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PEDIATRIC STEM CELLS -THE FUTURE AHEAD

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ABSTRACT

Stem cell therapy has been used around the world to treat life threatening conditions, and the full promise of stem cell therapy has only been glimpsed so far. Discoveries in stem cell research presents an opportunity for scientific evidences that stem cells, whether derived from adult tissues or the earliest cellular forms hold great promise that go far beyond regenerative medicine.

Pediatric stem cells or SHED are 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.

Keywords: stem cells, pediatric, SHED, stem cells, tooth banking

1. Introduction

Stem cell therapy has been used around the world to treat life threatening conditions, and the full promise of stem cell therapy has only been glimpsed so far. Discoveries in stem cell research presents an opportunity for scientific evidences that stem cells, whether derived from adult tissues or the earliest cellular forms hold great promise that go far beyond regenerative medicine.

Stem cells are a class of undifferentiated cells that are able to differentiate into specialized cell types[1]. According to the ability and potency to differentiate into different cellular types, three types of stem cells have been established: (1) totipotent stem cells – each cell has the capability of developing into an entire organism, (2) pluripotent stem cells – embryonic stem cells that are grown *in vivo* under induced conditions and are capable of differentiating into all types of tissue, and (3) multipotent stem cells – postnatal stem cells or adult stem cells with the capability of multilineage differentiation[2].

Dentists are at the forefront of engaging their patients in potentially life-saving therapies derived from their own stem cells located either in deciduous or permanent teeth.Postnatal stem cells have been isolated from various dental tissues[3]. So far, five types of dental stem cells have been identified: dental pulp stem cells (DPSC), stem cells from exfoliated deciduous teeth (SHED), stem cells from apical papilla (SCAP), periodontal ligament stem cells (PDLSC), and dental follicle progenitor cells (DFPC)[4,5,6,7,8]. Dental stem cells belong to the multipotent stem cell population[9].

Recent studies have shown that SHED have the ability to develop into more types of body tissues than other types of stem cells[5,10,11] and there is an abundant source of adult Stem cells in the Human Exfoliated Deciduous teeth *(SHED)*[5] .Researchers have found the pulp of exfoliated deciduous teeth to contain chondrocytes, osteoblasts, adipocytes, and mesenchymal stem cells[5,10]. 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 to name a few and to grow replacement teeth and bone[11-18]. 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[15-17].

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[5]. Keeping this premise in mind, the concept of tooth banking has also 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.

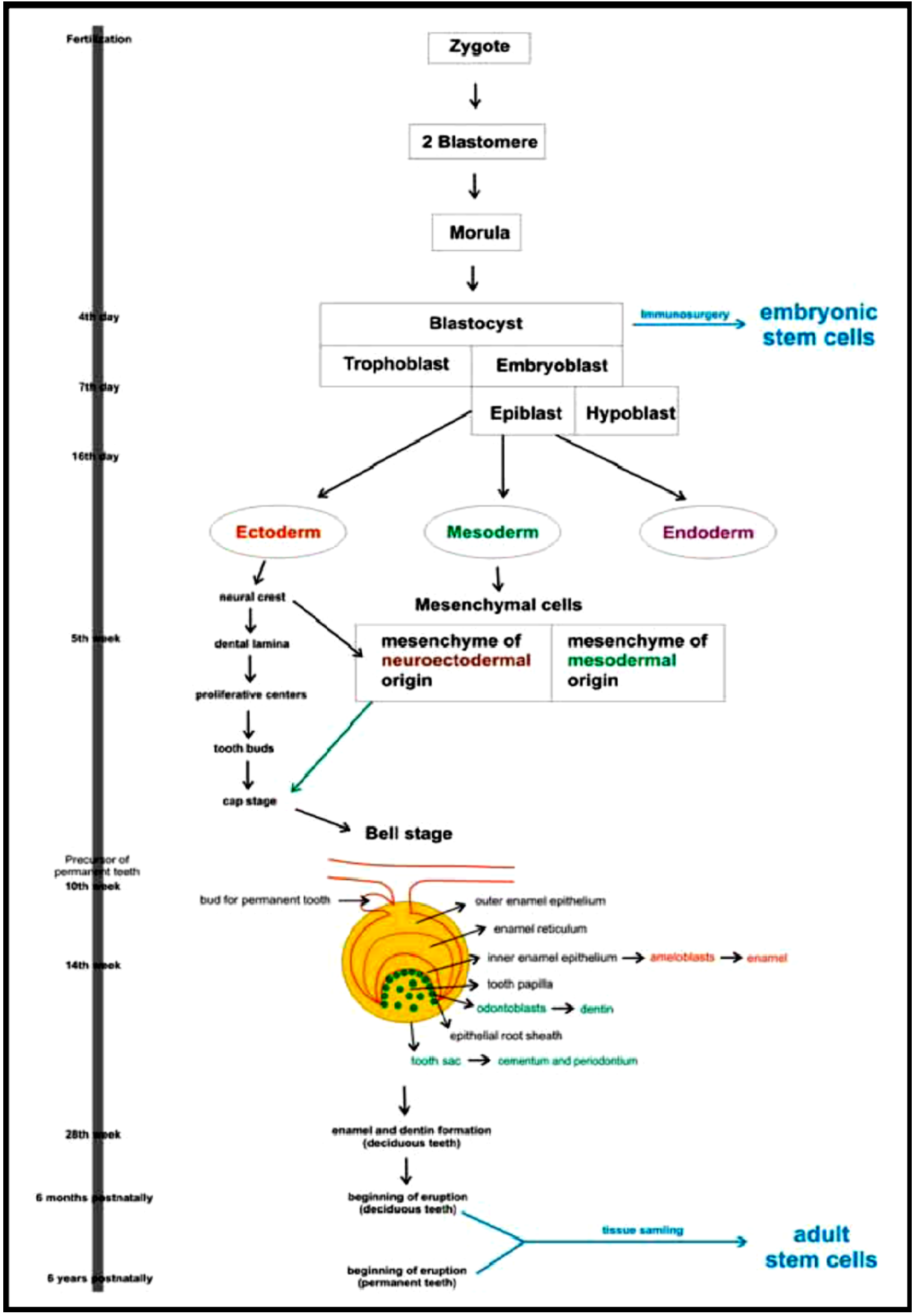

Fig. 1 Embryogenesis and odontogenesis[19]

2. Stem cells from Human Exfoliated Deciduous teeth (SHED)

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 cell 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[5]. 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[5]. 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[5,19]. Giordano G *et al(*2011)[20] stated that Dental pulp from adult as from deciduous teeth seems to be the most valuable form of stem cells due to the pluripotential type of cells.

Abbas *et al* (2008)[21] 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*. 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. 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)[5,21]. In fact, SHED differentiates 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[5,21]. SHED cells have been shown to express factors that induce bone formation and assist with the guidance of the eruption of the permanent teeth[5,22]. Shi S *et al* (2005)[22] 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 clongenic 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.*

Wang X *et al* (2012)[23] studied the characterization of stem cells from human exfoliated deciduous teeth (SHED) in comparison with dental pulp stem cells (DPSCs) to certify SHED as a key element in tissue engineering. SHED showed a higher proliferation rate and differentiation capability in comparison with DPSCs *in vitro*, and the results of the in vivo transplantation suggest that SHED have a higher capability of mineralization than the DPSCs.

3. 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[14].

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[5,10,11].

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.[5,15,16,22,24] Mesenchymal stem cells have more therapeutic potential than other type of adult stem cells[5,17,22].

Role of SHED in Craniofacial Tissue Engineering

Breakthrough lifesaving medical treatments using stem cells are being discovered and will continue to emerge in the decades to come. Koyama N *et al*(2009)[26] demonstrated that pluripotential cells isolated from the pulp of human teeth expanded *in vitro* and differentiated into osteoblasts, chondrocytes, and adipocytes. DPSC and SHED are not only derived from a very accessible tissue resource but also capable of providing enough cells for potential clinical applications.

SHED may be used to regenerate bone and correct craniofacial defects[10,11]. Both *in vitro* studies and *in vivo* research in animal models have shown that tooth-derived adult stem cells can be used to re grow 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[17].

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. Vakhrushev IV *et al* (2012)[26] characterized Cultures of multipotent mesenchymal stromal cells from the pulp of human deciduous teeth (SHED cells). The cells were used for population of 3D biodegradable polylactoglycolide scaffolds; their osteogenic potential was preserved under these conditions. Implantation of the scaffolds to mice induced no negative reactions in the recipients. These results suggested that the use of polylactoglycolide scaffolds populated with SHED cells is a promising approach for creation of implants for bone defect replacement.

Zheng Y *et al* (2009)[27] examined the efficacy of utilizing SHED in regenerating orofacial bone defects. The results indicated that stem cells from miniature pig deciduous teeth, an autologous and easily accessible stem cell source, were able to engraft and regenerate bone to repair critical-size mandibular defects at 6 months post-surgical reconstruction. In a similar study,Shen YY (2010)[28] evaluated the capacity of the stem cells derived from human exfoliated deciduous teeth in *in vitro* differentiation into osteoblasts The purified CD34(+)/CD117(+) stem cells derived from exfoliated deciduous teeth of healthy children possess the capacity to differentiate into osteoblasts and form calcium deposits and mineralized nodules *in vitro*. Li B *et al* (2012)[29] stated that Stem cells from human exfoliated deciduous teeth (SHED) are a unique postnatal stem cell population capable of regenerating mineralized tissue and treating immune disorders. However, the mechanism that controls SHED differentiation is not fully understood. Here, we showed that basic fibroblast growth factor (bFGF) treatment attenuated SHED-mediated mineralized tissue regeneration through activation of the extracellular signal-regulated kinase (ERK) 1/2 pathway.

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 *et al*(2008)[30] 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. 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.

Hara K *et al* [31] focussed on the characterization of SHED as compared with bone marrow-derived mesenchymal stem cells (BMMSCs). By using the gene expression profiles, this study indicated that SHED is involved in the BMP signaling pathway and suggests that BMP-4 might play a crucial role in this. These results might be useful for effective cell-based tissue regeneration, including that of bone, pulp, and dentin, by applying the characteristics of SHED.

In a study, De Mendonça Costa A *et al* (2008)[10] evaluated the capacity of human dental pulp stem cells (hDPSC), isolated from primary teeth, to reconstruct large-sized cranial bone defects in non immunosuppressed (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. Mao JJ *et al* (2006)[32] 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.

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.

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 motor neurons following stroke or injury. Stem cells from third molars release chemicals that may allow the remaining nerves to survive the injury[15]. Future research will investigate if using tooth-derived stem cells can be used to regenerate neurons following spinal cord injury.

Inoue T *et al* (2012)[33] reported that dental pulp stem cells (DPSC) ameliorated ischemic tissue injury in the rat brain and accelerated functional recovery after middle cerebral artery occlusion (MCAO). SHED-CM promoted the migration and differentiation of endogenous NPC, induced vasculogenesis, and ameliorated ischemic brain injury after pMCAO as well as DPSC transplantation. Taghipour Z *et al*(2012)[34] assessed the potential in addition to neural induced SHED (iSHED) for functional recovery when transplanted in a rat model for acute contused spinal cord injury (SCI). These findings have demonstrated that transplantation of SHED or its derivatives could be a suitable candidate for the treatment of SCI as well as other neuronal degenerative diseases .Karaöz E *et al*(2011)[35] stated that SHED not only differentiate into adipogenic, osteogenic, and chondrogenic lineage, but also share some special characteristics of expressing some neural stem cell and epithelial markers. Under defined conditions, hDP-SCs are able to differentiate into both neural and vascular endothelial cells *in vitro*. Dental pulp might provide an alternative source for human MSCs.

hDP-SCs with a promising differentiation capacity could be easily isolated, and possible clinical use could be developed for neurodegenerative and oral diseases in the future

Nourbakhsh N *et al*(2011)[36] provided an evidence that SHED can differentiate into neural cells by the expression of a comprehensive set of genes and proteins that define neural-like cells *in vitro*. SHED cells might be considered as new candidates for the autologous transplantation of a wide variety of neurological diseases and neurotraumatic injuries.Razieh Alipour *et al* (2010)[37] studied to examine and compare the expression of important stem cell surface markers on two populations of mesenchymal stem cells, one derived from human exfoliated deciduous teeth and the other derived from human adipose tissue. These new stem cells will offer a promising avenue for prevention and reversal of many human diseases such as type 1 diabetes and prevention of liver fibrotic process.

Wang J *et al* (2010)[38] reported that SHED were able to differentiate into neural cells based on cellular morphology and the expression of early neuronal markers when cultured under neural inductive conditions. This study therefore investigated the therapeutic efficacy of SHED in alleviating Parkinson's disease (PD) in a rat model. They found that SHED could be induced to form neural-like spheres in a medium optimized for neural stem cells *in vitro*.. Moreover, transplantation of SHED spheres into the striatum of parkinsonian rats partially improved the apomorphine-evoked rotation of behavioral disorders compared to transplantation of control SHED. Our data indicate that SHED, potentially derived from neural crest cells, may be an optimal source of postnatal stem cells for PD treatment.

Yamaza T *et al* (2010)[39] characterized mesenchymal stem cell properties of SHED in comparison to human bone marrow mesenchymal stem cells (BMMSCs).They utilized systemic SHED transplantation to treat systemic lupus erythematosus (SLE)-like MRL/lpr mice.They found that SHED are capable of differentiating into osteogenic and adipogenic cells, expressing mesenchymal surface molecules (STRO-1, CD146, SSEA4, CD73, CD105, and CD166), and activating multiple signaling pathways, including

TGFbeta, ERK, Akt, Wnt, and PDGF. Recently, BMMSCs were shown to possess an immunomodulatory function that leads to successful therapies for immune diseases. We examined the immunomodulatory properties of SHED in comparison to BMMSCs and found that SHED had significant effects on inhibiting T helper 17 (Th17) cells *in vitro*. Moreover, we found that SHED transplantation is capable of effectively reversing SLE-associated disorders in MRL/lpr mice. At the cellular level, SHED transplantation elevated the ratio of regulatory T cells (Tregs) via Th17 cells.It was suggested that SHED are an accessible and feasible mesenchymal stem cell source for treating immune disorders like SLE.

Ueda M & Nishino Y (2010)[40] investigated the interaction between stem cells from human exfoliated deciduous teeth (SHEDs)-derived growth factors and human dermal fibroblast (HDF) as the application of SHEDs for dermal wound healing remains speculative. Stem cells from human exfoliated deciduous teeth have effects on HDFs by increasing collagen synthesis and by activating proliferation and migration activity of HDFs, suggesting that SHEDs or SHED-derived conditioned medium (SH-CM) can be used for the treatment of photoaging. They suggested that SHEDs and SH-CM should be constitutionally suited for photoaging treatment. Mainly with secreted growth factors or extracellular matrix proteins, SHEDs contribute to enhanced wound-healing potential of HDFs.

Further mechanism studies using neutralizing antibodies against each growth factor may clarify the role of soluble factors of SHEDs in wound-healing process. In a similar study,Nishino Y *et al* (2011)[41] examined the effect on wound-healing promotion with unique stem cells from deciduous teeth as a medical waste. An excisional wound-splinting mouse model was used and the effect of wound healing among SHED, human mesenchymal stromal cells (hMSCs), human fibroblasts (hFibro) and a control (phosphate-buffered saline).SHED and hMSCs accelerated wound healing compared with hFibro and the control. This implies that SHED might offer a unique stem cell resource and the possibility of novel cell therapies for wound healing in the future.

5. Limitations in Clinical use

No doubt SHED 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. Morever, 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.

6. 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.
- 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.
- 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 ableto regenerate solid tissue types that cord blood cannot - such as potentially repairing connective tissues, dentaltissues, neuronal tissue and bone.
- SHED may also be useful for close relatives of the donor such as grandparents, parents, uncles, and siblings.

7. 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.21 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 projectbetween the Norwegian Institute of Public Health and the University of Bergen.

8. 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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