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Teeth - A New Source of Stem Cells "Stem Cell Revolution Lurks In Your Mouth"

Dr.Seema D.Pathak[1], Dr.ShraddhaGite[2,] Dr.Pradnya Bansode[3], Dr.Shirish Khedgikar[4], Dr.Madhri Wavdhane[5]

[1](*Associate Professor , Department of Conservative Dentistry and Endodontics, GDC & Hospital, Aurangabad/ MUHS,INDIA)*

[2](*2nd year Postgraduate student,Department Dentistry and Endodontics, GDC & Hospital, Aurangabad/ MUHS,INDIA)*

[3](*Professor and Head of Department, Department of Conservative Dentistry and Endodontics, GDC & Hospital, Aurangabad/ MUHS,INDIA)*

[4](*Lecturer, Department of Conservative Dentistry and Endodontics, GDC & Hospital, Aurangabad/ MUHS,INDIA)*

[5](*Associate professor, Department of Conservative Dentistry and Endodontics, GDC & Hospital, Aurangabad/ MUHS,INDIA)*

Abstract: *Stem cell (SC) therapy has a promising future for tissue regenerative medicine. However, because SC technology is still in its infancy, interdisciplinary cooperation is needed to achieve successful clinical applications. Dental SCs have drawn attention in recent years because of their accessibility, plasticity, and high proliferative ability. Several types of dental SCs have been identified, including dental pulp SCs from adult human dental pulp, SCs from human primary exfoliated deciduous teeth(SHED), periodontal ligament SCs, and dental follicle SCs from human third molars. Similar to mesenchymal SCs, these dental SCs can undergo self-renewal and have multipotent differentiation ability, but do not have the ethical issues associated with other sources of SCs.They are also used to treat range of diseases like Type I DM,spinal cord injury,cardiovascular,Motor neuron diseases. Therefore, appropriate preservation procedures for dental SCs and teeth are now needed. Application of stem cells in dentistry is limited due to various parameters that are not yet under control such as rejection, cell behaviour, appropriate crown morphology and suitable color. Nevertheless, the development of biological approaches for dental reconstruction using stem cells is promising and remains as a challenge for years to come.*
Here,in this article we discuss the opportunities for tooth-banking (as it is now clinically feasible and commercially available), the sources,used, advantages and limitations of current cryopreservation techniques for dental SCs/teeth or tissues, and the current status of tooth banks.

Keywords: *deciduous teeth, SHED, cryopreservation; dental stem cell; stem cell therapy; tooth banking ,tissue regenerative*

I. Introduction

Stem cells are undifferentiated primitive cells that have the capacity to divide and differentiate into specialized cells, which has more importance in the field of medicine. Stem cells are derived from embryonic, foetal and adult tissues. In adults bone marrow, fat, brain tissue, human exfoliated deciduous teeth (SHED), dental pulp (DPSCS), periodontal ligament (PDLSCS) are the major sources.(Fig-1) Researchers believe that stem cells are capable of providing treatment options in a wide variety of the diseases. Stem cells (SCs) are undifferentiated cells capable of self-renewal and differentiation into multiple functional cell types[1,2]. These cells are widely used in injury, repair and tissue regeneration. Adult SCs have been isolated from a variety of tissues including bone marrow, brain, liver, lungs, breast, skin, skeletal muscles, hair follicles and teeth[3,4]. Dental-derived SCs have been isolated and identified as the cell sources for tooth repair and regeneration. These cells are named according to their anatomical locations, and are characterized by their SC markers, colony-forming ability, and dental regenerative function.Current research indicates that dental SCs may have the potential to regenerate bone, the periodontal ligament (PDL), and possibly teeth. Thus, appropriate cryopreservation of these dental cells, tissues and teeth are imperative to realize the opportunities of these SCs for medical applications, particularly for autotransplantation[5]. However, the optimal methods for tissue cryopreservation remain largely unknown. Masato et al described long-term tooth cryopreservation using a programmed freezer with a magnetic field, the so called Cell Alive System (CAS)[6] History -The term stem cell was proposed by Russian histologist ALEXANDER MAKSIMOV in 1908 at congress of hematologic

society in Berlin. During early 1960's the Canadian scientists came out with the good results on stem cells. (Becker et al., 1963)

II. Types Of Stem Cells

The two broad types of stem cells are embryonic stem cells and adult stem cells. Embryonic stem cells are derived from embryos (cells from inner cell mass of blastocyst) (Shapiro al., et 1998). These cells has the capacity of forming all the 3 germ layers and able to develop more than 200 cell types. To derive the embryonic stem cells an embryo has to be destroyed, hence this has ethical problems. Hence embryonic stem cells remain potential only therotically because of its abilities of unlimited expansion and pluripotency. Adult stem cells can be found in the blood of umbilical cord, bone marrow and blood. Pluripotent stem cells that are found in the blood of umbilical cord are only few in number. These adult stem cells have been used for many years successfully to treat leukemia and bone\blood cancers through bone marrow transplantation

2.1 Stem cells in Dentistry

In 2003 DR. SONGTAO SHI- a Pedodontist discovered baby tooth stem cells by using the deciduous teeth of his six year old daughter and he named them as stem cells from human exfoliated deciduous teeth (SHED). Many (Chamberlain et al., 2007). dental pulp stem cells can be found both in children and adults (JJiang et al., 2002). SHED are immature, unspecialized cells in the teeth that are able to grow into specialized cell types by a process known as "differentiation." SHED appear at the 6th week during the embryonic stage of human development. Scientists believe that these stem cells behave differently than post-natal (adult) stem cells.[7] SHED cells multiply rapidly and grow much faster than adult stem cells, suggesting that they are less mature, so they have the potential to develop into a wider variety of tissue types.[7,8,9] Abbas et al (2008) investigated the possible neural crest

2.2 Types of Stem Cells in Human Exfoliated Deciduous teeth[Fig-2]

- Adipocytes; Adipocytes have successfully been used to repair damage to the heart muscle caused by severe heart attack. There is also preliminary data to indicate they can be used to treat cardiovascular disease, spine and orthopedic conditions, congestive heart failure, Crohn's disease, and to be used in plastic surgery.[10,11]
- Chondrocytes and Osteoblasts: Chondrocytes and Osteoblasts have successfully been used to grow bone and cartilage suitable for transplant. They have also been used to grow intact teeth in animals.[7,12,13,14]
- Mesenchymal : Mesenchymal stem cells have successfully been used to repair spinal cord injury and to restore feeling and movement in paralyzed human patients. Since they can form neuronal clusters, mesenchymal stem cells also have the potential to treat neuronal degenerative disorders such as Alzheimer's and Parkinson's diseases, cerebral palsy, as well as a host of other disorders. Mesenchymal stem cells have more therapeutic potential than other type of adult stem cells.[7,14,15]

2.3Advantages of dental pulp stem cells

• It Provides a guaranteed matching donor (autologous transplant) for life. There are many advantages of autologous transplant including; no immune reaction and tissue rejection of the cells, no immunosuppressive therapy needed, and significantly reduced risk of communicable diseases.[15,16]
• Saves cells before natural damage occurs.
• Simple and painless for both child and parent
. • Less than one third of the cost of cord blood storage
. • SHED are adult stem cells and are not the subject of the same ethical concerns as embryonic stem cells.[15,16]
• SHED cells are complementary to stem cells from cord blood. While cord blood stem cells have proven valuable in the regeneration of blood cell types, SHED are able to regenerate solid tissue types that cord blood cannot such as potentially repairing connective tissues, dental tissues, neuronal tissue and bone.[17,18,19,20]
• SHED may also be useful for close relatives of the donor such as grandparents, parents, uncles, and siblings.[16]

2.4 Potential Clinical Applications of Stem Cell Therapy with SHED –

Stem cell-based therapies are being investigated for the treatment of many conditions, including -- Neurodegenerative conditions such as Parkinson's Disease and Multiple Sclerosis, liver disease, diabetes, cardiovascular disease, autoimmune diseases, musculoskeletal disorders and for nerve regeneration following brain or spinal cord injury. Currently, patients are being treated using stem cells for bone fractures, cancer (bone marrow transplants) and spinal fusion surgery. New stem cell therapies are continually under review, and some have already been approved by the U.S. Food and Drug Administration. As the number of people affected by

degenerative diseases continues to increase, there will be a greater need for new treatment options for the ever-growing aging population.

Harvesting and banking SHED now will ensure their availability in the future when they will be needed most. This comprehensive list of diseases and conditions currently being treated using stem cells include Stem Cell Disorders, Acute and chronic Leukemias, Myeloproliferative Disorders, Myelodysplastic Syndromes, Lymphoproliferative Disorders, Inherited Erythrocyte Abnormalities, Liposomal Storage Diseases, Histiocytic Disorders, Phagocyte Disorders, Congenital Immune System Disorders, Inherited Platelet Abnormalities, Plasma Cell Disorders and malignancies morbidity and mortality. [15,16,17,20]

III. Collection, Isolation And Preservation Of Shed [Fig-3]

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

3.1Step 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, indicating 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 to be necrotic and are no longer viable for recovery. Teeth that become mobile, either through trauma or disease (e.g. grade III or IV mobility), often have a severed blood supply, and are not candidates for stem cell recovery. So 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 (we can keep four teeth in the one vial). Placing a tooth into this vial at room temperature induces hypothermia. 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 temperature sensitive, and careful attention is required to ensure that the sample will remain viable. The time from harvesting to arrival at the processing storage facility should not exceed 40 hours.

3.2Step 2: Stem Cell Isolation [21]

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

A) Tooth surface is cleaned by washing three times with Dulbecco's Phosphate Buffered Saline without Ca++ and Mg++ (PBSA).

B) Disinfection is done with disinfection reagent such as povidone iodine and again washed with PBSA.

C) The pulp tissue is isolated from the pulp chamber with a sterile small forceps or dental excavator. Stem cell rich pulp can also be flushed out with salt water from the center of the tooth.

D) Contaminated Pulp tissue is placed in a sterile petri dish which was washed at least thrice with PBSA.

E) The tissue is then digested with collagenase Type I and Dispase for 1 hour at 37ºC. Trypsin- EDTA can also be used.

F) Isolated cells are passed through a 70 um filter to obtain single cell supensions. G) 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.

H) Different cell lines can be obtained such as odontogenic, adipogenic and neural by making changes in the MSC medium.

I) 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.
3.3Step 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

3.3.1Cryopreservation:

It is the process of preserving cells or whole tissues by cooling them to sub-zero temperatures.[16] At these freezing temperatures, biological activity is stopped, as are any cellular processes that lead to cell death.[22,23] 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.[24,25,26] Cells harvested near end of log phase growth (approximately. 80–90% confluent) are best for cryopreservation. The sample is divided into four cryo-tubes and each part is stored in a separate location in cryo-genic system so that even in the unlikely event of a problem with one of storage units, there will be another sample available for use. The cells are preserved in liquid nitrogen vapour at a temperature of less than -150ºC. This preserves the cells and maintains their latency and potency.

3.3.2Magnetic freezing :

Hiroshima University uses magnetic freezing rather than cryogenic freezing. This technology, is called CAS and 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 idea of CAS is to completely chill an object below freezing point without freezing occuring, 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 snap freezes.

3.4Tooth Eligibility Criteria for SHED Banking[16]

Not all teeth hold the same potential. The teeth especially primary incisors and canines with no pathology and at least one third of root left contain these unique types of cells in sufficient number. Primary teeth distal to the canine are generally not recommended for sampling. Primary molars have a broader root base, and therefore, are retained in the mouth for a longer period of time than anterior teeth. Eruption of the posterior permanent teeth generally takes a longer amount of time to resorb the primary molar roots, which may result in an obliterated pulp chamber that contains no pulp, and thus, no stem cells. In some instances, early removal of deciduous molars for orthodontic considerations (e.g. early intervention for space maintenance) will present an opportunity to recover these teeth for stem cell banking.

3.5Commercial Aspect of SHED Banking

Tooth banking is not very popular but the trend is catching up mainly in the developed countries. In the USA, BioEden(Austin, Texas), has international laboratories in the UK (serving Europe) and Thailand (serving South East Asia) with further expansion plans for Russia, Australia, India and the Middle East. StemSave (USA) and Store –A- Tooth (USA) are also 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" (Suri Buraketto) 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.[27] The Norwegian Tooth Bank set up in 2008 is collecting exfoliated primary teeth from 100,000 children in Norway. The Tooth Bank is a sub-project in the Norwegian Mother and Child Cohort Study (MoBa), and is a collaborative project between the Norwegian Institute of Public Health and the University of Bergen.[28]

IV. Figures

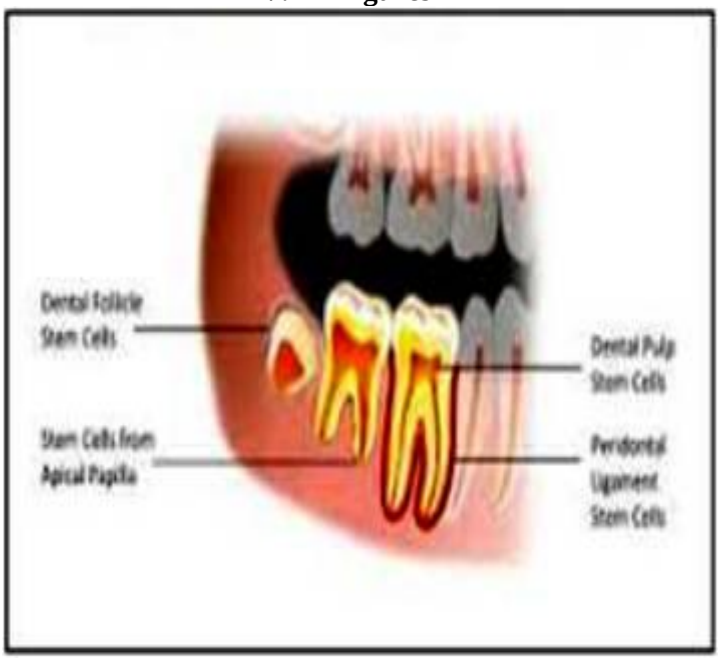

Fig-1 In adults bone, human exfoliated deciduous teeth (SHED,, dental pulp (DPSCS), periodontal ligament (PDLSCS) are the major sources.

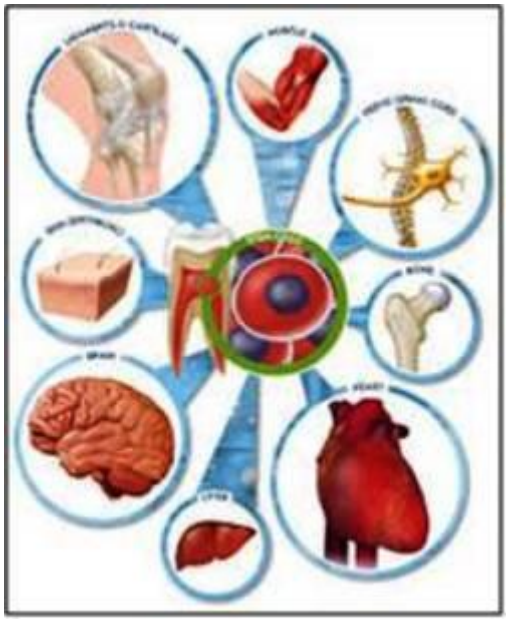

Fig-2 Applications of stem cells **(**Neurodegenerative conditions such as Parkinson's Disease and Multiple Sclerosis, liver disease, diabetes, cardiovascular disease, autoimmune diseases, musculoskeletal disorders and for nerve regeneration following brain or spinal cord injury. Currently, patients are being treated using stem cells for bone fractures, cancer (bone marrow transplants) and spinal fusion surgery)

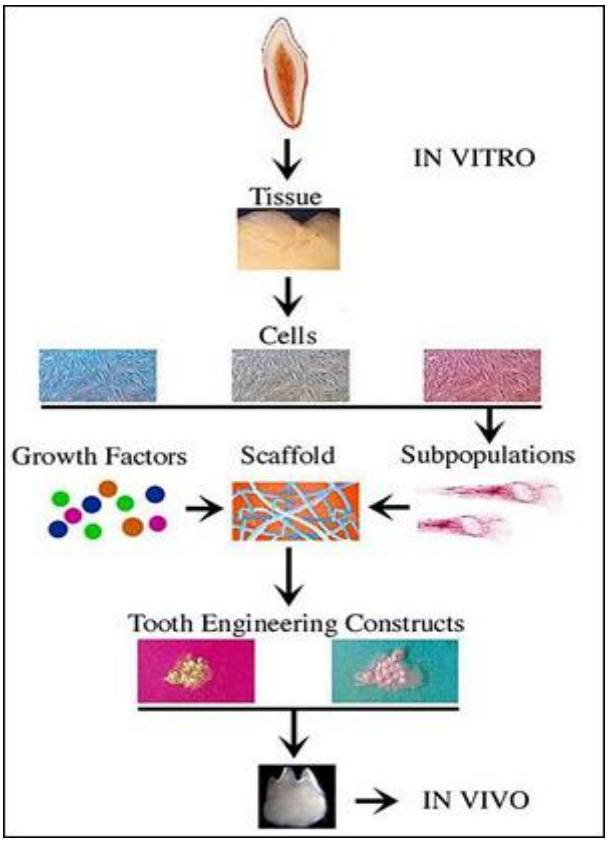

Fig-3 Steps in Stem cell culture

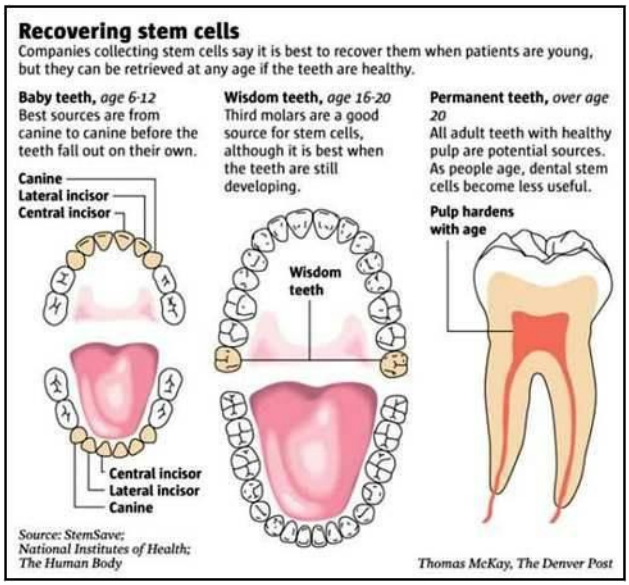

Fig-4

V. Conclusion

Stem cells can be used to treat various diseases like Parkinson's disease, cancer, spinal injuries, heart diseases, liver diseases, blindness, muscle damage, diabetes and many other diseases in the future. (Fiegel et al., 2006; Lindvall 2003; Goldman and Windrem 2006; Timper et al., 2006). In the dental field stem cells can correct periodontal problems, injured teeth and jaw bones. The hallmark of stem cells is the regenerative capacity to form entire tooth structure. Stem cell therapy is emerging as a revolutionary treatment modality to treat diseases and injury, with wide-ranging medical benefits. SHED are stem cells found in the exfoliated deciduous/ primary teeth of children. Recent studies show that SHED appear to have the ability to develop into more types of body tissue than other types of stem cells. This difference opens the door to more therapeutic

applications .There is much research left to be conducted, but the existing research has clearly shown that primary teeth are a better source for stem cells. While the promise of the immense scope and magnitude that stem cell therapies will have upon the population will only be fully realized in the future, Dental Professionals have realized that the critical time to act is now. The available opportunities to bank their patients'dental stem cells will have the greatest future impact.

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