Indian Journal of Contemporary Dentistry
Volume 11 No. 1, January-June 2023

Stem Cells: Impact on Regenerative Dentistry: A Review

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How to cite this article: Kunder AS, Holeyannavar RM, Babaji P, Shashibhushan KK. Stem Cells: Impact on Regenerative Dentistry: A Review.. 2023;11(1): 25-29.

ABSTRACT

The best possible treatment options for patients are now available thanks to the recent revolution in regenerative dentistry using stem cell therapy. The stem cells, which are unspecialized, highly proliferative, clonogenic, and capable of self-renewal, have opened the door to new methods of regenerating missing tissues, repairing cleft palates, regenerating periodontal and jaw bones, and, most importantly, remaking the entire tooth structure. The interest in clinical dentistry is growing daily thanks to recent advancements in stem cell therapy. Stem cell therapy therefore has a bright future in tissue regeneration dentistry. This review provides a concise summary of the background, current uses, and potential future of stem cells.

Keywords: Dental pulp tissue; Stem cells; Regenerative dentistry; Revascularization.

INTRODUCTION

Stem cells are undifferentiated cells of biological type, capable of differentiating into indefinite specialized cells which divide (through mitosis) to produce more cells of a similar type. It is regarded as the predecessor of the genealogical tree of related cell types.[1]

Based on their origin and capacity for differentiation, stem cells are divided into two groups: adult stem cells and embryonic stem cells. Mesenchymal stem cells, also known as MSCs, can be extracted from various tissues including the liver, synovial membrane, bone marrow, adipose tissue, amniotic fluid, umbilical cord, placenta, and teeth. The dental pulp of both deciduous and permanent teeth contains stem cells. Dental pulp stem cells (DPSCs) and stem cells from exfoliated deciduous teeth are the two types of stem cells found inside the tooth (SHED). The dental follicle, periodontal ligament (PDL), and apical papilla have all been found to be additional sources of dental stem cells. These cells have the ability to differentiate into a variety of cell types, including adipocytes, chondrocytes, osteoblasts, bone, new dental tissue, cartilage, muscle, and even nerve regeneration.[2,3]

Dr. Songtao Shi gave the acronym SHED after isolating stem cells from his 6-year-old daughter's deciduous teeth. These cells are of the mesenchymal type, which is highly proliferative and multipotent. Consequently, it is thought to be one of the candidate cell types for tissue regeneration.[1]

History of Stem Cells Research[4]

Early in the 1960s, James Till, Ernest McCulloch, and their University of Toronto

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colleagues learned about mouse reservoirs of cells that have characteristics similar to those of stem cells.

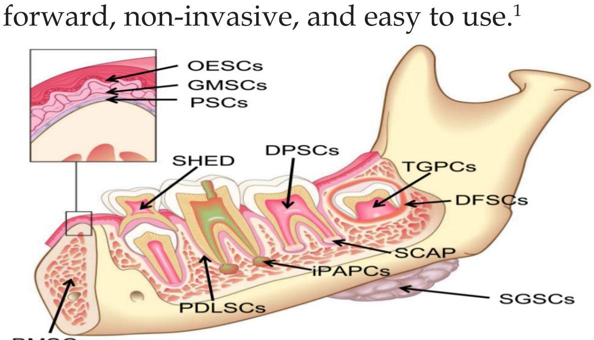
Mooney et al. (1996) performed the first in-vitro study to describe the procedure for regenerating new pulp-like tissues from cultured human pulpal fibroblasts. King et al and King and Hughes' periodontal studies suggest that the healthy PDL may be able to receive stimulation from a distance and move toward the injured, immature root apices, supporting the existence of stem cells in both the PDL and alveolar bone marrow. Following root canal procedures, Thibodeau et al. (2006) confirmed the existence of vital tissue within the root canal space. Gomez Flores et al. made an innovative attempt at in-vivo PDL regeneration using a multilayer human PDL cell sheets technique, which encouraged the formation of PDL and immature cementum-like tissue (2008).

Types of Dental Stem Cells

Diagrammatic representation of possible oral environments for post-natal stem cells. Dental pulp stem cells (DPSCs), bone marrow stem cells (BMSCs), stem cells of the apical papilla (SCAP), tooth germ progenitor cells (TGPCs), dental follicle stem cells (DFSCs), salivary gland stem cells (SGSCs), stem cells from human exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), gingival-derived mesenchymal stem cells (GMSCs (OESCs).[5,6]

SHED collection, separation, and preservation

The technique involves the collection, isolation, and storage of SHED and is straight forward, non-invasive, and easy to use.[1]



Step 1: Collection of tooth

A call is placed to the tooth bank or a dentist present at the bank after the exfoliated primary teeth are placed in the sterile saline solution.[7] In contrast to the grey colour of the pulp, which suggests that the pulpal blood flow has been compromised, necrotic, and is no longer viable for recovery, the pulp of an exfoliated tooth should be red in colour, indicative of cell viability.[8] Tumors or cysts that frequently have a severed blood supply, mobile teeth from trauma, teeth with apical abscesses, or teeth that are mobile due to trauma are not candidates for stem cell recovery. As a result, primary teeth are preferred over mobile teeth that are "hanging on by a thread" after extraction.[9] The tooth is placed into a vial filled with hypotonic phosphate buffered saline solution as it recovers to help prevent tissue desiccation while being transported (up to four teeth in one vial). The vial is then meticulously sealed, put into a thermite-based temperature phase change carrier, and finally placed into an insulated metal transport vessel that is kept hypothermic throughout transportation. This process is known as "Sustentation."[8] The time between harvest and arrival at the processing storage shouldn't be longer than 40 hours.

Step 2: Stem cell isolation[1]

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

With Dulbecco's Phosphate Buffered Saline (PBSA), the tooth surface is cleaned by washing it three times without Ca^{2+} and Mg^{2+}, disinfecting it with povidone-iodine, and then washing it again with PBSA. Using a small, sterile excavator or by directly flushing pulp-rich stem cells from the tooth's centre with salt water, the pulp tissue from the pulp chamber is isolated.[10] A sterile petri dish is used to contain the contaminated pulp tissue after it has been at least three times PBSA-washed. The tissue is then digested for an hour at 37 °C using Dispase and collagenase Type I. We can also use trypsin- EDTA.

Isolated cells are put through a 70 m filter to create single-cell suspension. Mesenchymal Stem Cell Medium (MSC), which contains alpha-modified essential medium with 2 mM glutamine, 15% foetal bovine serum (FBS), 0.1mM L-ascorbic acid phosphate, 100U/ml penicillin, and 100 g/ml streptomycin, is used to cultivate cells. MSC is maintained at 37 ^{0}C and 5% CO_2 in the air. Typically, isolated colonies become visible after 24 hours. The MSC medium can be changed to produce various cell lines.

If cultures are obtained using an unselected preparation, colonies of cells with a morphology similar to epithelial cells or endothelial cells can be established. Cells vanish as they move through successive cell passages. If contamination is severe, one of three techniques can be used:

1. Because epithelial or endothelial-like cells are more strongly attached to the culture flask or dish, culture is reserpinized for a brief period of time to ensure that only stromal cells are detached.
2. Because stromal cells adhere to the culture surface earlier than contaminating cells, the medium is changed 4-6 hours after subculture.
3. Use Fluorescence Activated Cell Sorting (FACS), in which STRO-1 OR CD146 can be used, to separate stem cells. This is thought to be the most trustworthy. The donor's parents receive confirmation of the current state of their child's health and cell viability.

Step 3: Stem cell storage

Stem cell storage can be done in two different approaches:

1. Cryopreservation

It is the process of cooling cells or entire tissues to below-freezing temperatures in order to preserve them. The biological activity or any cellular processes cease at this temperature, causing cell death. Cryopreservation allows for the storage of SHED for a longer period of time while maintaining its usability. Cells that are harvested near the end of log phase growth (roughly 80–90% confluent) are the best candidates for cryopreservation. In order to ensure that another sample will be available for use even in the unlikely event that one of the storage units experiences a problem, the sample is divided into four cryotubes and each part is stored in a separate location in the cryogenic system. The cells are kept alive in liquid nitrogen vapour at a temperature of less than -150 ^{0}C. This keeps the cells viable and maintains both their latency and potency. The ideal cell density in a vial is 1-2 x 10^6 cells in 1.5 ml of freezing medium. If the cell count is too low or too high, the recovery rate might be affected.[1]

Third molar pulp tissue may be a suitable source of multipotent stem cells even after cryopreservation for upcoming tissue engineering techniques and cell-based therapies, according to Zhang et al. (2006) who examined the differential potential of stem cells from the cryopreserved pulp of human third molars.[12]

2. Magnetic Freezing

This technology, known as CAS (Cell Alive System), takes advantage of the fact that when water or cell tissue is exposed to a weak magnetic field, the freezing point of those substances can drop by up to 6–7 ^{0}C. With CAS, an object is completely chilled below freezing without actually freezing. This prevents cell wall damage from ice expansion and nutrient loss from capillary action, both of which are common side effects of traditional freezing techniques. Once the object is uniformly chilled, the magnetic field is turned off, and the object snap-freezes.[1]

APPLICATIONS IN DENTISTRY

Pulp implantation:

In an open apex procedure, postnatal stem cells (obtained from skin, buccal mucosa, fat, and bone) are directly injected into clean root canal systems. This makes it easier to collect and administer autologous stem cells by syringe and increases their potential to promote new pulp regeneration. However, the drawbacks include the cells' low rate of survival and their migration to various parts of the body. To increase the likelihood that pulp regeneration will be successful, all three components (cells, growth factors, and scaffold) must be taken into account.[1]

Apexogenesis or Apexification

In younger patients with immature roots, a conservative approach is needed to retain some vital pulp tissue and allow continued root formation. In an immature permanent tooth, the regeneration of tissue into the apex may derive from stem cells already present in the vital pulp tissue, SCAP, PDL, or alveolar bone. Stem cells are used to promote pulpal regeneration so as to treat immature permanent teeth in a conservative approach. Regenerative endodontic increases root length and thickness leading to complete root formation.[4]

Pulp Revascularization

An immature tooth with pulpal necrosis (due to caries or trauma) may stop further root development, resulting in thin root canal walls susceptible to fracture and blunderbuss apices making it difficult to obtain a hermetic seal with conventional obturation techniques. Pulpal tissue regeneration of an infected immature tooth will take place only in the absence of intra-pulpal infection which favours the environment for repopulation of mesenchymal cells arising from dental papilla or apical periodontium.[4]

Whole Tooth Regeneration

By seeding various cell types on biodegradable scaffolds, tooth-like tissues have been regenerated. Cells can be harvested, grown, and differentiated in vitro before being seeded onto scaffolds and implanted in vivo. In certain circumstances, the scaffolds are reimplanted into the jaws or an extracted tooth socket.[4]

Dental or Tooth Regeneration, Why??

The ultimate goal of tooth regeneration is to have completely functioning bioengineered teeth that can replace the lost natural teeth. Recent advances like titanium dental implants do not function as identical as natural teeth, because they lack an intervening PDL after osseointegration. Hence, the regeneration of the tooth root using dental stem cells seem to be a more realistic and clinically approachable treatment modality.[4]

Hurdles for Regenerative Procedures

A major problem routinely encountered in the clinical application of tooth regeneration technology is the identification of the most appropriate and suitable autologous stem cell source in humans, but there are numerous hurdles with in-vitro regenerative procedures: Stem cells are avascular in nature, need to be grown and expanded before being implanted into the root canal, should have proper adherence to the disinfected root canal walls and the technical hurdle in replanting the regenerated pulp without harming the cells.[4]

Current Trends and Future Perspectives of Stem Cells Regeneration

Recently, it was developed to recreate a murine "Bioengineered tooth unit" in vivo using the same cell source as the bioengineered tooth. It's interesting to note that the unit also includes the alveolar bone in addition to a fully developed tooth and periodontal ligament. In a mouse model with a vertical alveolar bone defect, the unit promoted the regeneration of a fully functional tooth along with vertical bone growth. These findings gave rise to a novel idea for tooth regeneration therapy: The transplantation of a bioengineered tooth has unparalleled ability to both fully regenerate the tooth and to correct a severe alveolar bone defect.

In the future, it may be possible to regenerate dental tissue using genetically modified cells that deliver physiologically specific growth factors locally. Dental professionals have realised that the present is the crucial time to take action as the opportunities to bank patient stem cells will have the greatest future impact. Additional research must be done because the field of study is still in its infancy.[13]

CONCLUSION

Stem cell therapy is popular today as a treatment option for illnesses and injuries, and it has numerous health and dental advantages. The use of dental stem cells to treat diabetes mellitus, neurodegenerative diseases (Parkinson's disease, Alzheimer's disease), muscular dystrophy, lupus erythematosus, impaired vision, extensive burns, and cardiomyopathies is the focus of current research in regenerative medicine. Although there is still much research to be done, it is clear from the available evidence that primary teeth are a better source of stem cells and that dental stem cells have a huge potential for use in stem cell therapies.

CONFLICT OF INTEREST: Nil

SOURCE OF FUNDING: Self

ETHICAL CLEARANCE: Not applicable

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