a vial which is sealed and placed into the thermette (a temperature phase change carrier) and this is further transported in insulated transport vessel which maintains the sample in a hypothermic state during transportation. This procedure is described as Sustentation. The sample should reach the tooth bank facility before 40 hours. After tooth bank receives the vial, Stem cell isolation is done and they are stored by either

1) Cryopreservation

2) Magnetic Freezing techniques [26].

Cryopreservation or banking of stem cells maintains the viability of these cells indefinitely. "Cryo" means cold in Greek and cryopreservation is a process in which cells or whole tissues are preserved by cooling to subzero temperatures, typically −196°C[27]. This routine procedure generally involves slow cooling at 1 to 2 °C/min in the presence of a cryoprotectant, Dimethyl SulphOxide (DMSO) to avoid the damaging effects of intracellular ice formation which is considered standard [28].

Magnetic freezing uses the concept that applying even a weak magnetic field to water or cell tissue will lower the freezing point of that body by up to 6-7° C. It ensures distributed low temperature without the cell wall damage caused by ice expansion and nutrient drainage due to capillary action, as normally caused by conventional freezing methods. Then, once the object is uniformly chilled, the magnetic field is turned off and the object snap freezes[26].

DPSCs can be stored at -85°C or -196°C for at least six months without loss of functionality. These promising cryobiological properties as well as their multi-potentiality make DPSCs an ideal choice for clinical banking[25].

Banking of PDLSCs is done by CAS cryopreservation method. In this method, magnetic freezing rather than cryogenic freezing is used[26].

SCAFFOLDS FOR DENTAL STEM CELLS

Cells require interactions with their microenvironment to survive, proliferate and function. Scaffolds provide an environment that allows for the adhesion of cells and their proliferation, migration and differentiation until these cells and the host cells begin to secrete and shape their own microenvironment. Scaffolds are made of synthetic polymers. The most common synthetic polymers in tissue engineering are likely

- Poly-l-lactic acid (PLLA)
- Poly-glycolic acid (PGA) and
- Copolymer poly-lactic-co-glycolic acid (PLGA) [29].

Stem cells are loaded in an appropriate scaffold to close the defects or replace the organ. Scaffolds can be of different shapes, patterns and biomaterials [4].

APPLICATION OF DENTAL STEM CELLS IN REGENERATIVE MEDICINE

Regenerative medicine is a new scientific and medical discipline focused on harnessing the power of stem cells and the body's own regenerative capabilities to restore function of damaged cells, tissues and organs. Stem cells are being explored for a variety of chronic debilitating diseases that have so far escaped remedial measures of conventional treatment[30].

Regenerative medicine is defined as "the process of replacing or regenerating human cells, tissues or organs to restore or establish normal function". This field holds the promise of regenerating damaged tissues and organs in the body by replacing damaged tissue and/or by stimulating the body's own repair mechanisms to heal previously irreparable tissues or organs[5]. Regenerative medicine also includes the new field of tissue engineering. As stated by Langer and Vacanti, tissue engineering is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain or improve tissue function or a whole organ"[13].

Currently, there are two main branches of research of tooth tissue regeneration.

I. Partial tooth restoration:-

This line of research focuses on using existing reparative capacities of the tooth and/or use of tooth-related stem cells for repair of damaged tooth parts.

II. Whole tooth regeneration [15].

It requires the three key elements to combine together to form the basic framework for the formation of engineered tooth tissues

a. Progenitor/stem cells;
b. Inductive morphogenetic signals in an environment conductive to regeneration of a vital and functional tissue and/or organ; and
c. An extracellular matrix scaffold (which can be synthetic) [31].

Following are the possible applications of dental stem cells in tooth tissue engineering/regenerative medicine

I. Partial tooth restoration:-

Role in Regeneration of Dentin /Pulp

- DPSCs are capable of forming reparative dentin-like structure on the surface of human dentin, indicating the possibility and challenge of using DPSCs to repair tooth structures and to seal root canals[32].
- SHED can be used as a tool in dental pulp tissue engineering as it is proved that it can form pulp which is architecturally similar to normal pulp.

- Stem cells harvested from third molars can be utilized to regenerate the pulp of severely injured tooth thereby preventing the need for endodontic treatment in adults.

- Huang G.T. (2009) in his recent study demonstrated *de novo* regeneration of dental pulp in emptied root canal space using DPSC and SCAP isolated from human third molars. Moreover, a continuous layer of mineralized tissue resembling dentin was deposited on the existing dentinal walls of the canal[33].

Role in Periodontal Regeneration

- PDLSCs can differentiate into cells that can colonize on the biocompatible scaffold suggesting an easy and efficient autologous source of stem cells for regeneration of dental tissues.

- Patients treated with autologous DPSCs showed consistently enhanced regeneration of the bone defect as judged by gain of vertical bone height.

- Bone Marow Mesenchymal Stem Cells (BMMSCs) have been used by Kawaguchi et al for their capability to regenerate periodontal tissue and repair periodontal defects proving them an alternative source for the treatment of periodontal diseases[12].

II. Whole Tooth Regeneration

The regeneration of adult teeth would be possible in future with the newer advancement in the stem cell therapy and tissue engineering. This regenerative procedure may replace synthetic dental implants. Also, by providing a biological solution to the problem of tooth loss, the procedure would transform the prognosis for edentulous patients as well as for patients with severely diseased teeth or diminished jawbone.

Team of Malcolm Snead after series of experiments concluded that cap stage was the ideal point in tooth development for transplantation because it contains all the cell types and associated signals required to form the adult tooth, as well as the ability to induce the host to provide vasculature and innervation. According to them an approved clinical procedure of tooth regeneration, via an implanted bio manufactured cap stage, could be realized by 2017.

Proposed steps in Regenerating a Tooth via a Cap-Stage Implant are:-

1. Harvest adult stem cells (bone marrow stromal stem cells or tooth-derived postnatal stem cells) or employ NIH-approved human embryonic stem cells

2. Expand the cells in culture, with cell banking for future organ regeneration needs.

3. Seed the cells into scaffold that provides an optimized biochemical and biomechanical environment.

4. Instruct the cells with spatially targeted, soluble molecular signals and/or induce with porcine sources of odontogenic tissue.

5. Confirm that the gene expression profile of the cells demonstrates readiness for the next stage in the odontogenesis pathway.

6. Repeat these steps until the cells have expressed genes associated with the cap stage of odontogenesis[34].

Experimental studies with an animal model have shown that the crown tooth structure can be regenerated using tissue engineering techniques that combine stem cells and biodegradable scaffolds. Epithelial mesenchymal interactions are mandatory in tooth development[12].

Making entire teeth with enamel and dentin structures in vivo is a reality and not an utopia[9]. Some of the researches done in this direction are as follows:-

- Dualibi et al. in their experimental studies were able to form tooth structures from single cell suspensions of cultured rat tooth bud cells and bioengineered rat teeth developed in 12 weeks with scaffold.

- Honda et al. developed tissue engineered teeth using porcine tooth bud cells[22].

- Nakao *et al.*, using epithelial and mesenchymal cells seeded in collagen scaffold, in tooth cavity of adult mice showed the presence of all dental structures such as odontoblasts, ameloblasts, dental pulp, blood vessels, crown, periodontal ligament, root and alveolar bone[9].

Challenges in Tooth Regeneration

The most challenging part of tissue regeneration is perhaps the functional tissue engineering and regeneration. Following are some of the challenges in functional tooth regeneration:

a) Vascularization: One of the critical challenges of dental pulp tissue engineering is the generation of a functional vascular network, considering the anatomical constraints imposed by the fact that all vascularization must access the root canal through the apical foramen. To achieve a good blood supply, optimal cell density and the laying down of high quality extracellular matrix should occur. Synthetic scaffolds such as PLG can be fabricated with impregnated growth factors like VEGF, PDGF which can enhance and accelerate the pulp angiogenesis. Alternatively, the insertion of engineered pulp tissue may have to be separated into multiple steps.

b) Innervations: Since the newly generated dentin does not appear to have well organized dentinal tubules and is similar to reparative dentin, even if the regenerated A-δ fibers reach the pulp - dentin junction, it may not cause the normal dentin sensitivity as the natural teeth.

c) How to Regenerate Enamel: Enamel cannot self-regenerate; therefore, engineering approaches have to take place. Enamel regeneration has been tested by various acellular methods using recombinant enamel proteins amelogenin, surfactants or using simple chemicals like calcium phosphate containing solutions/paste to restore the enamel layer. However, these approaches appear to be difficult to apply clinically or can only produce minimal amount of regenerated enamel on existing natural enamel. Shinmura Y et al concluded that the epithelial rests of Malassez (ERM) are a group of cells that remain during root formation; thus, these cells are present in adult teeth and can be isolated and cultured. When ERM cells are maintained in vitro on feeder layers, they can be induced to form enamel-like tissues following recombination with primary (uncultured) dental pulp cells. Thus, the final step of restoring tooth after pulp and dentin regeneration is likely to still require the use of artificial materials[35].

Other possible Applications of DSCs in tissue engineering/regenerative medicine

DSCs in Nervous tissue regeneration

- DPSCs differentiate into functionally active neurons and implanted DPSCs induce endogenous axon guidance, suggesting their potential as cellular therapy for neuronal disorders[36].

- SHED holds enormous potential for therapeutic treatment of various diseases like:- neuronal degenerative disorders such as Alzheimer's, Parkinson's and also repair of motor neurons following stroke or injury. This all is due to their ability to produce and secrete neurotrophic factors.

- SHED could also be used for the treatment of paralysis due to spinal cord injury [37,38].

DSCs in Cardiac repair

- DPSCs can help cardiac repair after myocardial infarction due to its ability to secret different growth factors and cytokines.[9]

- SHED can also play an important role in treatment of Chronic heart conditions such as congestive heart failure, chronic ischemic heart disease[37,38].

DSCs in regeneration of osseous tissue

- DPSCs are able to differentiate in osteogenic lineage both in 2D and 3D surfaces, creating osteoblast-like cells that express specific osteogenic markers and produce mineralized ECM. This tissue engineering approach could be a promising tool to restore bone defects and deficiencies, congenital or acquired pathologies and secondary traumas characterized by critical bone mass defects.

- DPSCs can also be used to colonize prosthesis and orthodontic implants in order to optimize the osteo-integration and the efficiency of the implant itself[36].

- The ability to design anatomically viable and functional bone would have great potential for oromaxillofacial reconstructions of congenital defects and cancer resections[4].

Other applications

- Tissue engineered temporomandibular joint (created by making condyle shaped scaffolds and then stem cells seeded into them),[38] bone grafts, other engineered joints and cranial sutures can be developed with stem cell therapy[4].

- Experimental evidence indicates that SHED transplantation is an effective approach for treating immune disorders like SLE via improved immunomodulatory properties[37, 38].

- Role of SHED in wound healing: - YudaI Nishino et al have shown that SHED enhances wound healing by promoting re-epithelialization and the relationship with the extracellular matrix, especially hyaluronan. Deciduous teeth, which are considered to be medical waste, could provide novel therapeutic approaches for the treatment of wounds and novel stem-cell sources for wound healing [40].

- DFPCs could be a useful cell source for tissue engineering therapy[22]. These cells may be useful as a research tool for studying PDL formation and for developing regeneration therapies[13].

- DFPCs could be an interesting source of undifferentiated cells able to take up a mesenchymal pathway and would be the model of choice to test for the biocompatibility of several restorative dental materials[41].

CONCLUSION

In conclusion, stem cell-based dental tissue regeneration is a new and exciting field that has the potential to transform the way that we practice dentistry. The future of dentistry will be regenerative based, where patient's own cells can be used to treat diseases.

Dental stem cell research is not merely a science fiction, but something that one day will become a part of each dentist's clinical practice[31]. Over the last few years, dentistry has begun to explore the potential application of stem cells and tissue engineering towards the repair and regeneration of dental structures. It is becoming increasingly clearer that this conceptual approach to therapy, named "regenerative dentistry," will have its place in the clinical practice of dentistry in the future[29].

The field of stem cell-based regenerative dentistry is complex and multidisciplinary by nature. Progress will depend on the collaboration between clinicians and researchers from diverse fields (e.g., biomaterials, stem cell biology, endodontics) working together toward the goal of developing biological approaches to regenerate dental and craniofacial tissues[31].

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