

Tooth Banking- Risk Free Investment for a Healthy Future

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Abstract

Tooth banking is a process of banking stem cells isolated from dental pulp for future use when necessary. This concept arose following the path breaking advances in stem cell research which made it possible to extract and preserve stem cells and ultimately bank the smiles of the future. The purpose of this review is to present a comprehensive synthesis of current knowledge of tooth banking, the procedure involved, advantages and what this holds in future.

INTRODUCTION

Ever since the discovery and characterisation of mesenchymal stem cells (MSC), stem cell biology has become an evolving field in regenerative medicine and tissue engineering therapy. Until the documentation of identification of Stem cells from Human Exfoliated Deciduous Teeth (SHED) for the first time by Miura in 2003¹, the most common source of MSCs were umbilical cord, placenta, bone marrow, adipose tissues and skin². Oral and maxillofacial regions harbours several stem cell populations which include post natal dental pulp stem cells (DPSC), cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), dental follicle progenitor cells (DFPCs), alveolar bone derived mesenchymal stem cells (ABMSCs), stem cells from the apical part of the human dental papilla (SCAP), tooth germ progenitor cells (TGPCs) and Gingival mesenchymal stem cells (GMSCs)³.

These are derived from neural crest cell having the capacity of self-renewal and multi-lineage differentiation potential⁴. Numerous studies have been conducted on these dental stem cells due to their great clinical potential, easy accessibility and less invasive harvesting⁵. Keeping this premise in mind, tooth banking concept has popularized and further, to tap the potential of this new and innovative approach, various companies have set up tooth banks for preserving SHED and stem cells from other dental sources. This concept is based on the firm belief that personalized medicine is the most promising avenue for treating challenging diseases and injuries that could occur throughout lifetime⁶.

PROCEDURE/TECHNIQUE

STEP 1- Tooth Collection

Tooth Eligibility Criteria for SHED Banking

All teeth do not hold the same potential⁷. Primary incisors and canines having no pathology and at least one third of root left, contain these unique types of cells in sufficient

number and, are the most preferred ones. Generally, primary teeth distal to the canine are not recommended for sampling as these primary molars have a broader root base, and thus retained in the mouth for a longer period of time than anterior teeth. Also, primary molars resorb a little later, which may result in an obliterated pulp chamber that is devoid of pulp tissue, and thus, contain no stem cells. However, in some instances of early removal of deciduous molars for orthodontic considerations (eg. early intervention for space maintenance) will present an opportunity to recover these teeth for stem cell banking⁷. Pulp should appear red in color indicating that the pulp had received blood flow up until the time of removal, thus indicative of cell viability. Gray coloured pulp is likelihood of that compromised blood flow to the pulp and thus, making the stem cells prone to necrosis no longer available for recovery. Mobile teeth that due to trauma or disease (e.g. Class III or IV mobility), often have a severed blood supply, and are not candidates for stem cell recovery. Hence, recovery of stem cells from primary teeth is preferred after an extraction. Most importantly, they should not be harvested from teeth with apical abscesses, tumors or cysts⁸.

In case of an unscheduled event i.e. tooth extraction in the absence of a dental professional, parents are advised to give a call to tooth bank or attending dentist of the bank. Whereas, in the event of a scheduled procedure, the dentist visually inspects the freshly-extracted tooth to confirm the presence of healthy pulpal tissue⁹. The tooth or teeth is transferred into the vial (up to four teeth in the one vial) containing a hypotonic phosphate buffered saline solution, that provides nutrients and prevent the tissue from desiccation during transport. The vial is then sealed carefully and placed into the thermette (a temperature phase change carrier) after which the carrier is then placed into an insulated metal transport vessel, thereby maintaining the sample in a hypothermic state during transportation. This procedure is described as Sustentation¹⁰.

The collected specimen has to arrive at the processing storage facility within 40 hours¹⁰.

STEP 2- Stem cell Isolation

When the tooth bank receives the vial, the following protocol is followed¹¹. Initially, the tooth surface is cleaned by washing three times with Dulbeccos Phosphate Buffered Saline without Ca++ and Mg++ (PBSA) followed by disinfection with povidone iodine and again washed with PBSA. The pulp tissue is then isolated from the pulp chamber with a sterile small forceps or dental excavator or can also be flushed out with salt water from the centre of the tooth. If contaminated, then pulp tissue is placed in a sterile petri dish and washed at least thrice with PBSA. The tissue is then digested with collagenase Type I and Dispase for 1 hour at 37°C. Trypsin- EDTA can also be used.

Later, isolated cells are passed through a 70 um filter to obtain single cell suspensions and then 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 37°C and 5% CO₂ in air. Usually isolated colonies would be visible after 24 hours. We can obtain different cell lines such as odontogenic, adipogenic and neural by making changes in the MSC medium¹¹.

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 hours after subculture because stromal cells attach to culture surface earlier than contaminating cells.
3. Separate stem cells using Fluorescence 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 donors parents.

3. Stem Cell Storage

Two approaches have been practised for stem cell storage by various laboratories namely Cryopreservation and Magnetic Freezing¹². Cryopreservation is the process of preserving cells or whole tissues by cooling them to sub-zero temperatures¹³. Biological activity is stopped at these freezing temperatures¹². When needed, SHED cells can be carefully thawed to maintain their viability. For better cryopreservation, cells are harvested near end of log phase growth (approximately 80-90% confluent)¹³. Four sub samples are prepared from the initial collected sample and each stored in a separate location in the cryo-genic system so that there will be another sample available for use even in the unlikely event of a problem with one of storage units. In a vial, 1-2x 10⁶ cells in 1.5 ml of freezing medium is optimum. The cells are preserved in liquid nitrogen vapour at a temperature of less than -150°C, which preserves the cells and maintains their latency and potency¹³.

Magnetic freezing Magnetic freezing 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 water¹². This was first employed by the Hiroshima University¹⁴. This technology is based on 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. The object gets completely chilled without freezing occurring, thereby avoiding cell wall damage caused by ice expansion and nutrient drainage due to capillary action, as normally caused by conventional freezing methods. The magnetic field is switched off, once the object is uniformly chilled and the object snap freezes¹². They claim that this can increase the cell survival rate in teeth (83%) compared to 63% for liquid nitrogen, 45% for ultra-cold freezing (-80 degrees C) and just 21.5% for a household freezer (-20 degrees C)¹⁵.

ADVANTAGES

The remarkable advantages of dental stem cells are non-immunogenic, good match for the entire family, avoiding the risk of communicable diseases, easy accessibility, and remedy for organ shortage which is an expected future abdeciduous teeth provides a better source of stem cells because a significant decrease may occur in the number of stem cells with aging and stem cell quality within niches could be affected by genetic and/or environmental factors¹⁶.

Compared to other sources of stem cells, dental stem cells can be harvested in later part life even if parents missed the chance of storing umbilical cord. Also, it can be stored at various stages starting from deciduous teeth to the last tooth erupted. The whole modality is cost effective when compared to cord stem cell banking¹⁷.

They hold enormous potential for the therapeutic treatment of: Neuronal degenerative disorders such as Alzheimers, Parkinsons, and ALS (Amyotrophic Lateral Sclerosis or Lou Gehrigs Disease); chronic heart conditions such as congestive heart failure and chronic ischemic heart disease; periodontal disease and to grow replacement teeth and bone^{18,19,20}.

CONCLUSION

This new innovative procedure of Tooth banking definitely opens the door to a more risk free future via the 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. This promise of the immense scope and magnitude that stem cell therapies will have upon the population will only be fully realized in the future and we, Dental Professionals have a critical role to act is now. The available opportunities to bank their dental stem cells will have the greatest future impact if seized while patients are young and healthy.

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