Paving The Way For Future Solutions Through Human Exfoliated Deciduous Teeth (Shed)

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Citation

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Abstract

Mesenchymal stem cells from human exfoliated deciduous teeth (SHED) have a promising future for tissue engineering. These cells have generated a variety of interest in the field of health sciences to illustrate their therapeutic value. Since every child goes through the physiologic tooth exfoliation process, the same can be recovered and stored as a good source of stem cells. The purpose of this review is to discuss the various potential benefits, therapeutic uses of stem cells from human exfoliated teeth and also the advantages and limitations of the current status of tooth banking for regenerative medicine.

INTRODUCTION

The application of stem cell therapy using SHED to treat the diseases is currently being pursued by many researchers at the institutions around the world[1].Dental pulp appears to be an alternative and more readily available source of stem cells. Stem cells from the pulp of permanent teeth (dental pulp stem cells [DPSCs]) and from exfoliated deciduous teeth (SHED) have been identi□ed as a novel population of stem cells that have the capacity of self-renewal and multilineage differentiation[2,3]. Dental Pulp Stem Cells [DPSCs] are able to differentiate into odontoblastic and osteoblastic lineage and generate dentin/pulp complex and bone[2-5.]

The therapeutic potential of stem cells derived from human dental pulp since its discovery[2] in regenerative medicine has been extensively studied at several preclinical[6] and clinical levels[7]. The dental pulp tissue (DPT) appears to be an excellent source for stem cells because it can be obtained from the deciduous dentition requiring extraction as part of a planned serial extraction for management of occlusion and is originated from migrating neural crest cells during early development of embryos[8].

The Dental Pulp Stem Cells can be isolated from various age groups and teeth, for example, cells isolated from dental tissue of human impacted tooth germ are known as tooth germ progenitor cells (TGPCs)[9]; stem cells from human exfoliated deciduous teeth are known as SHED[10], and stem cells can also be isolated from human permanent teeth (impacted molar) (DPSCs)[4] or from apical papilla (SCAP)[11].

There is an abundant source of adult Stem cells in the Human Exfoliated Deciduous teeth (SHED).Recent studies have shown that SHED have the ability to develop into more types of body tissues than other types of stem cells[3]. Researchers have found the pulp of exfoliated deciduous teeth to contain chondrocytes, osteoblasts, adipocytes, and mesenchymal stem cells[3,12-13].In the past decade, many scientists have researched the potential of DPSCs to differentiate into osteo-/odontoblasts and adipocytes. In addition to the mesenchymal origins, DPSCs have recently even shown other cell lineage such as neuron[3,4,14,15,].

Tooth banking system is a new concept in which the stem cell are derived and stored under appropriate temperature. In case the need arises in future the same may be used for treating the diseases.

THE DIFFERENTIATION POTENTIAL OF STEM CELLS TYPES OF STEM CELLS

Stem cells are considered to be the most valuable cells for regenerative medicine. They have the ability to continuously divide to either replicate them or produce specialized cells that can differentiate into various other type of cells or tissues[16].

Totipotent or Early embryonic stem cells: These early stem cells posses the ability to become any kind of cell in body. The fertilized egg, capable of independently giving rise to all embryonic and extraembryonic tissues.

Pluripotent or Blastocyte embryonic stem cells: the inner cell mass of the blastocyst in the developing zygote and embryonic stem cells in culture, capable of giving rise to all embryonic cells and tissues. However, the sourcing of embryonic stem cells is controversial and assosciated with ethical and legal issue,thus reducing their application for development of new therapies.

Multipotent fetal stem cells: cells derived from the three embryonic germ layers (ectoderm, mesoderm and endoderm) that become more and more committed to generating particular cells as organs and tissues are formed.

Multipotent adult stem cells orPostnatal stem cells: thought to be tissue-specific and sometimes form only one type of cell (unipotent)[17].

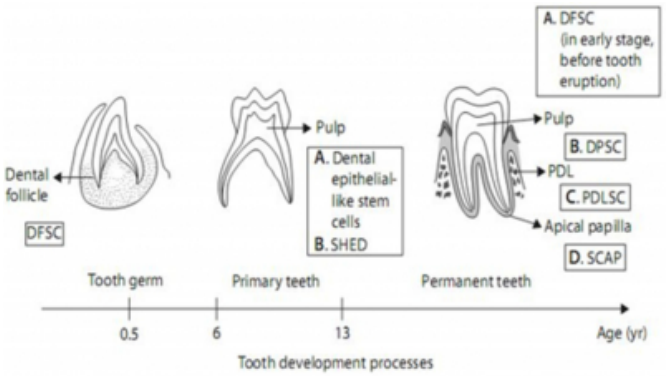
DENTAL DERIVED STEM CELLS

Dental Stem Cells have been found in several tissues and can be divided into:

- Dental mesechymal stem cells [MSCs]
- Dental epithelial stem cells.
- Derivation of dental derived stem cells:
- Dental Pulp Stem Cells (DPSC) and stem cells from human exfoliated deciduous teeth (SHED)
- Periodontal ligament stem cells (PDLSC)
- Dental follicle stem cells (DFSC)
- Dental Epithelial stem cells
- Stem cells from apical papilla (SCAP)

Figure 1

Fig: 1 Tooth developmental stages and derivation of dental derived stem cells. DFSC ? dental follicle stem cells; SHED ? stem cells from human primary exfoliated deciduous teeth; DPSC ? dental pulp stem cells; PDLSC ? periodontal ligament stem cells; SCAP ? stem cells from apical papilla.



KEY RESEARCH EVENTS

1908 - The term “stem cell” was proposed for scientific use by the Russian histologist Alexander Maksimov (1874–1928) at congress of hematologic society in Berlin. It postulated existence of haematopoietic stem cells.

1960s - Joseph Altman and Gopal Das present scientific evidence of adult neurogenesis and ongoing stem cell activity in the brain.

1963 - McCulloch and Till illustrate the presence of self-renewing cells in mouse bone marrow

1978 - Haematopoietic stem cells are discovered in human cord blood.

1981 - Mouse embryonic stem cells are derived from the inner cell mass by scientists Martin Evans, Matthew Kaufman, and Gail R. Martin. Gail Martin is attributed for coining the term “Embryonic Stem Cell”.

1992 - Neural stem cells are cultured in vitro as neurospheres.

1997 - Leukemia is shown to originate from a haematopoietic stem cell, the first direct evidence for cancer stem cells.

1998 - James Thomson and coworkers derive the first human embryonic stem cell line at the University of Wisconsin–Madison[19].

2000s - Several reports of adult stem cell plasticity are published.

2001 - Scientists at Advanced Cell Technology clone first early (four- to six-cell stage) human embryos for the purpose of generating embryonic stem cells[20].

2003 - Dr. Songtao Shi of NIH discovers new source of adult stem cells in children's primary teeth[21].

2004-2005 - Korean researcher Hwang Woo-Suk claims to have created several human embryonic stem cell lines from unfertilised human oocytes.

2005 - Researchers at Kingston University in England claim to have discovered a third category of stem cell, dubbed cord-blood-derived embryonic-like stem cells (CBEs), derived from umbilical cord blood. The group claims these cells are able to differentiate into more types of tissue than adult stem cells.

2005 - Researchers at UC Irvine's Reeve-Irvine Research Center are able to partially restore the ability of mice with paralyzed spines to walk through the injection of human neural stem cells.

August 2006 - Rat Induced pluripotent stem cells: the journal Cell publishes Kazutoshi Takahashi and Shinya Yamanaka[22].

October 2006 - Scientists at Newcastle University in England create the first ever artificial liver cells using umbilical cord blood stem cells[23].

January 2007 - Scientists at Wake Forest University led by Dr. Anthony Atala and Harvard University report discovery of a new type of stem cell in amniotic fluid[24]. This may potentially provide an alternative to embryonic stem cells for use in research and therapy[25].

June 2007 - Research reported by three different groups shows that normal skin cells can be reprogrammed to an embryonic state in mice[26]. In the same month, scientist Shoukhrat Mitalipov reports the first successful creation of a primate stem cell line through somatic cell nuclear transfer[27]

October 2007 - Mario Capecchi, Martin Evans, and Oliver Smithies win the 2007 Nobel Prize for Physiology or Medicine for their work on embryonic stem cells from mice using gene targeting strategies producing genetically engineered mice (known as knockout mice) for gene research[28].

January 2008 - Robert Lanza and colleagues at Advanced Cell Technology and UCSF create the first human embryonic stem cells without destruction of the embryo[29]

January 2008 - Development of human cloned blastocysts following somatic cell nuclear transfer with adult fibroblasts[30].

February 2008 - Generation of pluripotent stem cells from adult mouse liver and stomach: these iPS cells seem to be more similar to embryonic stem cells than the previous developed iPS cells and not tumorigenic, moreover genes that are required for iPS cells do not need to be inserted into specific sites, which encourages the development of non-viral reprogramming techniques[31].

March 2008-The first published study of successful cartilage regeneration in the human knee using autologous adult mesenchymal stem cells is published by clinicians from Regenerative Sciences[32].

October 2008 - Sabine Conrad and colleagues at Tübingen, Germany generate pluripotent stem cells from spermatogonial cells of adult human testis by culturing the cells in vitro under leukemia inhibitory factor (LIF) supplementation[33].

30 October 2008 - Embryonic-like stem cells was isolated from a single human hair[34].

1 March 2009 - Andras Nagy, Keisuke Kaji, et al. discover a way to produce embryonic-like stem cells from normal adult cells by using a novel “wrapping” procedure to deliver specific genes to adult cells to reprogram them into stem cells without the risks of using a virus to make the change[35-37]. The use of electroporation is said to allow for the temporary insertion of genes into the cell[38-40].

28 May 2009 - Kim et al. announced that they had devised a way to manipulate skin cells to create patient specific “induced pluripotent stem cells” (iPS), claiming it to be the 'ultimate stem cell solution'[41].

PROPERTIES OF SHED

In vitro Characterization of SHED—Sphere-like Cluster Formation: SHED proliferate faster with greater PDs than DPSCs and BMMSCs (SHED > DPSCs > BMMSCs). SHED form sphere-like clusters when cultured in neurogenic medium. This is due to the highly proliferative cells, which aggregate in clusters that either adhere to the culture dish or float freely in the culture medium. The sphere-like clusters can be dissociated by passage through needles and subsequently grown on 0.1% gelatin-coated dishes as

individual fibroblastic cells. This phenomenon suggests a high proliferative capacity analogous to that of neural stem cells[3].

Investigators subsequently also isolated SHED and termed the cells 'immature DPSCs' (IDPSCs). Besides confirming the findings described above, they found that IDPSCs express the embryonic stem (ES) cell markers Oct4, Nanog, stagespecific embryonic antigens (SSEA-3, SSEA-4), and tumor recognition antigens (TRA-1-60 and TRA-1-81)[42].

In vitro Multilineage Differentiation: As reported for DPSCs, SHED showed the capacity to undergo osteogenic and adipogenic differentiation. Cultured SHED readily expresses variety of neural cell markers. If stimulated with neurogenic medium, expression of ß III-tubulin, GAD, and NeuN is increased, whereas the other neural markers remain unchanged. Under neurogenic conditions, SHED also exhibit multicytoplasmic processes instead of the typical fibroblastic morphology[3]. Myogenic and chondrogenic potentials have also been demonstrated[42].

In vivo characterization of SHED—Production of dentin-pulp-like structures but without a complex formation: Ex vivo-expanded SHED transplanted into immune compromised mice yield human-specific odontoblast-like cells directly associated with a dentin-like structure. The regenerated dentin expresses dentin-specific DSPP. However, unlike DPSCs, SHED are unable to regenerate a complete dentin-pulp-like complex in vivo [3].

Osteo-inductive capacity: One striking feature of SHED is that they are capable of inducing recipient murine cells to differentiate into bone-forming cells, which is not a property attributed to DPSCs following transplantation in vivo. When single-colony-derived SHED clones were transplanted into immunocompromised mice, only one-fourth of the clones had the potential to generate ectopic dentin-like tissue equivalent to that generated by multicolony-derived SHED.

However, all single-colony-derived SHED clones tested are capable of inducing bone formation in immunocompromised mice. While SHED could not differentiate directly into osteoblasts, they appeared to induce new bone formation by forming an osteoinductive template to recruit murine host osteogenic cells[3].

With the osteo-inductive potential, SHED can repair criticalsized calvarial defects in mice with substantial bone formation. These findings imply that deciduous teeth may not only provide guidance for the eruption of permanent teeth, as generally assumed, but may also be involved in inducing bone formation during the eruption of permanent teeth[13].

In vivo neurogenesis in mouse brain: Neural developmental potential was studied by the injection of SHED into the dentate gyrus of the hippocampus of immunocompromised mice[3].

SHED can survive for more than 10 days inside the mouse brain microenvironment and express neural markers such as neurofilament M (NFM). This finding is similar to what was demonstrated for BMMSCs, which are capable of differentiating into neural-like cells after in vivo transplantation into the rat brain[43].

SHED appear to represent a population of multipotent stem cells that are perhaps more immature than other post-natal stromal stem-cell populations. SHED express neuronal and glial cell markers, which may be related to the neural-crest-cell origin of the dental pulp[44].

In vivo engraftment into different tissues: Three months following the injection of IDPSCs into the intraperitoneal space of nude mice, IDPSCs can be traced in various tissues and organs, including liver, spleen, and kidney, suggesting their potent differentiation plasticity[42].

Figure 2

Table No. 1: Properties of SHED

In Vitro Analysis	In Vivo Analysis
Multipotentiality	Ectopic tissue formation
Dentinogenic	Dentin Pulp like Tissue
Adipogenic	Odontoblast like cells
Chondrogenic	No dentin-pulp complex formation
Myogenic	Bone Formation
Neurogenic	
Osteo-Inductive	

USE OF SHED IN TISSUE ENGINEERING

SHED may be used to regenerate bone and correct craniofacial defects.[12, 13].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[46].

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[3,12,13,47-49].

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[47].

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[50].

Tissue-engineered bone grafts will be useful for practitioners in all of the dental specialities. 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 evaluated the capacity of human dental pulp stem cells (hDPSC), isolated from primary teeth, to reconstruct large-sized cranial bone defects in nonimmuno- suppressed (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[12].

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[51].

SHED isolated from exfoliated deciduous incisors forms adherent clusters and showed a higher rate of proliferation as compared to BMSSC and DPSC and express a number of markers[3,52]. After transplantation into immune compromised mice, they formed ectopic-like dentin-like tissue but were unable to regenerate the dentin pulp-like complex[3, 50]. These results suggested that SHED can differentiate into odontoblasts in vivo. SHED are also capable of repairing critical-size parietal defects in immunocompromised mice; however, the bone lacks hematopoietic marrow elements[13].

Thus, SHED is capable of forming bone and small amounts of dentin in vivo as compared to DPSC that form dentin/pulp complex. SHED was isolated for the first time in 2003 by Miura et al[3] who confirmed that they were able to differentiate into a variety of cell types to a greater extent than DPSCs, including neural cells, adipocytes, osteoblast-like and odontoblast-like cells. The main task of these cells seems to be the formation of mineralized tissue[53-55] which can be used to enhance orofacial bone regeneration[13]

The ethical constraints associated with the use of embryonic stem cells, together with the limitations of readily accessible sources of autologous postnatal stem cells with multipotentiality, have made SHED an attractive alternative for dental tissue engineering. The use of SHED for tissue engineering might be more advantageous than that of stem cells from adult human teeth; they were reported to have a higher proliferation rate than stem cells from permanent teeth[3] and can also be retrieved from a tissue that is disposable and readily accessible[50] Thus, they are ideally suited for young patients at the mixed dentition stage who have suffered pulp necrosis in immature permanent teeth as a consequence of trauma[56].

TOOTH BANKING METHODS

The term tooth bank was raised in 1966[57].To emphasize the use and preservation of dental stem cells for medical application. With the advancement of cryo preservation technology first commercial tooth bank was established in 2004 at National Hiroshima University, Japan[58]. “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[18].

TOOTH ELIGIBILITY CRITERIA FOR SHED BANKING

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 because 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[46].

Various tooth banking methods used worldwide (Table-2)

Figure 3

Location	Indication	Cryopreservation Method	Tooth-derived stem cells	Tooth Preserved
United States[57]	Tissue culture Autotransplantation	Solution: Saline/antibiotic/glycerol Control Temperature:-20ºC for 25 min Dr ice & alcohol bath for 15min to reach -80ºC Storage Temperature:-80ºC	–	–
Denmark[59]	Autotransplantation Replantation	Solution: DMEM culture medium 10% human serum 10% DMSO Control Temperature:-1.2ºC/min to - 40ºC 6ºC/min to -100ºC	–	+
Korea[60]	PDL cell viability	Solution:- DMEM:F-12=3:1 10%FBS 10%DMSO Control Temperature:-196ºC Storage Temperature:-196ºC (LN)	–	–
Japan[28]	PDL cell viability	Solution:- BAMBANKER2 10% DMSO Control Temperature:-Programmable freezer (ABI Corp. Ltd.,Chiba,japan) 75mA electric current to generate a magnetic field -5º C for 15 min 0.5º C/min to -30º C Storage Temperature:-150º C	–	+
Taiwan[61]	Autotransplantation DPSC isolation PDLSC isolation	Solution:BAMBANKER2 10%DMSO Control temperature: Programmable freezer(ABL Corp.Ltd) 75mA electric current to generate a magnetic field -5º C for 15 min 0.5º C/min to -30º C Storage temperature:-150º C	+	+

DMEM=Dulbecco's modified Eagle Medium, DMSO=dimethyle sulfoxide, LN=liquid nitrogen, PDL=periodontal ligament, FBS=fetal bovine serum, DPSC=dental pulp stem cells, PDLSC=periodontal ligament stem cells.

CONCLUSION

Tissue engineering using the triad of dental pulp progenitor/stem cells, morphogens, and scaffolds may provide an innovative and novel biologically-based approach for generation of clinical materials and/or treatments for dental disease.

Although the full possibilities of tissue regeneration in humans using tooth derived stem cells are not well-known, these cells potentially play an essential component of the armamentarium for regenerative medicine, specifically in the reconstruction of the craniofacial region. Future directions to solve these technical challenges and further understanding of SHED will facilitate translation of these advances in stem cell research into clinical practice.

It is also important to include tooth banking for dental SC preservation as a preventive treatment plan. It is highly likely that tooth banking will be the future of the SC era. It is now accepted that the dental pulp harbors several niches of multipotential stem cells capable of self-renewal and differentiation.

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