

Dental Stem Cells- Application In Dental Research And Therapeutic Implication

Research Article

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Abstract

Stem cells are "special cells" that possess pluripotent abilities – they are able to self-renew themselves and maintain the same properties as their progenitor cells. Stem cells have the ability to differentiate into multiple cell lineages. Stem cells are fascinating cells as both embryonic and somatic stem cells when introduced into a specific part of the body can differentiate into the required cell type. Thereby, help in replacing damaged tissue and restoring tissue function.

There are a multitude of cells that are a part of the embryogenesis of the orofacial region that retain pluripotent ability. The dental stem cells are an easily accessible source for stem cells. These cells, when isolated and cultured can be stimulated to proliferate and form viable tissues through chemical or virally induced methods.

However, the potential of dental stem cells in treating dental disorders and diseases is less explored. The objective of this review is to recapitulate the various cells of the orofacial region with pluripotent potential and their application in treating oral and dental disorders. This paper also renders an insight into Biobanking and tissue engineering pertaining to dental tissues. A greater insight and a better understanding of the cellular features and functional properties of dental stem cells will help in developing treatment strategies for dental diseases.

Keywords: Dental Stem Cells; Pluripotency; Regeneration; Biobanking; Tissue Engineering.

Introduction

Stem cells are undifferentiated and pluripotent cells that can produce new stem cells (self-renewal) and in a particular condition or in response to specific signals they can exit from self-renewal and engage in differentiation into specialized cells with specific function [1]. The stem cells are either embryonic stem cells or adult stem cells or perinatal stem cells or induced pluripotent stem cells (iPS). Several populations of stem cells have been isolated from a tooth and from the orofacial region. With advances in technology the pluripotent ability of the stem cells is harnessed to transform them into specialized cells. This has paved the way for stem cells to be used in tooth repair, restoration and in tissue regeneration. Regenerative dentistry is an up-and-coming specialty which involves the replacement of damaged tissue with regenerated hu-

man stem cells. Here we review the various sources of dental stem cells and their application in dental research and their therapeutic application.

Stem Cells Derived From Oral And Maxillofacial Region

Adult stem cells are also known as somatic stem cells or postnatal stem cells, and they are found in most of the tissues and organs. The adult stem cells which reside in several mesenchymal tissues, are jointly referred to as mesenchymal stem cells or multipotent mesenchymal stromal cells (MSCs). These cells can differentiate into limited cell types or cells of mesenchymal origin. MSCs are seen in dental tissues, including dental pulp, periodontal ligament, dental papilla, and dental follicle. These stem cells can be isolated

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and grown under defined tissue culture condition. Once the cells are cultured, they are used in tissue engineering [2].

Most of the adult stem cells of the oral and maxillofacial region are MSC's. The different Mesenchymal stem cells occupy "stem cell niches" in the oral and maxilla-facial region. These MSC's include dental pulp stem cells (DPSCs), stem cells from exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from the apical papilla (SCAP), Tooth germ progenitor cells (TGPCs) Gingiva- derived Mesenchymal Stem cells (GMSCs), dental follicle progenitor cells (DFPCs) and Bone Marrow stem cells (BMSCs), salivary gland stem cells (SGSC's) and Oral Epithelial stem cells [3]. The origin of mesenchymal stem cells of the oral and maxillofacial region was unknown for a long time. It was considered that perivascular cells form mesenchymal stem cells in most tissues. Recent studies have shown that glial cells generate multipotent mesenchymal stem cells that produce pulp cells and odontoblasts. This is consistent with the embryogenesis of tooth formation wherein neural crest cells migrate to form the dental ectomesenchyme [4].

Dental Pulp stem cells

The Dental pulp stem cells can be isolated from the permanent third molar teeth. They exhibit high proliferation rate and a high frequency of colony formation that produces densely calcified nodules [5]. The dental pulp stem cells can form dentin- pulp complexes [6].

Stem cells from exfoliated deciduous teeth

Stem cells are derived from the pulp of human exfoliated deciduous teeth (SHED). These stem cells have a higher proliferation rate, increased cell doubling time and a better osteo-inductive ability compared to Dental Pulp stem cells. [7]. These cells can differentiate into functional odontoblasts and are capable of generating the formation of tubular dentin and angiogenic endothelial cells [8].

Periodontal ligament stem cells

It is well established that the periodontal ligament contains a population of progenitor cells. This population of stem cells are able to differentiate into cementoblast-like cells, adipocytes and connective tissue that is rich in collagen I in both in vitro and in vivo settings [9].

Stem cells from the root apical papilla

Stem cells from the root apical papilla is an unique population of cells that are located at the tip of the developing tooth roots. Recently it has been determined that SCAPs possess the capacity to differentiate into multiple cell types such as osteoblasts, odontoblasts, neural cells, adipocytes, chondrocytes, and hepatocytes [10].

Gingiva mesenchymal stem cells

Gingiva mesenchymal stem cells/progenitor cells are isolated from the lamina propria of the gingiva. These cells are vested with substantial regenerative and immunomodulatory properties [11].

Dental follicle stem cells

The dental follicle is known to contain progenitors for cementoblasts, fibroblasts and osteoblasts. These stem cells can be isolated from the third molar tooth. These cells have the ability to form compact calcified nodules. These cells are typified by their rapid rate of attachment in the culture and their expression of Nestin and Notch -1 [12].

Tooth germ progenitor cell

TGPCS are found in the mesenchyme of wisdom teeth during the late bell stage of odontogenesis [13]. TGPCs can differentiate into dental tissues and non-dental mesenchymal tissues [14].

Oral epithelial stem cells

The basal layer of the oral epithelium contains a stem cell compartment from which the oral mucosa is rejuvenated. These cells expression of several stem cells markersThe basal layer serves as a reservoir of cells with properties of self -renewal [15].

Periosteal derived stem cells

Alveolar bone stem cells (ASMSCs) reside within the osseous tissue. Extracted ASMSCs displayed plastic adherence, colony formation, and spindle-shaped fibroblast-like morphology. ABMSCs had the potential to differentiate into adipocytes and chondrocytes similar to those of other DSCs [16].

Salivary gland stem cells

In the human salivary gland, stem cells reside in the ductal region, as indicated by the co-localization of stem cell markers [17]. The intercalated ducts of the adult salivary glands has been recognized to harbor a stem cell population capable of giving rise to both acini and ducts [18].

The molecular characteristics of the dental stem cells is given in Table 1.

Induced Pluripotent Stem Cells

Takashi and Yamanaka found that somatic cells can be genetically reprogrammed to achieve a pluripotent capacity by introducing defined factors such as Oct3/4, SO2, Klf4 and c-Myc [21].

By introducing 3-4 transcription factors, the oral mesenchymal cells such as dental pulp stem cells, stem cells from exfoliated deciduous teeth, SCAP and stromal cells derived from the third molars can be stimulated to generate iPS [22, 23]. Fibroblasts from the gingiva is a good source for iPS. These cells are easily available from patients and they have a high reprogramming capacity [24]. Adult human gingival fibroblasts can be reprogrammed without using the transcription factor c-myc [25].

Induced pluripotent stem cells (iPS) in regenerative dentistry

There are studies that have been carried out where in iPS cells have been used to produce cementum, alveolar bone and ameloblasts [26, 27]. Ning et al stated that for formation of tooth to

Table 1. Molecular characteristics of dental stem cells.

Dental Stem cells	Source	Biomarker expressed	Authors
Dental pulp stem cells	Dental Pulp	CD105 ⁺ CD 13 ⁺ CD 73 ⁺	Yang et al [19]
Stem cells from human exfoliated deciduous teeth	Human exfoliated deciduous tooth	STRO-1 ⁺ CD 146 ⁺ CD 44 ⁺	Yang et al [19]
Periodontal ligament stem cells	Periodontal Ligament	STRO-1 ⁺ CD 146 ⁺	Yang et al [19]
Stem cells from the Apical Papilla	Apical Papilla	STRO-1 ⁺ CD 146 ⁺	Yang et al [19]
Gingival stem cells	Gingival	CD 146 ⁺ CD 105 ⁺	Yang et al [19]
Dental Follicle stem cells	Dental follicle	CD 44 ⁺ CD 90 ⁺ CD 150 ⁺ STRO-1 ⁺	Yang et al [19]
Oral Epithelial stem cells	Basal layer of the Oral Epithelium	CD44, Bmi1 SOX 2 Keratin 14	Papagerakis et al [15]
Dentin follicle stem cells	Dental Follicle	Nestin Notch	Lin et al [12]
Periodontal ligament stem cells	Periodontal ligament	CD90	Kadkhoda et al [20]
Stem cells from human exfoliated deciduous teeth	Dental Pulp	TGF β	Nakumara et al [7]

take place there is a need for an interaction of epithelium derived from the ectoderm with the mesenchymal cells that are derived from neural crest cells. Hence the cells that are induced to have a pluripotent capacity should be able to differentiate into two lineages with one being odontogenic in nature [28]. Through the combination of iPS cells and enamel matrix derivatives, formation of cementum, bone and periodontal ligaments has been achieved [27]. This expertise can reform the field of medicine because of IPS cells have the capacity to develop into all tissues/organs. These IPS cells can play a vital role in the developing field of “tailored medicine” which uses a patient’s own cells to provide biologically compatible tissues [29].

Dental stem cell banking

The advantages of dental stem cells include guaranteed matching of donors, cells are saved before they are damaged, acquiring them is a simple and a painless procedure, they are less expensive and there are less ethical issues related to their clinical application. The disadvantages of dental stem cells are that only a few cells are available for isolation and that there is no proper research directed towards understanding their long-term efficiency [30]. With the realization that dental pulp stem cells are a major source of stem cells and that they can be utilized for tissue engineering and in the treatment of dental and medical diseases “tooth stem cell banks” have emerged [31, 32].

The fundamental steps in dental biobanking involves sample collection (whole tooth under sterile conditions), stem cell isolation,

stem cell storage and stem cell usage [33]. The various sources of dental stem cells and the avenues where they can be utilized is given in Fig 1. The benefits of biobanking of stem cells is given in Fig 2.

Application Of Dental Stem Cells

Dental Material research

Evaluation of biological effects of dental materials is empirical for its ultimate clinical usage. Several types of cells have been used for this purpose ranging from primary cultured fibroblasts to immortalized cell lines [34].

Regenerative Dentistry

Regenerative dentistry refers to designing biological alternatives for root canal treatments, regeneration of dental hard tissues, revascularization and regrowth of lost periodontal tissues [35].

Pulpstem cells

Owing to their highly proliferative and clonogenic nature, DP-SCs can differentiate into hard tissue forming cells. Damaged odontoblasts can be replaced with newly regenerated odontoblast-like cells that are derived from the DPSCs [36]. DPSCs have also been implicated in regeneration of tooth pulp following endodontic infection [37].

Figure 1. The origin of dental stem cells and their application in oral research and therapeutics.

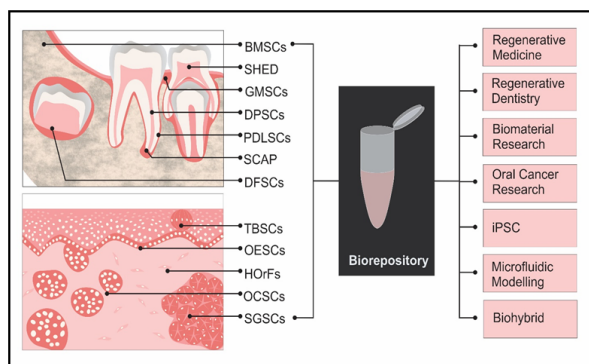
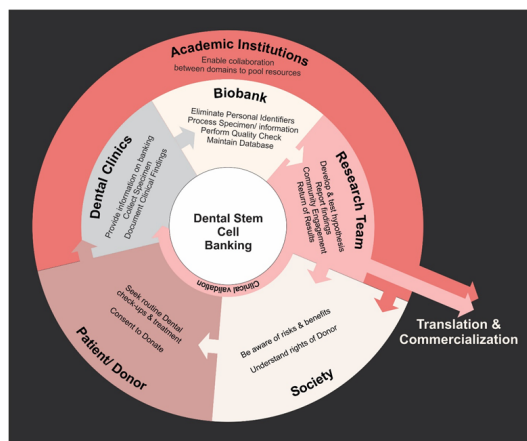


Figure 2. Application of Biobanking.



In another study a 3-dimensional cell construct comprising of DPSC's facilitated the regeneration of the dental pulp. When a 3-dimensional (3D) cell constructs composed of DPSC's was filled in a human root canal, pulp-like tissues with rich blood vessels were formed within the human root canal 6 weeks after the implantation [38].

Periodontal Ligament

Periodontal ligament stem cells that are isolated from miniature pig have similar properties as that human periodontal ligament stem cells. This implies that PDLSCs could assist in periodontal tissue regeneration in vivo. This kind of cellular therapies can be used to treat periodontal disease and to minimize tooth loss [39]. However, Li et al observed that PDLSCs derived from resorbed primary teeth (humans) expressed RUNX2, which upregulated RANKL and downregulated OPG. These imbalances between RANKL and OPG finally led to osteoclast differentiation and root absorption [40].

Salivary Gland

Salivary gland stem cells obtained from human submandibular salivary gland were cultured to form salispheres. When these cultured salispheres were transplanted into submandibular gland of irradiated (to ablate the salivary glands) mice. The transplanted stem cell salispheres were able to differentiate and produce saliva. This shows that human SGs contain stem/progenitor cells capable of self-renewal and differentiation to functional cells that can produce saliva. This can be very beneficial in treating patients with hyposalivation [41].

Dental stem cells and tissue Engineering

Tissue engineering comprises of three key elements: A scaffolding /extracellular matrix, stem cells/Progenitor cells and signaling molecules/ growth factor.

Scaffolds provide a structural integrity for cells until the newly formed tissue becomes auto sustainable. Synthetic scaffolds such a poly-(1-lactic acid) PLLA, Poly- (glycolic acid) PGA allow engineering of complex dental structures with characteristics that resemble the crowns of natural teeth [42].

When SHED cells were seeded in scaffolds surrounded with dentin, they differentiated into odontoblasts. These odontoblasts expressed DMP-1, sialophosphoprotein and matrix extracellular phosphoglycoprotein [43].

Bone morphogenic proteins, Dentin sialoproteins, Dentin phosphoproteins and Vascular endothelial factor that are present in the scaffold assist in differentiation of dental stem cells. It has been reported that SHED cells were able to differentiate into endothelial cells that make functional blood vessels that carry blood [8].

Whole tooth engineering

This technique refers to regeneration of an entire tooth complex of Enamel, dentin and cementum encasing the dental pulp with stroma blood vessels. Whole tooth engineering technology involves re-association between embryonic dental epithelial and mesenchymal cells. Many methods have been proposed to carry out this process. The hanging-drop technique provides a 3D architecture to enable cell-cell adhesion [44].

Regenerative Medicine

The stem cells of the orofacial region do not express HOX genes, and hence their cross-transplantation can result in the formation of mature bone directly without the intermediate stage of cartilaginous callus formation [45].

DPSCs were found to express a number of angiogenic factors such as platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF) [46]. Therefore, DPSCs are attractive stem cell source for the treatment for chronic wounds, myocardial infarction, and stroke.

3D cell culture

Jimenez et al carried out an in vitro study wherein dental pulp mesenchymal stem cells were cultured on 3D scaffolds of Polylactic and polyglycolic scaffolds incorporated with hydroxyapatite (HA) under osteogenic conditions. They found that the HA in the scaffold accelerated cell interaction and osteogenic differentiation of the hDPSC's. The cultured hDPSC's showed a high expression of osteogenic markers Runt-related transcription factor 2 (RUNX2), Osteopontin (OPN), Alkaline Phosphatase (ALP) and Collagen I (COL_1). Hence this technology can be effectively utilized in bone tissue engineering and regenerative medicine [47].

A fibrin-based bio-ink has been designed for human dental pulp stem cells (hDPSC). The morphology of the hDPSC was well-maintained even after 25 days of cell culture. The expression of DMP1 and DSPP (as evaluated through RT-PCR) by the hDPSC increased with the concentration of fibrin in the bio-ink. This indicates that Bio printing of hDPSC's can induce localized odontogenic differentiation [48].

Cell Sheet

Cell sheets technique is a process by which the cultured cells are harvested as intact sheets with an extracellular matrix. In a clinical study by Iwata et al, a three-layered periodontal ligament MSC's cell sheet was transplanted onto the clean root surface of teeth with periodontal disease in ten patients. A six month follow-up of these patients showed that there was reduced periodontal pocket probing depth, there was gain in the clinical attachment and an increase in the radiographic bone height in all the 10 patients [49].

Micofluidic modelling

The behaviour of stem cells in the orofacial region depends on the cellular, molecular and physiological conditions of their microenvironment, the stem cell niches. Micofluidic technique provides an environment for stem cells in culture media that mimics the microenvironment of stem cells niches of the orofacial region. This method provides a better microenvironment for cross-talk among stem cells. The dental stem cell cultured this way will be morphologically and functionally suitable for regeneration of damaged or pathological tissues and organs of the orofacial region [50].

Conclusion

To summarize, with a wide range of dental stem cell types that

have been identified and with advances in technology, numerous avenues are open for dental stem cells in dental research and therapy. Although most of the biological potential of dental stem cells has been deciphered through in vitro studies, the clinical trials that are being carried out are also showing positive results. The paramount target in dentistry is to be able to replace lost teeth using biological tissues; in essence a cell-based implant rather than a metal one. The task that lies ahead is to be able to generate a biologically functional root. Therefore, with advances in technology and empowered with skills in cell culture and molecular biology more research in the field of dental stem cells can be carried out. These research outcomes can greatly contribute towards treating dental and oral diseases with precision.

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