Review Article

STEM CELLS - HOPE OR HYPE….

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ABSTRACT: Recent and exciting new discoveries place dentists at the forefront of helping their patients benefit from potentially life-saving therapies derived from stem cells. Stem cells can be found in most tissues of the body, they are usually buried deep, are few in number, and are similar in appearance to surrounding cells. The tooth is store house for these precious stem cells, and there is an abundance of these cells in baby teeth, wisdom teeth, and permanent teeth. The stem cells have the ability to continuously divide and produce progeny that differentiate into hetrogenous types of cells or tissues. They are unspecialized and are capable of dividing and renewing themselves and can be readily recovered at the time of a scheduled dental procedure. Recent studies have demonstrated their wide range of plasticity and their potential use for regenerative medicine and dentistry. In this review, we describe the current knowledge about dental stem cells and discuss tissue engineering approaches and their significant benefits in the development of new medical and dental therapies. New treatment modalities such as stem cell therapy offer great opportunities for patients with novel therapeutic approaches and helps in unlocking life-saving secrets lying within a human tooth itself.

KEYWORDS: Tissue Engineering; Dental Stem Cells; Regenerative Medicine; Dentistry.

Introduction

Tooth loss is a redundant situation that occurs from numerous pathologies such as periodontal diseases, dental caries, injuries or even genetic alterations.[1,2,3] Since years, dentistry has dealt with the replacement of missing teeth with synthetic materials. Exsistent methodologies include the patient's own tissues, allogenic grafts, metallic alloys or synthetic implants.[4] The main drawback of these treatment strategies is the risk of failure and limited service time. Even though implants are biocompatible, their success greatly depends on osseointegration, quantity and quality of bone.

Thus, an implant can fail to remodel with host bone leading to aseptic loosening or infection resulting in its failure whereas the drawback of autologous tissue grafting is donor site trauma and morbidity.[5] Hence, the major interrogation is- "Is there any material that will replace the missing part and at the same time have better endurance over the conventional techniques" ? The justification to this question is stem cells. Stem cells show potential for many different areas of health and medical research, and studying them can help us understand how they transform into the dazzling array of specialized cells that make us what we are. This review highlights the biology, sources and different types of stem cells with emphasis on the potential application of dental stem cells in the fields of dentistry and medicine.

What is a stem cell?

Stem cells are the master cells of the body that meet the two conditions of self-replication and the ability to differentiate into at least two different types of cell.[5] The term stem cell was proposed for scientific use by Russian histologist Alexander Maksimov in1908. Research on stem cells grew out of the work of Canadian scientists in the 1960s.[6] The history of stem cell research includes work with both animal and human stem cell.[7]

Types of stem cells

There are two major types of stem cells are Embryonic Stem cells and Adult stem cells. Adult stem cells can also be of two types Mesenchymal stem cells and Hemopoietic stem cells.

Embryonic stem cells (esc)

Embryonic that are harvested from the first 50 –150 cells of a 4 – 5 old fertilized egg or blastocyst , which have extraordinary ability to form many cell types. Because of their totipotent nature, they serve as repository of cells and have a vast potential application in regenerative science.[7,8] Since ESCs can be obtained only from embryos, obtaining these cells will require destruction of an embryo and therefore are associated with ethical issues and technical issues.[9]

Adult stem cells (asc)

Adult stem cells are multipotent because their potential is normally limited to one or more lineages of specialized cells. However, a number of recent studies show that stem cells from one area may be manipulated to grow into cells types of a completely different tissue. This ability is called *transdifferentiation* or *plasticity*, and different types of adult stem cells have varying degrees of plasticity. Adult stem cells were originally thought to have constrained potential for generating new tissues; that is, hematopoietic stem cells could only make new blood cells. But studies suggest that, hematopoietic stem cells can also give rise to muscle and neuron-like cells in the brain. If this is possible, the adult stem cells will have the same potential as embryonic stem cells differentiating into many types of cells and thus overcoming the ethical issues associated with embryonic stem cells.[10,11]

Stem cells in orofacial tissues

Dr. Songtao Shi, pediatric dentist isolated stem cells by using the deciduous teeth of his 6-year-old daughter and named them as stem cells from human exfoliated deciduous teeth (SHED).[12] Dental stem cells can be isolated from different parts of teeth and includes stem cells derived from the apical papilla, stem cells from tooth germs and from human periodontal ligament.[13]

Dental stem cells isolated from different parts of teeth are:

1. Stem cells from human exfoliated deciduous teeth (SHED)
2. Adult dental pulp stem cells (DPSC)
3. Stem cells from the apical part of the papilla (SCAP)
4. Stem cells from the dental follicle (DFSC)
5. Periodontal ligament stem cells (PDLSC)

Stem cells from human exfoliated deciduous teeth

Miura and colleagues[12] named the cells **SHED**, which stands for Stem cells from human exfoliated deciduous teeth. SHED behaves much differently than dental pulp stem cells from permanent teeth. They exhibit an ability to grow much faster and double their populations in culture at a greater rate, suggesting SHED may be in a more immature state than adult stem cells. These cells could be prompted to express proteins on their surface indicative of stem cells that were in the process of switching into bone and dental pulp cells.[12]

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.[12] Vital anterior deciduous teeth from either the mandible or maxilla and deciduous molars for orthodontic considerations are considered as best samples. Deciduous teeth distal to the canine are not recommended for sampling, because of anatomical considerations. Eruption of the posterior permanent teeth generally takes a longer amount of time to resorb the deciduous molar roots, which may result in an obliterated pulp chamber that contains no pulp, and thus, no stem cells.[14]

Collection, isolation and preservation of shed

The technique is simple and non-invasive involving collection, isolation and storage of SHED.

Step 1: Tooth Collection :

Since, SHED banking is a proactive decision made by the parents, so ,the first step as informed to them is to put tooth fulfilling above mentioned criterias in sterile saline solution and give a call to tooth bank or attending dentist of the bank. The tooth exfoliated should have pulp red in color, indicat- ing that the pulp received blood flow up until the time of removal, which is indicative of cell viability. If the pulp is gray in color, it is likely that blood flow to the pulp has been compromised, and thus, the stem cells are likely necrotic and are no longer viable for recovery. Teeth that become very mobile, either through trauma or disease (e.g.Class III or IV mobility), often have a severed blood supply, and are not candidates for stem cell recovery. This is why recovery of stem cells from primary teeth is preferred after an extraction than the tooth that is "hanging on by a thread" with mobility. Pulpal stem cells should not be harvested from teeth with apical abscesses, tumors or cysts.

In the event of a scheduled procedure, the dentist visually inspects the freshly-extracted tooth to confirm the presence of healthy pulpal tissue and the tooth or teeth 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).

Placing a tooth into this vial at room temperature induces hypothermia.[15] The vial is then carefully sealed and placed into the thermette a temperature phase change carrier, after which the carrier is then placed into an insulated metal transport vessel. The thermette along with the insulated transport vessel maintains the sample in a hypothermic state during transportation. This procedure is described as **Sustentation**. Store-A-Tooth, a company involved in tooth banking uses the Save-A-Tooth device same as that used for transportation of avulsed teeth for transporting stem cells from the dental office to the laboratory. The viability of the stem cells is both time and tempera- ture sensitive, and careful attention is required to ensure that the sample will remain viable.[16] The time from harvesting to arrival at the processing storage facility should not exceed 40 hours. The same steps are performed by the attending assistant of the tooth bank if it is not a scheduled extraction for the collection of specimen.

Step 2: Stem Cell Isolation:

When the tooth bank receives the vial, the following protocol is followed.[16]

- Tooth surface is washed three times with Dulbecco's Phosphate Buffered Saline without Ca++ and Mg++ (PBSA).
- Disinfection is done with disinfection reagent such as povidone iodine and again washed with PBSA.
- The pulp tissue is isolated from the pulp chamber with a sterile small forceps. Stem cell rich pulp can also be flushed out with salt water from the center of the tooth.
- Contaminated Pulp tissue is placed in a sterile petri dish which was washed at least thrice with PBSA.
- The tissue is then digested with collagenase Type I and Dispase for 1 hour at 37ºC. Trypsin- EDTA can also be used.
- Isolated cells are passed through a 70 um filter to obtain single cell supensions.
- Then the cells are cultured in a Mesenchymal Stem Cell Medium(

MSC) medium which consists of alpha modified minimal essential medium with 2mM glutamine and supplemented with 15% fetal bovine serum (FBS),0.1Mm L- ascorbic acid phosphate,100U/ml penicillin and 100ug/ml streptomycin at 37ºC and 5% CO2 in air. Usually isolated colonies are visible after 24 hrs.

- Different cell lines can be obtained such as odontogenic, adipogenic and neural by making changes in the MSC medium.
- 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 course of successive cell passages. If contamination is extensive, three procedures can be performed:

1) Retrypsinizing culture for a short time so that only stromal cells are detached because epithelial or endothelial like cells are more strongly attached to culture flask or dish.

2) Changing medium 4-6 hrs after subculture because stromal cells attach to culture surface earlier than contaminating cells.

3) Separate stem cells using Fluorosence Activated Cell Sorting (FACS) , in which STRO-1 OR CD 146 can be used. This is considered most reliable.

Confirmation of the current health and viability of these cells is given to the donor's parents.

Step 3: Stem Cell Storage:

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

a) Cryopreservation.
b) Magnetic freezing.

Cryopreservation: It is the process of preserving cells or whole tissues by cooling them to sub-zero temperatures. At these freezing temperatures, biological activity is stopped, as are any cellular processes that lead to cell death.[16] SHED can be successfully stored long-term with cryopreservation and still remain viable for use. These cells can be cryopreserved for an extended period of time, and when needed, carefully thawed to maintain their viability.[17] Cells harvested near end of log phase growth (approximately. 80–90% confluent) are best for cryopreservation. The cells are pre- served in liquid nitrogen vapor at a temperature of less than -150ºC. This preserves the cells and maintains their latency and potency.

Magnetic freezing: Hiroshima University uses magnetic freezing rather than cryogenic freezing. This technology, exploits the little known phenomena 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 degrees Celsius. The Hiroshima University company is the first company to express this new technology. Maintaining magnetic freezing system is a lot cheaper than cryogenics and more reliable as well.[18]

Commercial aspect of SHED banking:

These cells can be best utilized for the patients from which they are harvested, and to a certain extent their immediate family and blood relatives. As such, it is inevitable that the key to successful stem cell therapy lies in being able to harvest the cells at the right point of development and to safely store them until accident or disease requires their usage. Needless to say, this means potentially storing for decades, and the cost and technical difficulty of doing this properly make stem cell therapy using one's own cells a still uncertain bet. This is one aspect but a strong lobby of researchers working with these cells considers banking of SHED as Biological Insurance and a ray of hope for the treatment of various ailments already discussed in the paper. Till date, tooth banking is not very popular but the trend is catching up mainly in the developed countries. StemSave (USA) and Store –A- Tooth (USA) are companies involved in banking tooth stem cells and expanding their horizon in other countries. In Japan, the first tooth bank was established in Hiroshima University and the company was named as “Three Brackets” in 2005. Nagoya University (Kyodo, Japan) also came up with a tooth bank in 2007.Taipei Medical University (TMU) in collaboration with Hiroshima University opened the nation’s first tooth bank in September, 2008 with the goal of storing teeth for natural implants and providing a potential alternative source for harvesting and freezing stem cells including SHED.[18]

Dental Pulp Stem Cells (DPSC)

DPSC generate a dentin, pulp like complex that is composed of mineralized matrix with tubules lined with odontoblasts. Studies have shown that DPSCs also differentiate into adipocytes and neural like cells.[17]

Stem Cells from Apical Part of the Papilla (SCAP)

Because of higher proliferative capacity, they are suitable for inducing roots. SCAPs can generate odontoblast like cells and produce dentin, thus help in formation of root dentine as in case of apexogenesis.[18]

Dental Follicle Precursor Cells (DFPC)

Dental follicle precursor cells (DFPC) differentiate into osteoblasts/cementoblasts, adipocytes, and neurons. DFPCs have increased their potential for use in tissue engineering applications, including periodontal and bone regeneration.[19]

Periodontal Ligament Stem Cells (PDLSC)

PDLSC are isolated from root surface of extracted teeth and differentiate into cells or tissues very similar to periodontium for tissue regeneration and periodontal repair.[20]

Implementatons of stem cells in oral diseases

Clinical applications of dental stem cells will continue to emerge in the near and longer term. Currently dental stem cell research focuses on:-

- Regeneration of dentine, pulp and teeth.
- Regeneration of periodontal ligament or bio-root engineering.
- Salivary gland regeneration after radiation therapy.
- Neural regeneration.
- Repair of craniofacial defects.
- Treatment of lichen planus.
- Applications in medical field.

Dental Pulp Regeneration

Tooth regeneration represents a new era in dentistry as the concepts of repair is being shifted to regeneration. Stem cells from human exfoliated deciduous teeth were able to differentiate into odontoblast-like cells, and also endothelial-like cells. In another study using dental pulp stem cells were supplemented with dentin matrix protein (DMP-1) and were able to regenerate pulp-like tissue.[21] These findings suggest that stem cells from these sources can be considered for dental pulp tissue engineering and regeneration.

Bio-Root Engineering

Sonoyama *et al.* demonstrated the use of mesenchymal stem cell populations for root/periodontal tissue regeneration. Periodontium can also be regenerated by culturing of non-dental stem cells such as bone marrow mesenchymal stem cells, and adipose-derived stem cells.

Salivary gland regeneration after radiation therapy

Studies have shown that stem cell therapy provides a means to reduce radiation-induced hyposalivation and improve the quality of life of the cancer patients.[22]

Neural regeneration

Cranial neural crest (CNC) cells represent an ideal source for neuronal differentiation and regeneration. The migrating CNC cells contribute to the formation of dental papilla, dental pulp, PDL and other tissues in the tooth and mandible.[18]

Lichen planus

Recent advance in the treatment of Oral lichen planus employ the use of immunomodulating agents such as tacrolimus which may treat the disease. In the past years, studies have concentrated on immunosuppressive features of mesenchymal stem cells on various immune cell types. Based on these studies it is nominated that mesenchymal stem cells can be utilized to treat oral lichen planus patients via systemic infusion or local application.[23]

Repair of craniofacial defects

Craniofacial defects results from post-cancer ablative surgery, infection, trauma, congenital malformations and progressively deforming skeletal diseases. Although autologous bone graft is considered the best option, it has the limitation of donor sites. Use of skeletal or dental stem cells may one day be used to repair craniofacial bone and may provide a promising alternative approach for reconstruction of craniofacial defects. Bone tissue engineering endeavors to repair large bone losses using three dimensional scaffolds to deliver vital cells to the defective site.[24]

Applications in medical field

Applications of regenerative medicine technology may offer new therapies for patients with injuries, end-stage organ failure, or other clinical problems such as cardiac infarcts, immune disorders, Parkinson's and liver disease.[22] Currently, patients suffering from diseased and injured organs can be treated with transplanted organs. However, there is a shortage of donor organs that is worsening yearly as the population ages and new cases of organ failure increase. Scientists in the field of regenerative medicine and tissue engineering are now applying the principles of cell transplantation, material science, and bioengineering to construct biological substitutes that will restore and maintain normal function in diseased and injured tissues. The stem cell field is a rapidly advancing aspect of regenerative medicine as well, and new discoveries here create new options for this type of therapy.

Non dental Stem Cells for Dental Applications

Just as dental stem cells have the potential for therapeutic use throughout the body, stem cells from outside the oral cavity may be used for regenerating tissue in the mouth. Bone marrow stem cells has been used for many years by neurosurgeons and orthopedic surgeons to generate bone for procedures such as spinal and tibia fusions. It is a minimally invasive, relatively painless outpatient procedure that takes between one and three hours to perform, providing faster healing, less pain and better results than other implant procedures.[25]

Conclusion: Stem cell research in dentistry has proven to be a milestone in regenerative dentistry and medicine. Advances in the isolation and understanding of dental stem cells have opened areas of research into the possibility to 'regrow' lost dental tissues. This may not only prevent tooth loss but also fundamentally change the concept and definition of a dental caregiver. Current research is exploring the capability of the dental stem cells to differentiate into non-dental tissues such as cardiac muscle. Previously untreatable patients may now be improved by using stem cells harvested from their own teeth.[25] With the advances in the stem cell biology, years from now dental stem cells will hopefully be able to correct cleft palate, save injured teeth and jaw bones, correct periodontal defects, and most strikingly regenerate the entire teeth structures. Although many studies have to be conducted before applying such therapeutic modalities, the dental stem cells represent a powerful tool which holds a significant potential for advancement in the field of regenerative dentistry and medicine.

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