REVIEW ARTICLE

Tooth Bank: Store a Tooth for New Life

Kislaya Kumar[1], Mohan Gundappa[2], Ranjana Mohan Gundappa[3], Neelakshi Singh Rallan[4], Karthik Krishna M[5]

1-UG Student, Teerthanker Mahaveer Dental College & Research Center, Moradabad. 2-Principal and Head of Department, Department of Conservative Dentistry and Endodontics, Teerthanker Mahaveer Dental College & Research Center, Moradabad. 3-Prof and Head, Department of Periodontology, Teerthanker Mahaveer Dental College & Research Center, Moradabad. 4-Reader, Department of Oral Pathology and Microbiology, Teerthanker Mahaveer Dental College & Research Center, Moradabad. 5-Professor, Department of Periodontology, Teerthanker Mahaveer Dental College & Research Center, Moradabad.

Correspondence to:
Dr. Kislaya Kumar, UG Student, Teerthanker Mahaveer Dental College & Research Center, Moradabad.
Contact Us: www.ijohmr.com

ABSTRACT

Teeth are the natural source of stem cells. These teeth could be deciduous teeth, and wisdom teeth . Stem cells are immature, unspecialized cells that have the ability to develop into many different cell via differentiation. Dental stem cells are easy and inexpensive to collect and store. Stem cell (SC) therapy has a promising future for tissue regenerative medicine. Several types of dental SCs have been identified which includes 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. Extraction of Stem Cells is due to unique receptors like OCT-4, TRA-1-60.

KEYWORDS: Dental Stem Cells, Tissue Culture Method, Regenerating Tissues, OCT-4, TRA-1-60

INTRODUCTION

A ‘‘stem cell’’ refers to an undifferentiated cell that is capable of self-replicating via differentiation.

Current research indicates that dental SCs may have the potential to regenerate bone, the periodontal ligament (PDL), and possibly teeth via cryopreservation. Masato et al described long-term tooth cryopreservation using a programmed freezer with a magnetic field, the so called Cell Alive System (CAS). Using the CAS method, the PDL showed good cell viability and differentiation capability.

The application of stem cell therapy using SHED (Stem cells from exfoliated human dentition) to treat these diseases is currently being pursued by many researchers at the institutions around the world. Now a days it is cleared that the primary teeth are a better source for therapeutic stem cells than wisdom teeth. With these features , the concept of tooth banking has popularized now a days as earlier the stem cells extracted from umblical cord was the only tools to be cured from future deadly diseases.

Current research indicates that dental SCs may have the potential to regenerate bone, the periodontal ligament (PDL), and possibly teeth. Thus, with appropriate cryopreservation method of these dental cells, we realize the opportunities of these SCs for medical applications, particularly for autotransplantation. Masato et al described long-term tooth cryopreservation using a programmed freezer with a magnetic field, the so called Cell Alive System (CAS).

Dental-derived SCs: Dental SCs have been found in dental mesenchymal SCs (MSCs) and dental epithelial SCs. MSCs from human dental tissues include dental pulp SCs (DPSCs) in human permanent teeth, SCs from human exfoliated deciduous teeth (SHEDs), periodontal ligament SCs (PDLSCs), and dental follicle SCs (DFSCs) from human third molar.

DPSCs AND SHEDs

DPSCs are SCs derived from dental pulp.

DPSCs show a multipotential differentiation ability, which is similar to that of Mesenchymal stem cells.

These DPSCs express Mesenchymal stem cells markers, including Stro-1 and CD146, which helps in undergoing colony forming in vitro. It can also regenerate the dentin/pulp complex in vivo. SHEDs are multiple SCs found in the pulp tissue of human exfoliated deciduous teeth. They were identified as an extensively proliferative clonogenic cells, which can differentiate plastically into neuronal cells,followed by adipocytes and odontoblasts and can form significant amounts of alveolar and orofacial bone for tissue regeneration.

DFSCs: Dental follicles comprise the neural crest cells, which is derived from ectomesenchymal tissue surrounding the developing tooth germ. After wisdom tooth extraction, human dental follicles can be isolated, and they play an important role in tooth eruption by regulating osteogenesis and osteoclastogenesis. After tooth eruption, the dental follicle differentiates into cells of the periodontium, including alveolar osteoblasts, the PDL, fibroblasts and cementoblasts. The pluripotency of DFSCs has also been demonstrated. For example, the neuronal - differentiation ability of DFSCs was

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documented using the neural progenitor cell markers Notch-1 and Nestin. Meanwhile, the adipocyte differentiation capability of DFSCs was demonstrated by cultivating dental follicle cells with an adipogenesis medium. These observations suggest the presence of pluripotent SCs in human dental follicles.

Dental epithelial SCs: The most mineralized tissue of the body is the Tooth Enamel which is first formed in the crown stage of dental development. Before the tooth erupts into the oral cavity, the ameloblasts are being broken down. The enamel is unable to regenerate itself. The apical bud is a condensed SC compartment responsible for replenishing the growing dentition when it interacts with mesenchymal cells.

TOOTH-BANKING: A PRELIMINARY STEP FOR FUTURE TISSUE REGENERATION

In older days, Extracted teeth are thought to be of medical waste because of the lack of knowledge about therapeutic potential of dental SCs and also there were no appropriate storage methods for donor teeth or SCs. Unlike embryonic SCs, which involve the destruction of human embryos, dental SCs are easily accessible and available. With advances in tissue engineering, dental SCs have shown their potential in regenerating odontoblasts,41 dentin/ pulp-like structure, and dentin. Furthermore, dental SCs can differentiate into adipocytes and neurons, which promote the proliferation and differentiation of endogenous neural cells.[2]

STEM CELL STORAGE-CRYOPRESERVATION AND MAGNETIC FREEZING

The process of preserving cells or whole tissues by cooling them to sub-zero temperatures is known as Cryopreservation. At these freezing temperatures, biological activity is stopped.. The best phase for Cryopreservation is harvesting of cells near end of log phase. The cells are preserved in liquid nitrogen vapor at a temperature of less than -150°C. This preserves the cells and maintains their potency. Ice injury is a major concern for tissue cryopreservation. Kawasaki *et al.* suggested that the slow and rate-controlling freezing reduced the ice injury of cryopreserved living cells.

Another storage process is Magnetic freezing, which is the Cell Alive System (CAS). Under the condition of CAS magnetic field energy, water clusters do not accumulate but remain in smaller groups, thus minimizing restraining the expansion of the water. This technology, is called CAS and uses the 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. Once the object is uniformly chilled, the magnetic field is turned off and the object snap freezes.[3]

PROCESS OF DENTAL STEM CELL BANKING

This involves the following steps:

Step 1: Tooth Collection: The first step is to place the tooth in sterile saline solution. The tooth exfoliated should have pulp which is red in color & non necrotic which indicates that the pulp received blood flow till the time of removal, this indicates the viability of cell . With the recovery of tooth it 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 hypothermic state during transportation and is described as Sustentation.

Step 2: Stem Cell Isolation: When the tooth bank receives the Kit or vial, all the cells are isolated and stringent protocol is followed for cleaning of tooth surface by various disinfectants, isolation of pulp tissue from pulp chamber and cells are 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 contamination is extensive, than a change in procedures can be performed: in which STRO-1 or CD 146 can be used.

Step 3: Tooth Cell Storage: The two approaches that are used for stem cell storage:-
a) Magnetic freezing
b) Cryopreservation

a) Magnetic freezing: The idea of this technique is based on to completely chill an object below freezing point, by using a magnetic field, 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 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 cheaper than cryogenics and more reliable as well.

b) Cryopreservation: "Cryo" means cold in Greek, and cryopreservation is a process in which cells or whole tissues are preserved by cooling to subzero temperatures, typically –196°C in reproductive medicine, cryopreservation plays a very important role in cell and tissue preservation. Cryopreservation of the tooth cells is done for future use.

The first 48 hours after the tooth is out of the mouth are critical. The tooth must be prepared, packaged and

received at laboratory during this time to maximize a successful isolation.

ADVANTAGES OF STEM CELL RECOVERY AND CRYOPRESERVATION FROM TEETH

- It is Accessible – The stem cells contained within teeth are recovered at the time of a planned procedure; for example, Extraction of wisdom teeth, baby teeth or other healthy permanent teeth.
- Affordable and less invasive - When compared with other methods of acquiring and preserving lifesaving stem cells it is less invasive.
- Convenience –it is so convenient that the recovery of stem cells from teeth can be performed in the doctor's office anytime when a healthy tooth is being extracted.

TYPES OF STEM CELLS IN PULP OF HUMAN TEETH

Adipocytes have successfully been used to repair damage to the heart muscle caused by severe heart attack or to treat congestive heart failure.

Chondrocytes and Osteocytes have been successfully used in growth of new bone and cartilage which is suitable for transplantion To repair spinal cord injury, Mesenchymal stem cells have successfully been used .They also have the potential to treat neuronal degenerative disorders such as Alzheimer's and Parkinson's diseases.[3]

TOOTH ELIGIBILITY CRITERIA FOR SHED BANKING

All the teeth have not the same potential to regenerate. The teeth especially primary incisors and canines with no any defect and at least one third of their root left contain these unique types of cells in sufficient number. Primary teeth distal to the canine are generally not recommended for sampling because the Primary molars have a broader root, 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.

Sample selection criteria:

Deciduous teeth

- The Pulp should be vital.
- Deciduous teeth with two third of root are preferred.
- Posterior deciduous teeth are not preferred because of less pulp and chance of infection due to longer retention in the mouth than anterior.
- Extracted teeth are preferred than loose teeth.
- Adult teeth
- Only vital teeth should be harvested.
- Teeth with infection and any pathology are avoided Masthan et al.
- Mobile teeth with devoid of blood supply can't be harvested.
- Teeth should have sufficient amount of pulp.

Steps in the dental clinic:

- Examine the tooth and find out any infection.
- proper Rinse the tooth.
- Transfer to transportation tube.
- Add saline solution.
- Wait for five minutes.
- Seal the tube.
- Transport under room temperature before 48 hrs.

Steps in the laboratory

- Identification of stem cells with markers.
- Separation of viable cells by centrifuge.
- Cryopreservation.
- Retrieval.

CONCLUSION

Dental stem cells are the cells that differentiate into different tissues, maintain their characteristics after cryopreservation. Stem cell therapy is evolving as an innovative management modality to treat diseases and injury. SHED are stem cells found in the exfoliated deciduous/ primary teeth of children. This difference opens the door to more therapeutic applications. This shows that primary teeth are a better source for stem cells.

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