ACTA SCIENTIFIC DENTAL SCIENCES (ISSN: 2581-4893)

Volume 3 Issue 10 October 2019

Review Article

Stem Cells from Human Exfoliated Deciduous Teeth (SHED) – Turning Useless into Miracle: A Review Article

Lucky Jindal[1]*, Nitish Bhat[2], Divya Vyas[3], Kalpna Thakur[4], Neha[5] and Silky Mehta[6]

[1]*Paedodontics and Preventive Dentistry, India*

[2]*Registrar, Department of Oral Pathology, Indira Gandhi Dental College and Hospital, Jammu, J and K, India*

[3]*Senior Lecturer, Department of Paedodontics and Preventive Dentistry, Himachal Institute of Dental Sciences, Paonta Sahib, H.P, India*

[4]*Lecturer, Department of Oral Pathology and Microbiology. H.P.Government Dental College and Hospital, Shimla, India*

[5]*PG Student, Department of Paedodontics and Preventive Dentistry, Himachal Institute of Dental Sciences, Paonta Sahib, H.P, India*

[6]*PG Student, Department of Paedodontics and Preventive Dentistry, J. N. Kapoor DAV (C) Dental College, Yamuna Nagar, Haryana, India*

***Corresponding Author:** Lucky Jindal, Paedodontics and Preventive Dentistry, India. **E-mail:** jindallucky30@gmail.com

Received: August 26, 2019; **Published:** September 20, 2019

DOI: 10.31080/ASDS.2019.03.0644

Abstract

Recent and exciting new discoveries place dentists at the forefront of helping their patients benefit from potentially life-saving therapies derived from stem cells. The tooth is store house for these precious stem cells, and there is an abundance of these cells in baby teeth, wisdom teeth, and permanent teeth. Obtaining stem cells from human exfoliated deciduous teeth (SHED) is simple and convenient, with little or no trauma. SHEDs exhibit higher proliferative rate than bone marrow-derived MSCs and greater osteogenic differentiation potency than human dental pulp stem cells.

Keywords: SHED; Regeneration; Isolation; Cryopreservation; Banking

Introduction

Stem cells are undifferentiated biological cells that are able to differentiate into specialized cells and has the ability to divide (through mitosis) to produce extra stem cells [1]. It is the ancestor at the top of the family tree of related cell types. Stem cells research is based on the knowledge about how a single cell leads to development of an organism and how damaged cells get replaced by healthy cells in adult organisms [2]. Based on the stage at which they are isolated stem cells can be categorized as embryogenic stem cells (ESCs) or adult stem cells. Embryogenic stem cells are totipotent being derived from the inner cell mass of blastocyst during gastrulation. Even after having the greatest biological potential, ethical issue on the use of ESCs has precluded their widespread study, especially in humans [3]. These are obtained from in vitro fertilization, or aborted embryos 3 or 4 day old embryo [4]. Adult stem cells are derived from postnatal fully developed tissue and are believed to renew cell populations, maintenance of tissue homeostasis and participate in repair of tissue following injury [3].

Various sources from which dental stem cells have been isolated include dental follicle progenitor cells (DFPCs), dental pulp stem cells (DPSCs), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), stem cells from apical papilla (SCAPs) [5].

Dr. Songtao Shi, isolated stem cells using the deciduous teeth of his 6 – year - old daughter and named them as stem cells from human exfoliated deciduous teeth (SHED) [6]. These cells have the ability of high proliferation potency and are multipotent mesenchymal stem cells. These cells not only differentiate into dental pulp-related cells, but also, other cell types such as osteoblasts, adipocytes, neuronal-like cells and endothelial cells [7]. Taking all these properties together, SHEDs are one of the candidate cell types for tissue regeneration study.

Discussion

SHEDs are progenitor cells that appear at 6th week during the embryonic stage of human development [8] and isolated from the pulpal remnants of exfoliated deciduous teeth [3]. Number of studies have shown more proliferation rate and higher capability for differentiation for SHEDs when compared to BMSCs and even DPSCs [6,9]. When compared to adult stem cells SHED may be in a more immature state. These cells could be prompted to express proteins on their surface indicative of stem cells that were in the process of switching into bone and dental pulp cells [10]. Following exfoliation of a deciduous tooth dental stem cells can be recovered immediately, but after the extraction of deciduous teeth they are best recovered as the teeth become mobile, still maintaining their circumferential gingival attachment [6].

Deciduous teeth differ significantly from permanent teeth with respect to developmental processes, tissue structure and function. So, it is not a surprise to find that SHED are different from DPSCs with respect to their higher proliferation rate, increased cell- population doublings, sphere-like-cell-cluster formation, osteoinductive capacity *in vivo*, but failure to reconstitute a dentin-pulp-like complex, perhaps in order to have more immature characteristics than other post - natal stem cell population [6]. Deciduous teeth that are distal to canine are not recommended for sampling, because of anatomical considerations. Posterior permanent teeth eruption generally takes a longer time to resorb the deciduous molar roots, resulting in an obliterated pulp chamber that contains no pulp, and thus, no stem cells [11].

Types of stem cells in human exfoliated deciduous teeth

Adipocytes

Repair of damaged heart muscle caused by severe heart attack have been done successfully by adipocytes. They can be used to treat cardiovascular disease, spine and orthopaedic conditions, congestive heart failure, Crohn's disease, and to be used in plastic surgery [12].

Chondrocytes and osteoblasts

They have been used to grow bone and cartilage suitable for transplant. Intact teeth in animals can also be grown by chondrocytes and osteoblasts [12].

Mesenchymal

Repair of spinal cord injury and restoration of feeling and movement in paralyzed human patients have been done successfully by mesenchymal cells. Because of their ability to form neuronal clusters, they 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. Mesenchymal stem cells have more therapeutic potential than other type of adult stem cells [12].

Collection, isolation and preservation of SHED

The technique is simple and non-invasive involving collection, isolation and storage of SHED [12].

Step 1: Tooth collection

As banking of SHED is a proactive decision made by the parents, so, at first, they are informed to put tooth in sterile saline solution and a call is given to tooth bank or a dentist attending the bank [11]. Pulp of exfoliated tooth should be red in color, indicative of blood flow received by pulp blood flow till the time of removal, indicative of cell viability. Gray color of pulp is suggestive that pulpal blood flow has been compromised, and thus, the stem cells are likely necrotic and are no longer viable for recovery [13]. Mobile teeth, either because of trauma or disease (e.g. Class III or IV mobility), often have a severed blood supply, so they are not candidates for stem cell recovery. It is because of this reason that stem cell recovery from primary teeth is preferred after an extraction than the tooth that is "hanging on by a thread" with mobility [12]. Pulpal stem cells from teeth with apical abscesses, tumors or cysts should not be harvested [11].

With tooth recovery, it is transferred into a vial containing hypotonic phosphate buffered saline solution, that helps to prevent drying of tissue during transport (up to four teeth in one vial). The vial is then carefully sealed and placed into a temperature phase change carrier i.e. thermite, after which into an insulated metal transport vessel, the carrier is placed. The sample is maintained in a hypothermic state during transportation in the thermite along with the insulated transport vessel and the procedure is described as Sustentation [13].

Stem cells viability is both time and temperature sensitive, and careful attention is required to ensure that the sample will remain viable. The time should not exceed 40 hours from harvesting to arrival at the processing storage facility [14].

Step 2: Stem cell isolation

When the tooth bank receives the vial, the following protocol is followed [11]:

1. Tooth surface is cleaned without Ca++ and Mg++ (PBSA) by washing three times with Dulbecco's Phosphate Buffered Saline.
2. Disinfecting reagent such as povidone iodine is used for disinfection and then again washed with PBSA.
3. With a sterile small forceps or dental excavator, the pulp tissue from pulp chamber is isolated. Pulp rich in stem cell can also be flushed out from centre of the tooth with salt water.
4. Pulp tissue which is contaminated is placed in a sterile petri dish which was washed at least thrice with PBSA.
5. Then, digestion of tissue is done with collagenase Type I and Dispase for 1 hour at 37ºC. Trypsin- EDTA can also be used.
6. To obtain single cell suspensions, isolated cells are passed through a 70 um filter.
7. 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 37ºC and 5% CO2 in air, cells are cultured. Usually after 24 hours, isolated colonies are visible.
8. By making changes in the MSC medium, different cell lines can be obtained such as odontogenic, audiogenic and neural.
9. Colonies of cells with morphology resembling epithelial cells or endothelial cells can be established, if cultures are obtained with unselected preparation. During course of successive cell passages, cells disappear. If contamination is extensive, three procedures can be performed:
    1. Culture is reserpinized 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. Medium is changed 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 cell viability is given to the donor's parents [11].

Step 3: Stem cell storage

Either of the following two approaches are used for stem cell storage:

- Cryopreservation
- Magnetic freezing

Cryopreservation

In this process cells or whole tissue is preserved by cooling them to sub-zero temperatures [15]. Biological activity is stopped at these freezing temperature, as are any cellular processes that lead to cell death [8]. With cryopreservation, SHED can be successfully stored long-term and still remain viable for use. Cryopreservation of these cells can be done for an extended period of time, and carefully thawed to maintain their viability, when needed. Cryopreservation is best done when cells harvested near end of log phase growth (approximately. 80 – 90% confluent). 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. In liquid nitrogen vapor at a temperature of less than -150 º C, the cells are preserved. This, not only preserves the cells but also maintains their latency and potency [12]. 1 – 2 x 10^6 cells in 1.5 ml of freezing medium is optimum in a vial. Recovery rate may decrease if cell number is too low or high [8].

Zhang., *et al.* (2006) evaluated the differential potential of stem cells from the cryopreserved pulp of human third molars and concluded that even after cryopreservation, third molar pulp tissue may serve as a suitable source of multipotent stem cells for future tissue engineering strategies and cell-based therapies [16].

Magnetic freezing

This technology is called CAS (Cell Alive System) and exploits the phenomena that even application of a weak magnetic field to water or cell tissue, freezing point of that body will get lower by

up to 6 - 7 degrees Celsius [12]. The idea of CAS is that without the occurrence of freezing, an object is completely chill below freezing point, 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, the magnetic field is turned off and the object snap freezes, once the object is uniformly chilled [8].

Commercial aspect of SHED banking

Utilization of these cells can be done best for the patients from which they are harvested, and to some 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, the cost and technical difficulty of storing for decades properly make stem cell therapy using one's own cells a still uncertain bet [11].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. Trend of tooth banking is catching up mainly in the developed countries but till date, it is not very popular [8].

Stem Save (USA) and Store –A- Tooth (USA) are companies involved in banking tooth stem cells and expanding their horizon in other countries. In Japan, the company was named as "Three Brackets" in which first tooth bank was established in Hiroshima University in 2005. In 2007, Nagoya University (Kyodo, Japan) also came up with a tooth bank. In September 2008 nation's first tooth bank was opened by Taipei Medical University (TMU) in collaboration with Hiroshima University with the goal of storing teeth for natural implants and providing a potential alternative source for harvesting and freezing stem cells including SHED [17].

In India, Stemade Biotech Pvt. Ltd. Has the licensed tooth stem cell banks in Delhi, Chennai, Chandigarh, Pune and Hyderabad [12].

Advantages of Banking SHED Cells [18]

- It provides a guaranteed matching donor for life.
- Before natural damage occurs, cells are saved.
- For both child and parent, it is simple and painless.
- Cost is less than one third of cord blood storage
- Like embryonic stem cells, SHED are not the subject of the same ethical concerns.
- SHED cells are complementary to stem cells from cord blood. SHED are able to regenerate solid tissue types that cord blood cannot- such as potentially repairing connective tissues, dental tissues, neuronal tissue and bone. Whereas cord blood stem cells have proven valuable in the regeneration of blood cell types
- For close relatives of donor such as grandparents, parents, uncles and siblings, SHED are useful.

Potential clinical applications of SHED

Investigation of stem cell based therapies is done for 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, using stem cells patients are being treated for bone fractures, cancer and spinal fusion surgery.

Treatment of diseases and conditions using stem cells include Acute and chronic Leukemia, 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 [12].

Applications in Dentistry

Based on the basic tissue engineering principles, Peter Murray., *et al.* identified several major areas of research that might have applications in the development of these techniques.

Root canal revascularization via blood clotting

Revascularization of the necrotic root canal systems by disinfection followed by establishing bleeding into the canal system via over instrumentation. Use of intracanal irritants (Na OCl and chlorhexidine) along with the placement of antibiotics (e.g., a mixture of ciprofloxacin, metronidazole, and minocycline paste), for several weeks, is a critical step, as it effectively disinfects the root canal systems and increases revascularization of the avulsed and necrotic teeth. There is negligible chances of immune rejection and pathogen transmission by revascularization, as patient's own blood cells results in regeneration [13].

Postnatal stem cell therapy

In this process, after the apex is opened, postnatal stem cells (derived from skin, buccal mucosa, fat, and bone) are injected into disinfected root canal systems. Several advantages of this process like the harvesting and delivery of autogenous stem cells by syringe, being relatively easy; and the potential of these cells to induce new pulp regeneration. However, this process has many disadvantages, like the low survival rate of cells and within the body the cells may migrate to different locations. To maximize the potential for success of pulp regeneration, all the three elements (cells, growth factors, and scaffold) must be considered [13].

Pulp implantataion

To transform two dimensional into three dimensional cell cultures, pulp cells can be grown on biodegradable membrane filters. For evaluation of cytotoxicity of test materials, the main advantage of this delivery system is the ease of growing these cells on filters in the laboratory. The problems with the implantation of sheets of cultured pulp tissue is that it requires specialized procedures for proper adherence to the root canal walls. With coronal canal systems filled with scaffolds capable of supporting cellular proliferation, only the apical portion of the canal systems will receive these cellular constructs as sheets of cells lack vascularity [19].

Scaffold implantation and delivery

A scaffold should contain growth factors, Bone Morphogenic Protein (BMP), fibroblast growth factors, and Vascular endothelial growth factors, which aid in stem cell proliferation and differentiation, apart from having nutrients that will promote cell survival and growth as well as antibiotics to prevent any bacterial in growth in the canal systems. The scaffold materials may be natural or synthetic, biodegradable or permanent. The synthetic materials like polylactic acid, polyglycolic acid, and polycaprolactone degrade within the human body and have been successfully used for tissue engineering purposes. Limitations consist of difficulties of obtaining high porosity and regular pore size [13].

Three-dimensional printing

With this technique, cells can be positioned precisely cells which create tissue constructs that will mimic the natural tooth pulp tissue structure. The prime requisite for the success of the technique is careful orientation of the pulp tissue construct during placement into the cleaned and shaped root canal systems in accordance with its apical and coronal asymmetry is [20].

Gene therapy

This technique involves a gene encoding a therapeutic protein being introduced into the cells, which can then express the target protein. In his study, Rutherford used ferret pulps with cDNA (complementary DNA) transfected mouse BMP - 7, but no reparative response was produced, which suggest that further research is required in the potential of pulp gene therapy. Even after the successful use of viral delivery systems in a broad range of tissues, there are serious health hazards which include the risks for mutagenesis, carcinogenesis, and invoking immune reactions in response to viral infection or viral proteins [13].

Huang., *et al.* explored that stem cells from apical papilla and dental pulp give rise to odontoblast-like cells, producing dentin-like tissue on the existing dentinal walls via stem/progenitor cell-based approaches and tissue engineering technologies, pulp-like tissue can be regenerated de novo in an emptied root canal space in mice [21].

Future prospectives

Further investigation of SHEDs must be done in rhesus monkeys, whose alveolar bone microenvironments are more similar to humans. To examine methods to regulate the balance between the osteogenesis and osteoclast genesis processes, further studies are required. Additionally, a series of standardized operation procedures and a safety evaluation system must be established to guarantee the feasibility of the clinical application of SHEDs.

Summary and Conclusion

With extraordinary advances in the prevention, diagnosis and treatment of human diseases, Stem cell research is being pursued in the hope of achieving major medical breakthroughs. With tissues grown from stem cells, scientists are striving to create therapies that rebuild or replace damaged cells and offer hope to people suffering from various ailments. Recently, for the in vitro culture of stem cells, various techniques have been developed that will provide unprecedented opportunities for studying and understanding human embryology. With the advances in the stem cell biology, dental stem cells will hopefully be able to correct cleft palate, save injured teeth and jaw bones, correct periodontal defects, and most importantly regenerate the entire teeth structures.

Bibliography

1. Singh H., *et al.* "Stem Cells: An Emerging Future in Dentistry". *International Journal of Advanced Health Sciences* 1.2 (2014): 17-23.

2. Mihaylova Z., *et al.* "Platelet – derived products and Periodontal Ligament Stem Cells". *Archives of Stem Cell Research* 2.1 (2015): 1-7.

3. Park YJ., *et al.* "Regenerative Applications Using Tooth Derived Stem Cells in Other than Tooth Regenration: A Literature Review". *Stem Cells International* (2016): 1-12.

4. Fouad SAA. "Dental Stem Cells: A Perspective Area in Dentistry". *International Journal of Dental Sciences and Research* 3.2A (2015): 15-25.

5. Venkatesh D., *et al.* "Gingival Mesenchymal Stem Cells". *Journal of Oral and Maxillofacial Pathology* 21.2 (2017): 296-298.

6. Miura M., *et al.* "SHED: Stem cells from human exfoliated deciduous teeth". *Proceedings of the National Academy of Sciences of the United States of America* 100.10 (2003): 5807-5812.

7. Sukarawan W and Osathanon T. "Stem Cells from Human Exfoliated Deciduous Teeth: Biology and Therapeutic Potential". *Intech Open* (2017): 55-76.

8. Arora V., *et al.* "Banking stem cells from human exfoliated deciduous teeth (SHED): Saving for the future". *Journal of Clinical Pediatric Dentistry* 33.4 (2009): 289-294.

9. Wang J., *et al.* "Stem cells from human exfoliated deciduous teeth can differentiate into dopaminergic neuron-like cells". *Stem Cells and Development* 19.9 (2010): 1375-1383.

10. Shetty RM., *et al.* "SHED (Stem Cells from Human Exfoliated Deciduous teeth) - A new source of stem cells in dentistry". *Chatt Journal of Health Sciences* 1.1 (2013): 66-68.

11. Kohli A., *et al.* "Stem Cells – Hope or Hype". *Rama Univ Journal of Dental Sciences* 2.1 (2015): 34-41.

12. Pathak SD., *et al.* "Teeth – A New Source of Stem Cell Stem Cell Revolution Lurks in our Mouth". *IOSR Journal of Dental and Medical Sciences* 15.1 (2016): 17-23.

13. Rai S., *et al.* "Applications of Stem cells in Interdisciplinary Dentistry and Beyond: An Overview". *Annals of Medical and Health Science Research* 3.2 (2013): 245-254.

14. Cordeiro MM., *et al.* "Dental pulp tissue engineering with stem cells from human exfoliated deciduous teeth". *Journal of Endodontics* 34.8 (2008): 962-969.

15. Reznick JB. "Continuing Education: Stem cells: Emerging medical and dental therapies for the dental professional". *Dental Town Magazine* (2008): 42-53.

16. Zhang W., *et al.* "Multilineage differentiation potential of stem cells derived from human dental pulp after cryopreservation". *Tissue Engineering* 12.10 (2006): 2813-2823.

17. Sonoyama W., *et al.* "Mesenchymal Stem - Cell Mediated Functional Tooth Regeneration in Swine". *Plos One* 1.1 (2006): 1-8.

18. Zhu W and Liang M. "Periodontal Ligament Stem Cells: Current Status, Concerns and Future Prospects". *Stem Cells International* (2015): 1-12.

19. Jindal L., *et al.* "Stem cells- the tiny procreators: A review arcticle". *Asian Pacific Journal of Health Science* 6.1 (2019): 118-123.

20. Barron JA., *et al.* "Laser printing of single cells: Statistical analysis, cell viability, and stress". *Annals of Biomedical Engineering* 33.2 (2005): 121-130.

21. Huang GT., *et al.* "Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model". *Tissue Engineering Part A* 16.2 (2010): 605-615.

Volume 3 Issue 10 October 2019