STEM CELLS: MILESTONE IN REGENERATIVE DENTISTRY

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ABSTRACT

Stem cells offer an amazing potential for tooth homeostasis, repair, and regeneration. Manipulating dental stem cells in situ and expanding them ex vivo by using specific signaling molecules is an exciting outcome. Nevertheless, stem cell based tooth repair is not devoid of challenges that need to be solved prior to any clinical application. For example, it is crucial to identify the different types of dental stem/ progenitor cells and their niches in teeth in order to understand the mechanisms that support stem cell survival. This knowledge will guarantee the success of stem-cell-based therapeutic approaches in dentistry.

Key words: *Stem cells, Dental stem cells, Tooth repair, Regeneration, Dentistry.*

INTRODUCTION

Stem cells are unspecialized/ primitive cells found in all multi-cellular organisms that are characterized by self-renewal and the capacity to differentiate into diverse specialized cell types.[1,2] These are obtained either from the embryo (ESCs) or from the adults (ASCs). ESCs are initially derived from the inner cell mass of the blastocyst from which many tissues of the embryo arise. They can differentiate into each of the more than 200 cell types of the adult body and are involved in the correction of genetic alterations.[3] ASCs can be defined as self renewing cells, which can differentiate into several functional cell types in vitro and after their reintroduction into an injured organ they are able to functionally restore tissues environment throughout their differentiation into definitive functional cell type. These can be categorized based on their origin into two main groups: germ line and somatic stem cells (Figure1).[4] In addition, induced pluripotent stem cells (iPSCs) also exist that are generated artificially by reprogramming adult somatic cells so that they behave like ESCs.[1]

Bone marrow has been used as an ultimate source of adult mesenchymal stem cells (MSCs) from past several years for various stem cell therapies. Due to certain limitations like tissue site morbidity, low cell numbers and painful procedure for procuring the tissue, alternative sources of MSCs which are often highly vascularized sites including skin, hair follicles, bone marrow, intestine, pancreas, and more recently, dental pulp have been identified.[5]

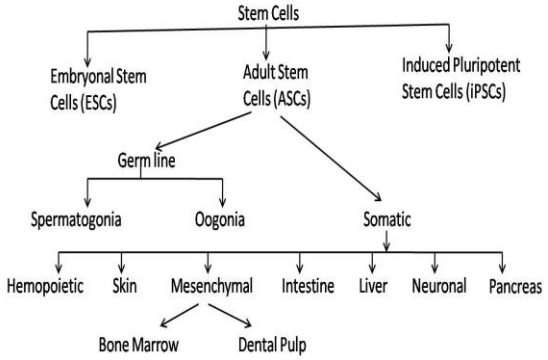

Fig. 1: Flowchart of ASCs based on their origin

DENTAL PULP STEM CELLS (DPSCs)

DPSCs were the first type of dental stem cells to be isolated which were obtained by enzymatic digestion of the pulp tissue. These are fibroblast-like in morphology, clonogenic in nature and can maintain their high proliferation rate even after extensive subculturing.[7] Historically, dental stem cells were first isolated by Gronthos and co-workers from the dental pulp and exfoliated deciduous teeth. Dental stem cells can also be extracted from the apical papilla of shed primary teeth.[8] They are thought to originate from the cranial neural crest which explains their multi- differentiation potential, with the capacity to give rise to various cell lineages such as adipocytes, osteocytes, chondrocytes, and myocytes; odontoblasts, and neuronal cells. DPSCs improve angiogenesis and play a role in cardiac repair. Animal studies revealed osteogenic regenerative potential of DPSCs and clinical studies also characterized their role in bone augmentation in tooth extraction sockets. Moreover, it has been reported that DPSCs display increased immunosuppressive activity. Because of the multipotent nature and immuno-modulatory

properties of DPSCs they may be an important source of MSCs for stem cell based therapies.[5,9-11]

Source of DPSCs

Five different human dental stem/progenitor cells have been isolated and characterized:

1. Human adult dental pulp stem cells (ADPSCs) (Gronthos et al., 2000) [7,12]
2. Stem cells from human exfoliated deciduous teeth (SHEDs) (Miura et al., 2003) [7,12]
3. Periodontal Ligament Stem Cells (PDLSCs) (Seo et al., 2004) [7,12]
4. Stem Cells of Apical Papilla (SCAPs) (Sonoyama et al., 2008) [7,12]
5. Dental Follicle Precursor Cells (DFPCs) (Morsczeck et al., 2005) [7,12]

Human adult dental pulp stem cells (ADPSCs) and stem cells from human exfoliated deciduous teeth (SHEDs) are self-renewing stem cells residing within the perivascular niche/ cell rich zone of the dental pulp. ADPSCs and SHEDs can be obtained without any complications from impacted adult wisdom teeth and naturally exfoliated deciduous respectively. Culturing ADPSCs with various differentiation media demonstrated their dentinogenic, osteogenic, adipogenic, neurogenic, chondrogenic and myogenic differentiation capabilities. Following their trans-plantation in animal models, ADPSCs were able to maintain their self renewal and to form pulp-like tissue, odontoblast- like cells, ectopic dentin as well as reparative dentin-like and bone-like tissues.[7] Deciduous or primary teeth are considered to be the finest source for stem cells.[5,9] In contrast to ADPSCs, the isolated SHEDs did not grow as individual cells, but clustered into several colonies which, after separation, grew as individual fibroblast-like cells. The multi lineage differentiation potential of SHEDs was demonstrated under different inductive conditions. They have the ability to differentiate *in vitro* to neuron-like cells, odontoblasts, osteoblasts, and adipocytes. SHEDs also have a higher proliferation rate and a higher number of colony forming cells which might indicate a more immature form than ADPSCs.[1,7]

Heterogeneity and continuous remodeling of PDL is an indication for the presence of progenitor cells which can give rise to specialized cell types PDLSCs were isolated using the same methodology for ADPSCs and SHED, but this time, the tissue used was from the separated PDL of the roots of impacted human third molar.[7] The isolated cells were able to undergo osteogenic, adipogenic and chondrogenic differentiation when they were cultured with the appropriate inductive medium. Stem cells from the apical papilla (SCAPs) are found in the papilla tissue in the apical part of the roots of developing teeth. The third molars and teeth with open apices are an important source of SCAPs. These cells have the potential to differentiate into osteoblasts, odontoblasts, and adipocytes and show higher rates of proliferation *in vitro* compared with DPSCs. Transplantation of SCAPs and periodontal ligament stem cells (PDLSCs) into tooth sockets of mini pigs allowed the formation of dentin and periodontal ligament (Sonoyama et al., 2006).[1,7]

Dental follicle stem cells (DFSCs) have also been isolated from the follicles of developing third molars. They can differentiate into osteoblasts, adipocytes, and nerve like cells *in vitro* (Kémoun et al., 2007; Couraetal., 2008; Yaoetal., 2008) and form cementum and periodontal ligament *in vivo* (Handaetal., 2002; Yokoietal., 2007).[1,7]

Method of isolation of dental pulp stem cells:

1. After extraction keep the tooth in saline/ fresh milk in a container with frozen gel packs for delivery to the laboratory.
2. Then, it is transferred to the tooth bank into a vial containing hypotonic phosphate buffered saline solution (to prevent the tissue from drying during transport). The vial is then carefully sealed and placed into a thermette, which is then placed into an insulated metal transport vessel.
3. At the tooth bank a stringent protocol is followed for cleaning the tooth surface by various disinfectants.
4. The pulp tissue is isolated from the pulp chamber and the cells are then cultured in a mesenchymal stem cell (MSC) medium. Different cell lines (odontogenic, adipogenic, and neural) can be obtained by making various changes in MSC medium.[13]

Drawbacks of using dental pulp stem cells:

1. Stem cells may have a low survival rate.
2. Transport and isolation of stem cells is critical.
3. Challenges in methodology and clinical translation.
4. Chances of contamination are high.
5. Trained expertises are required.
6. Cost is way to high.[13,14]

Uses of dental pulp stem cells in interdisciplinary dentistry:

1. Dental pulp revascularization via blood clotting in regenerative endodontic to 'revitalize' root canal–treated teeth (pulp regeneration).
2. Tooth and root regeneration.
3. Regeneration of periodontal tissue/ periodontal tissue engineering.
4. TMJ reconstruction.
5. Repair of craniofacial bone and even the replacement or regeneration of oral tissues.
6. Alveolar ridge augmentation and long-bone defects.
7. Mandible reconstruction.

8. Cell and organ models for studying molecular physiology behind processes like tooth eruption.
9. Forensic dental profiling.
10. Identification of a rare subpopulation of cancer cells, termed cancer stem cells, from oral squamous cell carcinoma (OSCC) facilitates the monitoring, therapy, or prevention of OSCC.[13-16]

CONCLUSION

1. Dental pulp is a remarkable site of stem cells;
2. Collecting stem cells from dental pulp is a noninvasive practice that can be performed in the adult during life and in the young after surgical extraction of wisdom teeth, a common surgical practice;
3. Tissue sacrifice is very low when collecting dental pulp stem cells;
4. Several cytotypes can be obtained from dental pulp stem cells owing to their multipotency;
5. 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;
6. Dental pulp stem cells can be cryopreserved and stored for long periods;
7. Dental pulp is ideal for tissue engineering and for clinical use in several pathologies requiring bone tissue growth and repair. In addition, tooth extraction is a clinical/ therapeutical need. 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. The good results obtained up to now reinforce this thought.

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