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STEMS CELLS IN ENDODONTIC THERAPY - A LITERATURE REVIEW

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ABSTRACT

Stem cells havea prominent role in developing different types of cell in the body. It is a repair system of the body, which can divide into other cells in humans without any limit. However, In replacing the damaged or lost tooth, many options are available in the stem cell and tissue engineering. Every year, the treatment of endodontics is helpful in saving millions of teeth, now the regenerative ideas for the treatment of disease pulp are being implemented. The process is done to replace the damaged structures like root, dentin, and cells in the dentin - pulp complex which is biologically done is called regenerative endodontic procedures(REPS). The major elements used in regenerative endodontics are the utilization of stem cells and tissue engineering. Tissue engineering is one of the branches in which biology, bioengineering, biotechnology, and clinical sciences are combined for the production of new cells. In dentistry, regeneration of the lost tissue is a developing process. The main aim of stem cell therapy is to treat the not fully developed permanent teeth or the death of the cells and tissues in pulp by regeneration and revascularization. The goal of this review is to discuss the potential impact of stem cells on endodontics therapy.

INTRODUCTION

The process is done to replace the damaged structures like root, dentin, and cells in the dentin - pulp complex which is biologically done is called regenerative endodontic procedures(REPS). (1)The main aim of stem cell therapy is to treat the not fully developed permanent teeth or the death of the cells and tissues in pulp by regeneration and revascularization. (2).
The major elements used in regenerative endodontics are the utilization of stem cells and tissue engineering. Tissue engineering is one of the branches in which biology, bioengineering, biotechnology, and clinical sciences are combined for the production of new cells. (3)

STEM CELLS
Stem cells are defined as clonogenic cells capable of both self- renewal and multilineage differentiation since they are thought to be undifferentiated cells with varying degrees of potency and plasticity. (4) they differentiate into one daughter stem cell and one progenitor cell.

CLASSIFICATION OF STEM CELLS:(6)
Stem cells can be classified according to their mobility:
i) Totipotent stem cell-can differentiate into all embryonic and extraembryonic cell types.
ii) Pluripotent stem cells- can differentiate into all types of cells except cells of the embryonic membrane.
iii) Multipotent stem cells - can differentiate into more than one mature cell

Stem cells can be classified based on the origin (7)
a) Embryonic stem cells - They are the stem cells derived from the undifferentiated inner mass of the human embryo, and they derived from the inner mass of the blastocyst stage of development. It has the capacity to regenerate and repair the organs and tissues which are affected in the body.
b) Postnatal stem cells/ Adult stem-cells -They are also known as somatic stem cells, It is undifferentiated cells which are found throughout the body that divide to replenish and regenerate the damaged stem cells.
Stem cells are often categorized by their source.(7)
a) Autologous stem cells - are obtained from the same individual to whom they will be implanted.
b) Allogeneic stem cells - originate from a donor of the same species.
c) Xenogeneic cells - are those isolated from individuals of another species
d) Syngenic: obtained from genetically identical organisms

Table 1: Classification of stem cells (8)

TYPES OF STEM CELLS		
CLASSIFICATION BASED ON THEIR PLASTICITY		
1.	Totipotent stem cells	*They differentiate into all embryonic and extraembryonic cell types.
2.	Pluripotent stem cells	*They differentiate into all types of cells except cells of the embryonic membrane.

3.	Multipotent stem cells	*They differentiate into more than one mature cell.
CLASSIFICATION BASED ON ORIGIN		
1.	Embryonic stem cells	*They are the stem cells derived from the undifferentiated inner mass of the human embryo, and they derived from the inner mass of the blastocyst stage of development.
2.	Postnatal stem cells/Adult stem cells	*They are also known as somatic stem cells, It is undifferentiated cells which are found throughout the body that divide to replenish and regenerate the damaged stem cells.
CLASSIFICATION BASED ON THEIR SOURCE		
1.	Autologous stem cells	*Are obtained from the same individual to whom they will be implanted.
2.	Allogenic stem cells	*Originate from a donor of the same species.
3.	Xenogeneic cells	*Are isolated from individuals of another species.
4.	Syngenic	*Obtained from genetically identical organisms.

Adult multipotent mesenchymal stem cells are seen in the pulp of deciduous and permanent teeth. Blood vessels and a huge network of nerves are situated in the central area of the pulp. Those areas were lined by odontogenic area peripherally which has three layers

1.Cell rich zone - The layer consists of fibroblast and undifferentiated mesenchymal cells that are present in the innermost area of the pulp.

2. Cell-free zone (zone of Weil) - the intermediate zone which has plexus of Rashkow and is rich in both nerve networks and capillaries.

3. Odontoblastic layer - The layer which lies next to the predentin and mature dentin consists of odontoblasts cells that were in the outer area.

MATERIALS AND METHODS

This study included 35 Articles from various search engines. The search engines used for the present study include PubMed, Google Scholar, Cochrane, MesH core, BioRxiv.The articles were collected from the duration of 2000 to till date. However, a few articles due to unavailability in recent years were also selected for the study.

For the selection of articles,5 steps were involved - Identification of articles with a clear objective, Identification of relevant articles, selection of articles, Data extraction, analysis and report

Dental stem cells: Dental stem cells can be isolated from dental pulp. The dental stem cells which are successfully found are of 5 types, they are

- Dental pulp stem cells.
- Stem cells from human exfoliated deciduous teeth.
- Stem cells from apical papilla.
- Periodontal ligament stem cells
- Dental Follicle progenitor cells.

Table 2: Types of dental stem cells(9)

Type of Dental stem cells	Definition
Dental pulp stem cells (DPSs)	They are isolated from the pulp tissue of extracted human third molars.
Dental Follicle progenitor cells (DFPCs)	The dental follicle helps in the formation of progenitor cells of the periodontal ligament which is surrounded by the tooth germ.
Stem cells from apical papilla (SCAP)	Mesenchymal stem cells are present in the apical papilla of permanent immature teeth
Periodontal ligament stem cells (PDLSCs)	They are stem cells present in the perivascular space of the periodontium, the tissue that surrounds the teeth. They are responsible for the regeneration of periodontal components including periodontal ligament, alveolar bone
Stem cells from human exfoliated deciduous teeth(SHED)	They are mesenchymal cells present within exfoliated deciduous tooth pulp tissue that can different cell types

THE TECHNIQUE OF STEM CELL IDENTIFICATION:

Staining the cells with specific antibody markers and using a flow cytometer, in a process called fluorescent antibody cell sorting (FACs). (10)

Flow cytometry has advantages such as the ability to escape dead cells, to identify the cell by size and structure. and multi-color analysis to measure several antigens simultaneously. with cone-beam computed tomography, In a root canal, the thickness of dentin is checked after the cleaning and shaping (11)

- Immuno-magnetic bead selection - Immunomagnetic separation (IMS) is a method that deals with the isolation of cells, proteins, and nucleic acids within a cell culture or body fluid through the specific capture of biomolecules through the attachment of small-magnetized particles, beads, containing antibodies and lectins.(12)
- Immunohistochemical staining - Immunostaining is any use of an antibody-based method to detect a specificprotein in a sample. (13)
- Physiological and histological criteria - including phenotype, chemotaxis, proliferation, differentiation, and mineralizing activity

DENTAL PULP STEM CELLS (DPSC):

Dental stem cells contain a population of stem cells which is the Mesenchymal stem cells that are isolated from the dental pulp of permanent teeth are termed as Dental Pulp Stem Cells (DPSC). (14)They are also referred to as odontoblastic cells because these cells appear to synthesize and secrete dentin matrix like the odontoblast cells that they replace. (15)

Dental pulp stem cells, in vitro culture, have osteogenic and chondrogenic potential *in vitro* and have a capability to differentiate into dentin, whereas in vivo culture, it differentiates into dentin-pulp-like complex(16).

In the study done by Ming Yan *et al*, for the reconstruction of biotooth and pulp dentin complexes, DPSCs are useful. Human teeth are made up of enamel, dentin, cementum, and pulp tissue. Enamel is formed by ameloblasts cells, dentin is made by odontoblasts cells, and cementum is made by cementoblast cells. In this all types of stem cells, stem cells are differentiated. Hence from stem cells, bioteeth was manufactured. (17).

The advantages of DPSCs were, it forms neurons than other stem cells.they are more easily isolated than other stem cells, such as MSCs from the bone marrow and NSCs from cadavers, and are stored in the medium (18) and there are fewer ethical considerations than those which should other stem cells. (19).

The factors which make dental stem cells unique are Dental stem cells are highly proliferative, growing better in culture than many other types of adult stem cells. Dental stem cells are adult stem cells and are not the subject of the same ethical concerns as embryonic stem cells.(20,21)

STEM CELLS FROM HUMAN EXFOLIATED DECIDUOUS TEETH

Mesenchymal stem cells that are isolated from the dental pulp of exfoliated deciduous teeth are termed as Stem cells from Human Exfoliated Deciduous teeth (SHED). (22)SHED belongs to the highly proliferative clonogenic cell which has the ability to differentiate into a variety of cell types including neural cells, adipocytes, and odontoblasts. (23)In the process of repairing damaged tooth structures, to induce regenerating the bone, and in treating of neuronal tissue injury or degenerative disease the ideal source of stem cells was Deciduous teeth (24)

SHED cells are less mature and have the potential to grow into a large variety of tissue types and can multiply rapidly and grow much faster than adult stem cells. (25,26). Further, SHED cells can differentiate not only into dental pulp, bone, and dentin but also into neural and fat cells because they were able to express proteins on their cell surfaces. ((27)

The major variations between dental stem cells and cord blood stem cells were, Dental stem cells consist of mesenchymal stem cells whereas cord blood

contains mainly of hematopoietic stem cells, likely calcified tooth and bone marrow consists of both types of stem cells. (28)SHED cells have more proliferation rate, increased cell population doublings,(5) viability, osteoinductive capacities and failure to reconstitute a dentin pulp like complex so that these cells were said to be distinct from DPSC (29)
Types of Stem Cells in Human Exfoliated Deciduous teeth (SHED):(30)
Adipocytes; It has been helpful in treating cardiovascular disease, spine and orthopedic conditions, congestive heart failure, Crohn's disease, and plastic surgery.
Chondrocytes and Osteoblasts: successfully used for the growth of bone and cartilage suitable for transplant. They are also used to grow the intact teeth
Mesenchymal cells: Mesenchymal Stem cells have more therapeutic potential than other types of adult stem cells.

PERIODONTAL LIGAMENT STEM CELLS

From the root of the extracted teeth, human Periodontal ligament stem cells have been isolated (31).In 2004, Seo et al was the first to isolate and characterize the stem cells present in it. (32) PDLSCs has the capability to form cell lineages of other types and has an ability to differentiate into adipocytes, cells like cementoblast and in vivo, collagen forming cells whereas in in-vitro, they have the capacity to form Periodontal ligament type of cells. (33)) The studies done by Kawanabe *et al* found that human periodontal ligaments have high proliferating stem cells. (34)

The PDL is a specialized tissue located between the cementum and the alveolar bone and has as a role the maintenance and support of the teeth. These cells could be isolated as, colony-forming cells,plastic-adherent whereas in in-vivo conditions they show low potency of osteogenic differentiation. These stem cells have a capacity to differentiate in cells or tissues which are closely related to periodontium.but display a low potential for osteogenic differentiation under in vitro conditions. PDL stem cells differentiate into cells or tissues very similar to the periodontium. (35)

STEM CELLS FROM THE APICAL PAPILLA (SCAP):

Stem cells from the apical papilla (SCAP) are the mesenchymal cells that are isolated from the developing apical ends of the tooth. (36)It has a high capability to differentiate into odontoblast when compared to stem cells of the periodontal ligament. (37)
In vivo conditions, odontoblast-like cells are formed which produce dentin and for the formation of root dentin, they are likely to be the source of the cell of odontoblast. These support apexogenesis, which mostly present in immature permanent teeth which have periradicular periodontitis and abscess. (38) Due to close proximity to periapical tissue vasculature, they are capable of surviving in pulp necrosis. After the disinfection of endodontic, they are able to generate odontoblasts and under the influence of Hertwig epithelial root sheath, complete root formation occurs.(39,40)Mostly, SCAP was isolated from the third molars of humans.

STEM CELLS IN ENDODONTICS

Stem cells in the dental pulp: Multipotent stem cells in the dental pulp have their phenotype in perivascular niches and their fraction is small and the location of these cells was not clearly known. The DPSCs and SHED develop from the same origin that is dental pulp, but show slight differences. (41) For example, during osteogenic differentiation, SHED presents higher levels of alkaline phosphatase activity and osteocalcin production, and higher proliferative rate than DPSC. SHED and DPSC cells are capable of regenerating dentin and pulp-like tissues in vivo(42).

Stem cells in pulp angiogenesis: The inducer of endothelial cell differentiation and survival, and it is the most effective angiogenic factor is Vascular endothelial growth factor (VEGF). (43), In the inflammations, matrix metalloproteinase genes are involved in which VEGF also plays a critical role in the control of vascular permeability during physiological and pathological events. VEGF is strongly expressed by odontoblasts and in the sub-odontoblastic layer in vivo(44)

POTENTIAL APPLICATIONS OF DENTAL STEM CELLS

In recent years, many researchers focused mainly on the regenerations of damaged pulp, dentin, root resorption and the regeneration and repair of the periodontium

Whole tooth regeneration to replace traditional dental implants is also in the pipeline. (45)

To exploit oral microbial colonization procedures and salivary proteins, methods like gene transfer were mostly used to promote faster healing of ulcers and wounds in the oral cavity is done by the applications of tissue engineering of dental stem cells. (46)

Over the past few years and the research has been done in the regenerative potential of adult stem cells like dental tissues have the following (47)

- Regeneration of damaged coronal dentin and pulp
- Regeneration of resorbed root, cervical or apical dentin, and repair perforations
- Periodontal regeneration.
- Repair and replacement of bone in craniofacial defects.
- Whole tooth regeneration.

Table 3: Clinical Applications of dental stem cells(48)

Cell type	Possible clinical applications
DPSCs	i) Regenerative endodontics ii) Bone regeneration
SHED	i) Regenerative endodontics ii) Bone regeneration
PDLSCs	i) Regenerative endodontics ii) Bone regeneration

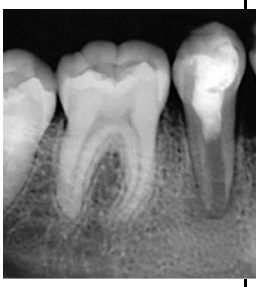
DFSCs	i) Periodontal regeneration ii) Bone regeneration
SCAP	i) Periodontal regeneration ii) Bone regeneration

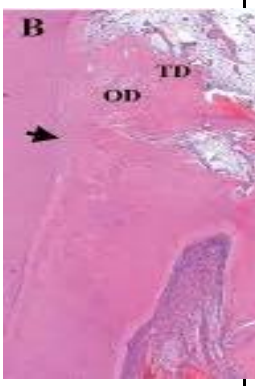
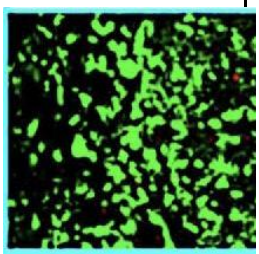
ROLE OF DENTAL STEM CELLS IN REGENERATIVE MEDICINE

Regenerative medicine is to replace and regenerate the tissues which are fully functional and have a capacity to repair the damaged ones which occur during diseases, injury. In regenerative medicine and tissue engineering, they have potential use and features in dental stem cells. (49)

1. Dental Pulp Regeneration- It is a biological process, where the continuity of the disrupted or the lost tissues and it is used to restore diseased dental pulp and help in healing. This process should implement the dentin repair with the use of tooth regeneration scaffolding which contains collagen, fibrin, and man-made polymers, a biodegradable substance that will encourage the tooth to repair itself. (50,51)
2. Bio tooth engineering - The process in which the natural teeth can be remodeled into a similar type of tooth and which has a capacity to replace permanently like dental implants. (52) The bioengineered teeth which were functional were resembling the both anisotropic, structural, mechanical properties of original teeth have become a major problem.(53,54)

Table 4: Developmental approaches for regenerative endodontic **techniques**(55)

Technique	Image	Advantages	Disadvantages
Root canal revascularization : Open up to tooth apex to 1mm to allow bleeding into root canals		• Lowest risk of immune injection • Lowest risk of pathogenic infection	• Minimal case reports published to date • The potential risk of necrosis if tissue becomes infected
Stem cell therapy: Autologous or allogeneic stem or cells are delivered to teeth via injectable		• Quick • Easy delivery • Least painful • Cells are easy to harvest	• Low cell survival • Cells do not produce new functional pulp • High risk of

matrix			complications
Pulp implant: pulp tissue is grown in the laboratory in sheets and implanted surgically		• Sheets of cells are easy to grow • More stable than an injection of dissociated cells	• Sheets lacks vascularity so only small constructs are possible
Scaffold implant : Pulp cells are seeded onto a 3D scaffold made of polymers and implanted surgically		• Structure supports cell organization • Some materials may promote revascularization.	• Low cell survival after implantation • Must be engineered to fit root canal precisely
3D cell printing : It is used to combine cells, growth factors, biomaterials to fabricate them. It is an Ink jet-like device.		• Multiple cell types can be precisely positioned	• Must be engineered to fit root canal precisely

Injectable scaffolds: With the injections, cells are suspended along with hydrogels		• Easy delivery • May promote regeneration by providing a substitute in the extracellular matrix	• Limited control over tissue formation • Low cell survival
Gene therapy: Mineralizing genes are transfected into vital pulp cells of necrotic and symptomatic teeth		• May avoid cleaning and shaping root canals • May avoid the need to implant stem cells	• Difficult to control • Risk of health hazards • Not approved by the FDA

FUTURE SCOPE

For regeneration of the dental pulp, fabrication of vascularized scaffolds is likely a key requirement.Advances in growth factors or drugs to control the activity of cells must be sought out.

CONCLUSION

Stem cells are critical for the dental pulp and for the response of this tissue to injury. Many findings displayed that in the case of reversible pulpitis, stem cells have the potential to implement.Importantly, these cells may become the primary strategy for the revitalization of necrotic immature permanent teeth. Such discoveries have the potential to fundamentally change the paradigms of conservative vital pulp and root canal therapy, and perhaps allow for the treatment in the future of conditions that are presently untreatable in Dentistry. Therefore, endodontists should recognize the potential of the emerging field of regenerative endodontics and the possibility of obtaining stem cells during conventional dental treatments that can be banked for autologous therapeutic use in the future.

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