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Stem Cells In Dentistry

[1] Meetali Guleria
[2] Harpreet Dua
[3] Sushant Rohila
[4] Anil Kumar Sharma
[1] PG Department of Oral Pathology
[2] Reader
[3] Reader
Department of Prosthetics
HIDS Paonta Sahib.
[4] Dentist, ECHS, Panchkula.
Address For Correspondence:
Dr. Meetali Guleria
PG Student, Dept. of Oral Pathology
Himachal Institute of Dental Science, Paonta Sahib

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Abstract
Stem cells constitute the source of differentiated cells for the generation of tissues during development, and for regeneration of tissues that are diseased or injured postnatally. Several types of dental SCs have been identified, including dental pulp SCs from adult human dental pulp, SCs from human primary exfoliated deciduous teeth, periodontal ligament SCs, and dental follicle SCs from human third molars In recent years, stem cell research has grown exponentially owing to the recognition that stem cell-based therapies have the potential to improve the life of patients with conditions that span from Alzheimer's disease to cardiac ischemia to bone or tooth loss. 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 pulp is considered a rich source of mesenchymal stem cells that are suitable for tissue engineering applications. It is known that dental pulp stem cells have the potential to differentiate into several cell types, including odontoblasts, neural progenitors, osteoblasts, chondrocytes, and adipocytes. The dental pulp stem cells are highly proliferative. This characteristic facilitates ex vivo expansion and enhances the translational potential of these cells. Notably, the dental pulp is arguably the most accessible source of postnatal stem cells. Collectively, the multipotency, high proliferation rates, and accessibility make the dental pulp an attractive source of mesenchymal stem cells for tissue regeneration.

Key Words
Stem Cells, Embryonic, Oral and Maxillofacial Region

Introduction

The human body performs a variety of functions essential for its survival and healthy existence which is made possible by the ability of the tissues to undergo renewal or regeneration following trauma or disease. This renewal or regeneration of tissues is possible due to the existence of a unique set of unspecialized cells – "The Stem Cells". These cells are unique because of their potential ability for unlimited replication and capacity for differentiation into other cell types from the same embryonic germ layer or to transdifferentiate into cells from a different germ layer.[1]

What Are Stem Cells

The term stem cell was proposed for scientific use by Russian histologist Alexander Maksimov in 1909. Alexander Maximov 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 microecological niches that would allow them to proliferate and differentiate along specific. While research on stem cells grew out of findings by Canadian scientists in the 1960s. Based on their origin, there are two main types of stem cells: embryonic stem cells (ES cells) and postnatal or adult stem cells (AS cells). Embryonic stem cells were harvested from embryos, they are cells derived from the inner cell mass of the blastocyst (early stage embryo, 4-5 days old, consist of 50-150 cells) of earlier morula stage embryo. In other words these are the cells that form the three germ layers, and are capable of developing more than 200 cell types. In 1998 the first human embryonic stem cell line was derived at university of Wisconsin-Madison[2].

Stem cells should possess two characteristic features: first, they must be able to renew themselves and, second, they must have the capacity for multilineage differentiation. However, stem cells have varying degrees of potential. This ranges from the totipotency (ability to form the embryo and the trophoblast of the placenta) of the fertilised oocyte (the zygote), to the pluripotency (ability to differentiate into almost all cells that arise from the three germ layers) of ES cells, to the multipotentiality (capability of producing a limited range of differentiated cell lineages appropriate to their location) of most tissue based stem cells, and lastly to the unipotentiality (only able to generate one cell type) of cells such as the epidermal stem cells and the spermatogonial cells of the testis.[3]

Classification of Stem Cells

Depending on their residency, stem cells are classified in two categories: ESCs and adult stem cells, which are also called tissue-specific stem cells (TSSCs), derived either from a fetus or a postnatal individual.

a) Embryonic stem cells: refer to the cells of the inner cell mass of the blastocyst during embryonic development. ES are particularly notable for their two fundamental properties: the capacity to differentiate into any cell type in the body and the ability to self replicate for numerous generations. One potential disadvantage of human ES, besides ethical issues, is precisely their virtually unlimited proliferation and differentiation capacity. The clinically observed teratoma is an example of ES growing into wrong tissues. To date, little attempt has been made towards the use of ES in dental, oral and craniofacial regeneration.
b) Adult stem cells are undifferentiated multipotent or unipotent cells that are found in most adult tissues.They have

beenharvested from different kind of tissues like bone marrow,umbilical cord, amniotic fluid, brain tissue, liver, pancreas cornea, dental pulp, and adipose tissue. Adult stem cells are comparatively easier to isolate and do not have any ethical issues. Immune rejection and teratoma formation is also rare with adult stem cells. Adult stem cells are commonly used in current day practice.[4]

Sources Of Stem Cells

The oral and maxillofacial region provides sources of stem cells from the following region:-

- Stem cells in the dental follicle
- Stem cells in the dental pulp
- Stem cells from human exfoliated deciduous teeth
- Stem cells in the periodontium
- Stem cells from the Apical Papilla

Stem cells in the dental follicle

One important biological function of the dental follicle is the coordination of tooth eruption. Moreover this tissue harbors progenitor cells for the periodontal ligament. The differentiation and function of dental follicle cells are controlled by a network of regulatory molecules including growth factors and cytokines. It is thought that the dental follicle cells near the forming root (innermost) differentiate into cementum forming cementoblasts and that cells towards the alveolar bone (outermost) differentiate to osteoblasts secreting bone matrix. Dental follicle cells found centrally between the cementoblast and osteoblast precursor cells develop into fibroblasts producing the extracellular matrix of the periodontal ligament. Morsczeck and co-workers reported the isolation of precursor cells derived from dental follicle of human third molar teeth. These fibroblast-like, colony forming and plastic adherent cells expressed putative stem cell markers; Notch-1 and Nestin. It has also been shown that long-term cultures with dexamethasone produced compact calcified nodules or appeared as plain membrane structures of different dimensions consisting of a connective tissue like matrix encapsulated by a mesothelium-like cellular structure. Therefore, these results demonstrated that cultured precursor cells are unique undifferentiated lineage committed cells residing in the periodontium prior or during tooth eruption. In a recent study, it was hypothesized that stem cells may be present in the dental follicle and be capable of differentiating into cells of the periodontium. At the same field of another study, it was demonstrated that human third molar pad possesses neural crest-derived cells that represent multipotent stem/progenitor cells. For future studies, however, the dental follicle's stem cells will need to be further characterized.[5]

Stem cells in the dental pulp

The dental pulp is the part in the center of a tooth made up of living soft tissue and cells called odontoblasts. The central region of the coronal and radicular pulp contains large nerve trunks and blood vessels. This area is lined peripherally by a specialized odontogenic area which has three layers which are (from innermost to outermost): cell rich zone, cell free zone and odontoblastic layer.

During tooth formation, interactions between epithelial and dental papilla cells promote tooth morphogenesis by stimulating a subpopulation of mesenchymal cells to differentiate into odontoblasts, which in turn form primary dentin. These odontoblasts are thought to arise from the proliferation and differentiation of a precursor population, residing somewhere within the pulp tissue.[6]

It has been speculated that adult dental pulp tissue might also contain a population of multipotential stem cells, and that postnatal dental pulp contains cells that are clonogenic, highly proliferative, and capable of regenerating a tissue, properties that effectively define them as stem cells. It has also been shown that Dental Pulp Stem Cells (DPSCs) were capable of forming ectopic dentin and associated pulp tissue in vivo. Stromal-like cells were re-established in culture from primary DPSCs transplants and re-transplanted into immunocompromised mice to generate a dentin-pulp like tissue, demonstrating their self renewal capability.[7]

Stem cells from human exfoliated deciduous teeth

Exfoliating deciduous teeth contain living pulp remnants are good sources of cells which are highly proliferative, clonogenic and have multi-differentiation potential These cells have been termed as SHED and were isolated and characterized by Miura et al, SHED offers attractive advantages over other post natal stem cells, as they are derived from a source which is non-invasive, readily accessible, naturally being disposed and with very limited ethical or legal concerns. SHED shows a higher proliferation rate, increased cell population doublings, sphere-like cell-cluster formation, osteoinductive capacity, more immature and higher selfrenewal capabilities. They exhibit differentiation ability to convert into adipocytes, neural cells, odontoblasts and osteoblasts. They however exhibit an osteoinductive potential in which the host cells are stimulated to differentiate into bone forming cells. Miura et al also demonstrated the inability of SHED to generate complete dentin-pulp like tissue indicating that perhaps they are immature cells. SHED could not differentiate directly into osteoblasts but did induce new bone formation by forming an osteoinductive template to recruit murine host osteogenic cells. This indicates that deciduous teeth may not only provide guidance for the eruption of permanent teeth, as generally assumed, but may also be involved in inducing bone formation during the eruption of permanent teeth.[8]

They are long lived, grow rapidly in culture, and, with careful prompting in the laboratory, have the potential to induce the formation of specialized dentin, bone, and neuronal cells. If follow up studies extend these initial findings, the scientists speculate they may have identified an important and easily accessible source of stem cells that possibly could be manipulated to repair damaged teeth, induce the regeneration of bone, and treat neural injury or disease.[9]

Dental stem cells can be recovered immediately following exfoliation of a deciduous tooth, but are best recovered after the extraction of deciduous teeth as the teeth become mobile, but still maintain their circumferential gingival attachment. SHED apparently represent a population of multipotent stem cells that are perhaps more immature than postnatal stromal stem-cell populations. Deciduous teeth may not only provide guidance for the eruption of permanent teeth, as generally assumed, but may also be involved in inducing bone formation during the eruption of permanent teeth.[8]

Stem cells in the periodontium

The periodontal ligament is a group of specialized connective tissue fibers that essentially attach a tooth to the alveolar bone within which it sits. These fibers help the tooth withstand the naturally substantial compressive forces which occur during chewing and remain embedded in the bone. Another function of the PDL is to serve as a source of proprioception, or sensory innervations, so that the brain can detect the forces being placed on the teeth and react accordingly.[10]

The periodontal ligament contains a unique assortment of cells that are capable of generating and maintaining three distinct tissues, namely the ligament itself as well as the mineralized tissues on either side of it, i.e. the cementum and the alveolar bone. The major cell types of the periodontal ligament include: Fibroblasts, macrophages and undifferentiated ectomesenchymal cells, cementoblasts and cementoclasts, osteoblasts and osteoclasts, cell rests of Malassez, vascular and neural elements PDL contains heterogeneous cell populations that can differentiate into either cementum-forming cells (cementoblasts) or bone-forming cells (osteoblasts).[11]

Recent findings suggest that PDL cells have many osteoblast- like properties, including the capacity to form mineralized nodules in vitro, expression of the bone-associated markers alkaline phosphatase and bone sialoprotein, and response to bone-inductive factors such as parathyroid hormone, insulin-like growth factor 1, bone morphogenetic protein 2 and transforming growth factor β1. The presence of multiple cell types within PDL has led to speculation that this tissue might contain progenitor cells that maintain tissue homoeostasis and regeneration of periodontal tissue. Using a methodology similar to that utilized to isolate Mesenchymal Stem Cells (MSCs) from deciduous and adult dental pulp, multipotent postnatal stem cells from human periodontal ligament or Periodontal Ligament Stem Cells (PDLSCs) have also been isolated and described. Cultured cells were expanded from single cell suspensions derived from periodontal ligament tissue and the presence of stem cells was determined using antibodies such as STRO-1 and CD146. Under defined cultured conditions, PDLSCs were able to differentiate into cementoblast-like cells, adipocytes and collagen-forming cells.[12]

Stem cells from the Apical Papilla

Amongst various post natal stem cells, stem cells from the apical portion of the dental papilla of human immature permanent teeth happen to be a newly discovered population of stem cells by Sonoyama et al who termed them as Stem Cells of Apical Papilla (SCAP) and studied their physical and histological characteristics. It is known that dental papilla participates in tooth formation and later evolves into dental pulp. In immature teeth, when the roots are still developing, dental papilla assumes a position apical to the pulp tissue and the epithelial diaphragm. This apical papilla is loosely attached to the apex of the root from where it can be easily detached. In between the apical papilla and the overlying dental pulp is present a layer of highly populated cell rich zone. Apical papilla is less cellular and vascular compared to dental pulp but SCAP compared to DPSCs shows a proliferation rate higher by two to three fold.[13]

New Harvesting Techniques

New harvesting techniques are crucial to successful stem cell research because they provide greater opportunities to treat diseases in a more unique case-by-case basis. They also provide ways to overcome challenges with current techniques as well as extending stem cell therapies to diseases that may otherwise have been untreatable by current therapies.

- Altered Nuclear Transfer
- Blastomere Extraction[14]

Stem Cell Culturing

Various techniques for culturing stem cells are:

- Petridishes
- Spinner Bottles
- Rotating Bioreactor
- Hollow Fiber Module
- Perfusion Containers
- Tissue Carriers[15]

Stem Cell Banking

Obtaining stem cells from human exfoliated deciduous teeth (SHED) is simple and convenient, with little or no trauma. Every child loses primary teeth, which creates the perfect opportunity to recover and store this convenient source of stem cells – should they be needed to treat future injuries or ailments and presents a far better alternative to simply discarding the teeth or storing them as mementos from the past. The key to successful stem cell therapy is to harvest cells and store them safely until accident or disease requires their usage. Tooth banking is not very popular, but the trend is catching up, mainly in the developed countries.[16]

Step 1: Tooth collection

With prior informed consent, the first step is to place the tooth in a sterile saline solution or in fresh milk in the storage container along with frozen gel packs. The kit is then ready for delivery to their lab. The tooth exfoliated should have pulp which is red in color, and not necrotic, thus indicating that the pulp received blood flow till the time of removal, which is indicative of cell viability. After its recovery, the tooth is transferred into the vial containing a hypotonic phosphate buffered saline solution, which provides nutrients and helps to prevent the tissue from drying out during transport (up to four teeth in the one vial). The vial is then carefully sealed and placed into the thermette, a temperature phase change carrier, which is then placed into an insulated metal transport vessel. This procedure maintains the sample in the hypothermic state during transportation and is described as sustentation.[17]

Step 2: Stem cell isolation

When the tooth bank receives the kit or vial, all the cells are isolated and the stringent protocol is followed for cleaning of the tooth surface by various disinfectants; isolation of pulp tissue from pulp chamber and cells is then cultured in a mesenchymal stem cell medium (MSC) under appropriate conditions. By making changes in the MSC medium different cell lines can be obtained such as odontogenic, adipogenic, and neural. If cultures are obtained with unselected preparation, colonies of cells with morphology resembling epithelial cells or endothelial cells can be established.Usually cells disappear during the course of successive cell passages. If contamination is extensive, then changes in procedure can be performed: in which STRO-1 or CD 146 can be used. This is considered as most reliable. The time from harvesting to arrival at the processing storage

facility should not exceed 40 hours.[16]

Step 3: Stem cell storage

In the light of present research, either of the following two approaches is used for stem cell storage:

a. Cryopreservation
b. Magnetic freezing

Cryopreservation

Cells are preserved by cooling them to subzero temperatures, at which biological activity is stopped. The cells are preserved in a liquid nitrogen vapor at a temperature of less than - 150° C. This preserves the cells and maintains their latency and potency.[18]

Magnetic Freezing

Hiroshima University uses magnetic freezing rather than cryogenic freezing. The idea of this technique is to completely chill an object below the freezing point, by using a magnetic field, without freezing, thus ensuring, 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 snaps freezes. Using CAS, Hiroshima University claims that it can increase the cell survival rate in teeth to a high of 83%. This system is a lot cheaper than cryogenics and more reliable as well.[19]

Licensed tooth banks are the following

- In Japan, the first tooth bank was established in Hiroshima University and the company was named as 'Three Brackets' (SuriBuraketto).
- BioEden (Austin, Texas), StemSave, and Store-a-Tooth (USA)
- Reliance life sciences, Delhi
- Life call, Chennai.
- Stemade Biotech Pvt Ltd. India.
- The Norwegian Tooth Bank.[20]

Clinical Applications Of Stem Cells

Regrowing Dental Enamel from Cultured Cell

Dental enamel is the hardest tissue produced by the body. It cannot regenerate itself because it is formed by a layer of cells that is lost by the time the tooth appears in the mouth. The enamel spends the remainder of its lifetime vulnerable to wear, damage, and decay.

Although researchers have experienced some success in producing enamel like and tooth like tissues, problems remain to be solved before the technology can be tested in humans. One of the issues has been how to produce, in culture, a sufficient number of enamel-forming cells. There are reports that a new technique is being developed for culturing cells that have the capacity to produce enamel.[21]

Regeneration Of Dentin, Pulp

Dental pulp tissue has the regenerative potential to form dentin in response to any injury. Tubular dentin formation was observed when human pulp stem cells with scaffold (hydroxyapatite/tricalcium phosphate) were implanted in immunocompromised mice. Reparative dentin formation on amputed pulp was found when stem cells were combined with recombinant human bone morphogenetic protein 2 (BMP 2) in experimental studies on animal models.[22]

Regeneration of the pulp inside the damaged tooth can be the basic clinical application of stem therapy in dentistry. Root canal treatment in a young permanent molar will stop Tooth's continuous maturation process there by leaving thin egg shell like weak tooth that is susceptible to fracture. Regeneration of pulp with stem cell therapy will be a better option. Stem cells harvested from the pulp of unwanted teeth like third molar can be utilized to regenerate the pulp of severely injured tooth thereby preventing the need for endodontic treatment in adults.[23]

Stem Cells In Periodontal Regeneration

The use of growth and differentiation factors for regenerating periodontal tissue is the most popular tissue engineering approach. To date several growth factors including Transfoming growth factors-β (TGF-β) superfamily members, such as, bone morphogenetic protein-2 (BMP-2), BMP-6, BMP-7, BMP-12, TGF-β, basic fibroblast growth factors (bFGF), and platelet derived growth factors (PDGF), have been used as a protein-based approach to regenerate periodontal tissues.[24]

Nagatomoet al. in their experimental studies found that PDL cells having stem cell properties can regenerate periodontium. Transplantation of PDL derived cells into animal models were shown to regenerate periodontal tissue.[25]

Iwata et al. harvested and expanded primary canine PDL cells in vitro and also made into transplantable constructs containing PGA Scaffold and PDL cell sheets. The transplantable constructs in combination with porous bTCP(b-tricalcium phosphate) induced regeneration of periodontal structures, including alveolar bone, cementum, and periodontal fibers.[26]

Alveolar Ridge Augmentation

Only 9 years after the first published literature involving dental pulp stem cells, dental stem cells were used in humans to regenerate dental bone in human clinical studies. Defects of at least 1.5 cm in the alveolar ridge of 17 human volunteers were filled with a construct of stem cells collected from third molars and seeded onto a collagen matrix. One year later in many cases, the gap was filled with bone.[27]

Regeneration of Craniofacial Defects

Stem cells can be useful in the regeneration of bone and to correct large craniofacial defects due to cyst enucleation, tumor resection, and trauma. The closure of a bone defect is commonly carried out with the transfer of tissue, which have disadvantages like, not able to restore the unique function of the lost part, donor site morbidity, accompanied by scarring, infection and loss of function. Adipose derived stem cells were used to treat the calvarial defect (120 cm2) of a 7-year-old girl who had severe head injury. Autologous adipose stem cells were extracted from gluteal region along with iliac crest bone graft. Autologous fibrin glue that holds the cells in place was prepared by cryoprecipitation. This successful technique has given new rays of hope that ADSCs can be used for difficult reconstructive procedures.[28]

Conclusion

Although many challenges remain, stem-cell-based tissue engineering of teeth could be a choice for the replacement of missing teeth in the future. Developments in dental stem cell research are taking place in such a way that they are beyond our expectation at present. However, it is a long journey, there are certain milestones to be passed and need to surmount obstacles

encounter in the way before the use of stem cells in a clinical setting to transform dentistry as we know it, and even revolutionize contemporary dentistry. Along with technical aspects, various other issues like social, political, ethical, and religious viewpoints need to be addressed in the scientific and clinical use of stem cells. Dental precursor cells are attractive for novel approaches to treat diseases like periodontitis, dental caries or to improve dental pulp healing and the regeneration of craniofacial bone and teeth. Further, dental stem cells can be utilized to regenerate different tissues like nerve and bone. Even though most of these modalities are still in infancy, it is evident that the 21st century dentist is going to play a critical role in the field of medicine

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