

# Banking Stem Cells from Human Exfoliated Deciduous Teeth (SHED): Saving for the Future

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*Tooth derived cells are readily accessible and provide an easy and minimally invasive way to obtain and store stem cells for future use. Banking ones own tooth-derived stem cells is a reasonable and simple alternative to harvesting stem cells from other tissues.*

*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. Furthermore, using ones own stem cells poses few, if any, risks for developing immune reactions or rejection following transplantation and also eliminates the potential of contracting disease from donor cells. Stem cells can also be recovered from developing wisdom teeth and permanent teeth. Individuals have different opportunities at different stages of their life to bank these valuable cells. It is best to recover stem cells when a child is young and healthy and the cells are strong and proliferative.*

*The purpose of this review is to discuss the present scenario as well as the technical details of tooth banking as related to SHED cells.*

**Keywords:** deciduous teeth, SHED, banking  
J Clin Pediatr Dent 33(4): 289–294, 2009

## INTRODUCTION

Tooth banking is based on the firm belief that personalized medicine is the most promising avenue for treating challenging diseases and injuries that could occur throughout lifetime. Stem cell therapy has been used around the world to treat such conditions, and the full promise of stem cell therapy has only been glimpsed so far. There is an abundant source of adult Stem cells in the Human Exfoliated Deciduous teeth (SHED).<sup>1</sup> Recent studies have shown that SHED have the ability to develop into more types of body tissues than other types of stem cells.<sup>1,2,3</sup> Researchers have found the pulp of exfoliated deciduous

teeth to contain chondrocytes, osteoblasts, adipocytes, and mesenchymal stem cells.<sup>1,2</sup> All of these cell types hold enormous potential for the therapeutic treatment of: Neuronal degenerative disorders such as Alzheimer's, Parkinson's, and ALS (Amyotrophic Lateral Sclerosis or Lou Gehrig's Disease); chronic heart conditions such as congestive heart failure and chronic ischemic heart disease; periodontal disease and to grow replacement teeth and bone.<sup>3,4,5,6,7,8,9</sup> One of the most important potential applications using these cells is for the treatment of paralysis due to spinal cord injury which has already been done using mesenchymal stem cells from other sources.<sup>7,8,9</sup> The application of stem cell therapy using SHED to treat these diseases is currently being pursued by many researchers at the institutions around the world. There is much research left to be conducted, but the existing research has clearly shown that primary teeth are a better source for therapeutic stem cells than wisdom teeth, and orthodontically extracted teeth.<sup>1</sup> Keeping this premise in mind, the concept of tooth banking has popularized and various companies have set up tooth banks to tap the potential of this new and innovative approach for preserving SHED and stem cells from other dental sources. This review will focus on the potential applications of SHED as well as the techniques adopted for their preservation.

## Stem cells from Human Exfoliated Deciduous teeth (SHED)

Breakthrough lifesaving medical treatments using stem

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cells are being discovered and will continue to emerge in the decades to come. While 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. Until recently, stem cells harvested from umbilical cord blood was the only storage option to guard against future illness or disease. Unfortunately, the cord cell harvesting and storage process is beyond the reach of many people.

With the documented discovery of SHED in 2003 by Dr. Songtao Shi, an accessible and available source of stem cells has been identified which can be easily preserved and used for future cure of ailments.<sup>1</sup> 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.<sup>1</sup> 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.<sup>1,9,10</sup>

Abbas *et al* (2008) investigated the possible neural crest origin of dental pulp stem cells from exfoliated deciduous teeth (SHED). Neural crest cells are multipotent cells that are capable of self-renewal and multi-lineage differentiation and play a major role in tooth development as they give rise to mesenchymal components of teeth including odontoblasts, pulp, apical vasculature and periodontal ligament. They found that SHED are heterogeneous population that shares common molecular characteristics with neural crest cells and stem cells *in vitro*.<sup>10</sup>

This ability to grow and regenerate tissues is the focus of the emerging field of personalized medicine which uses a patient's own stem cells for biologically compatible therapies and individually tailored treatments.<sup>9</sup> Further, SHED are able to express proteins on their cell surfaces that allows them to not only differentiate into dental pulp, bone and dentin, but also into neural and fat cells (adipocytes).<sup>1,10</sup> In fact, SHED differentiate into nerve cells more readily than adult stem cells isolated from permanent teeth. SHED express a variety of neuronal and glial cell markers which directly reflects the embryonic neural crest origin of dental pulp.<sup>1,10</sup> SHED cells have been shown to express factors that induce bone formation and assist with the guidance of the eruption of the permanent teeth.<sup>1,11</sup>

Shi S *et al* (2005) conducted a study on stem cells in adult human dental pulp (dental pulp stem cells, DPSC), human primary teeth (stem cells from human exfoliated deciduous teeth, SHED), and periodontal ligament (periodontal ligament stem cells, PDLSC) by their capacity to generate clonogenic cell clusters in culture. *Ex vivo* expanded DPSC, SHED, and PDLSC populations expressed a heterogeneous assortment of makers associated with mesenchymal stem cell, dentin, bone, smooth muscle, neural tissue, and endothelium. Xenogeneic transplants containing HA/TCP with either DPSC or SHED generated donor-derived dentin-pulp-like tissues with distinct odontoblast layers lining the mineralized dentin-matrix. They concluded that the presence

of distinct stem cell populations associated with dental structures have the potential to regenerate living human dental tissues *in vivo*.<sup>11</sup>

No doubt stem cells of dental origin have got multiple applications, there are certain limitations as well. The oncogenic potential of these cells is still to be determined in long term clinical studies. Moreover, till date the research is mainly confined to animal models and still human research trials are needed to document same results in humans. Another main issue to consider is the difficulty to identify, isolate, purify and grow these cells in lab as these cells are required in large numbers to be therapeutically used. Immune rejection is also one of the issues which requires a thorough consideration. Lastly, these are comparatively less potent than embryonic stem cells.

### Types of Stem Cells in Human Exfoliated Deciduous teeth

**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.<sup>6,12</sup>

**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.<sup>1,2,3,11</sup>

**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.<sup>1,5,7,8,11,12</sup> Mesenchymal stem cells have more therapeutic potential than other type of adult stem cells.<sup>1,8,11</sup>

### Role of SHED in Craniofacial Tissue Engineering

SHED may be used to regenerate bone and correct craniofacial defects.<sup>2,3</sup> Both *in vitro* studies and *in vivo* research in animal models have shown that tooth-derived adult stem cells can be used to regrow tooth roots in the presence of proper growth factors and a biologically compatible scaffold. Regenerative therapy is less invasive than surgical implantation, and early animal studies suggest comparable results in strength and function of the biological implant as compared to a traditional dental implant.<sup>9</sup> SHED are capable of extensive proliferation and multipotent differentiation, which makes them an important resource of stem cells for the regeneration and repair of craniofacial defects, tooth loss and bone regeneration.<sup>1,2,3,7,10,11</sup> Given their ability to produce and secrete neurotrophic factors, SHED cells may also be beneficial for the treatment of neurodegenerative diseases and the repair of motoneurons following stroke or injury. Stem cells from third molars release chemicals that may allow the remaining nerves to survive the injury.<sup>7</sup> Future

research will investigate if using tooth-derived stem cells can be used to regenerate neurons following spinal cord injury.

SHED can be directly implanted into the pulp chamber of a severely injured tooth to regenerate the pulp inside the damaged tooth, preventing the need for endodontic treatment. Cordeiro (2008) evaluated morphologic characteristics of tissue formed when SHED seeded in biodegradable scaffolds prepared within human tooth slices were transplanted in immunodeficient mice. They observed that resulting tissue presented architecture and cellularity closely resembling that of a physiologic pulp.<sup>13</sup>

Tissue-engineered bone grafts will be useful for practitioners in all of the dental specialties. Future applications may also include engineered joints and cranial sutures, which would be especially helpful to craniofacial and oral maxillofacial surgeons. Recently in a study, De Mendonça Costa A *et al* (2008) evaluated the capacity of human dental pulp stem cells (hDPSC), isolated from primary teeth, to reconstruct large-sized cranial bone defects in nonimmunosuppressed (NIS) rats. They found that hDPSC is an additional cell resource for correcting large cranial defects in rats and constitutes a promising model for reconstruction of human large cranial defects in craniofacial surgery.<sup>2</sup> Mao JJ *et al* (2006) in a review discussed that adult stem cells have been isolated from the dental pulp, the deciduous tooth, and the periodontium. Several craniofacial structures—such as the mandibular condyle, calvarial bone, cranial suture, and subcutaneous adipose tissue—have been engineered from mesenchymal stem cells. They stated that Craniofacial tissue engineering is likely to be realized in the foreseeable future, and represents an opportunity that dentistry cannot afford to miss.<sup>14</sup>

### Advantages of banking SHED 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.<sup>8,9</sup>
- 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.<sup>8,9</sup>
- 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.<sup>7,11,13,14</sup>
- SHED may also be useful for close relatives of the donor such as grandparents, parents, uncles, and siblings.<sup>9</sup>

### 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.<sup>9</sup>

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.<sup>7,8,9,14</sup>

### 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, 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 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. 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.

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<sup>15</sup>

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  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  (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 37°C. Trypsin- EDTA can also be used.
- F) Isolated cells are passed through a 70  $\mu\text{m}$  filter to obtain single cell suspensions.
- 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 37°C and 5%  $\text{CO}_2$  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 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 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.<sup>9</sup> At these freezing temperatures, biological activity is stopped, as are any cellular processes that lead to cell death.<sup>16,17</sup> 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.<sup>18,19,20</sup> 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 vapor at a temperature of less than -150°C. This preserves the cells and maintains their latency and potency. In a vial, 1-2x 10<sup>6</sup> cells in 1.5 ml of freezing medium is optimum. Too low or high cell number may decrease recovery rate.

Suchánek J *et al* (2007) established a protocol of Dental Pulp Stem Cells (DPSCs) isolation and to cultivate DPSCs either from adult and exfoliated tooth, and compared these cells with mesenchymal progenitor cell (MPCs) cultures. In comparison with bone marrow MPCs, DPSCs shared similar biological characteristics and stem cell properties. The results proved that the DPSCs and MPCs were highly proliferative, clonogenic cells that can be expanded beyond Hayflick's limit and remain cytogenetically stable.<sup>18</sup>

Zhang *et al* (2006) evaluated the differential potential of stem cells from the cryopreserved pulp of human third molars and concluded that the pulp tissue of the third molar may serve as a suitable source of multipotent stem cells for future tissue engineering strategies and cell-based therapies, even after cryopreservation.<sup>19</sup>

Papaccio G *et al* (2006) studied the differentiation and morpho-functional properties of cells derived from stem

cells after long-term cryopreservation to evaluate their potential for long-term storage with a view to subsequent use in therapy. They concluded that dental pulp stem cells and their osteoblast-derived cells can be long-term cryopreserved and may prove to be attractive for clinical applications.<sup>20</sup>

**Magnetic 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 occurring, 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. The Hiroshima University company is the first expression of this new technology. Using CAS, Hiroshima University claims that it can increase the cell survival rate in teeth to a high of 83%. This compares to 63% for liquid nitrogen (-196 degrees C), 45% for ultra-cold freezing (-80 degrees C), and just 21.5% for a household freezer (-20 degrees C). Maintaining a CAS system is a lot cheaper than cryogenics and more reliable as well.<sup>21</sup>

#### Tooth Eligibility Criteria for SHED Banking<sup>9</sup>

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.

#### 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.

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.<sup>21</sup>

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.<sup>22</sup>

#### Summary

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 if seized while patients are young and healthy.

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