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TISSUE ENGINEERING AND REGENERATIVE ENDODONTICS -A REVIEW

[1] Mohammed Ghazanfaruddin [1] Reader

[1] Department of Conservative Dentistry and Endodontics, GITAM Dental College and Hospital, Rushikonda, Visakhapatnam-530045, Andhra Pradesh, India.

ABSTRACT:. Conventional endodontic therapy provides a high success rate in pulpless tooth but immature pulpless tooth provides potential treatment complications. The ideal treatment objective would be to replace a tissue with a similar tissue. Pulpal regeneration is a novel way to restore the function of a non-vital tooth. Tissue engineering involves the development of functional tissue with the ability to replace missing or damaged tissue.

KEYWORDS: Embryonic stem, Periodontal ligament cells, Scaffold

INTRODUCTION

Dental pulp is a highly specialized connective tissue. The damage to dental pulp by mechanical, chemical, thermal and microbial irritants activates various types of inflammatory responses involving complex vascular, lymphatic and local tissue reaction[1]. Diseased dental pulp with its limited reparative potential leaves very less treatment alternatives and the dental restorations or the prosthesis have a limited life. One novel approach to restore the tooth structure is based on biology; regenerative endodontic procedures by application of tissue engineering[2].

The term "Tissue engineering" was coined by Langer and Vacanti in 1993 to describe the process by which the tissues and organs are generated by cell transplantation with or without a scaffold[3].

In endodontics the objectives of tissue engineering are

1. To regenerate the lost tissues
2. To repair the damaged tissues
3. To replace the missing tissues

The key elements involved in tissue engineering are [1,2,3]

1. Cells
2. Scaffolds
3. Morphogenes

I. Cells

They are undifferentiated cells with varying degree of potency and plasticity, capable of self-renewal and multi lineage differentiation [1,4]. Depending on origin, stem cells are classified into two basic types:

1. Embryonic stem
2. Post natal stem cells

Use of embryonic cells is limited by ethical issues, postnatal stem cells constitutes more favourable cellular source to be used in tissue engineering. These can be isolated from various tissues including brain, skin, hair follicles, skeletal muscles bone marrow and dental tissues.[5]: five different human dental stem cells have been isolated and they are[6]

1. Dental pulp stem cells(DPSCs)
2. Stem cells from exfoliated deciduous teeth(SHED)
3. Periodontal ligament stem cells(PDLSCs)
4. Stem cells from apical papilla(SCAP)
5. Dental follicle progenitor cells(DFPCs)

Dental pulp stem cells (DPSCs)

During pulpal injury these undifferentiated mesenchymal cells migrate from deeper region of the pulp and replace the degenerated odontoblasts[7]. It has been demonstrated that they have capacity to adhere to scaffold and they can also differentiate into odontoblastic lineage cells.[6] The ability of DPSCs to produce a tooth- like tissue expressing genes that are consistent with odontoblastic differentiation was demonstrated when these cells were transplanted into immunocompromised mice.[8]

Stem cells from exfoliated deciduous teeth (SHED)

In 2003 Dr. Songtao shi isolated stem cells from SHED of his 6 year old daughter.SHED showed higher proliferation rates than DPSCs.[9] and demonstrated formation of well organised pulpal tissue and differentiation of functional odontoblasts that are capable of generating new dentin. The advantage of these cells are that they are easy to harvest and abundantly available.

Periodontal ligament cells

These are multipotent cells having greatest potential for osteogenic, adipogenic and chondrogenic differentiation.

Stem cells from apical papilla(SCAP)

Apical portion of growing roots have specialised cells which have capacity to differentiate into various cell colonies. They have capacity to produce pulp like tissue [10]

Dental follicle progenitor cells (DFPCs)

Human dental follicle is a tissue of tooth germ which can be isolated after third molar extraction. Human DFPC have the ability to differentiate toward alveolar osteoblasts, PDL fibroblasts, cementoblasts, adipocytes, and neuron-like cells[11,12,13]

II. Scaffold

These are three dimensional structures that provide physico-mechanical and biological environment for cell growth and differentiation. *Natural* and *Synthetic* scaffolds can be used that have biocompatibility, non-toxicity and proper physical and mechanical strength.(14). *Natura*l and *synthetic* scaffolds can be used. Natural polymers such as collagen and glycosaminoglycans are used and synthetic polymers like polyLactic acid(PLA), poly glycolic acid (PGA), and their co polymers polylactic-co-glycoloc acid (PLGA). Synthetic hydrogels include poly ethelene glycol (PEG) based polymers, and those modified with cell surface adhesion peptides such as arginine glycine and aspartic acid that can improve cell adhesion and matrix synthesis with three dimensional network. [15]

III. Morphogenes

Stem cells require external stimuli to undergo differentiation. Morphogenes or the signalling molecules are protein in nature that binds to the specific cell membrane receptor and induce a cascade of processes that result in generation of new tissue[1,16)]. Morphogenes regulates the rate of tissue proliferation, cell differentiation into another cell type and matrix production.

Growth factors play an important role in regenerative endodontics. Dentin contains many proteins capable of stimulating tissue responses. Demineralization of dentinal tissue can lead to release of growth factors following the application of cavity etching agents, restorative materials and even caries.[17] List of growth factors are elaborated in table **(Table-I)**

Growth factors especially belonging to transforming growth factor beta (TGFβ) family, are important in cellular signalling for odontoblast differentiation and stimulation of dentin matrix secretion. [17] Another important family of growth factors in tooth development[18] and regeneration[19] consists of bone morphogenic protein.(BMP'S). Seven types of bone morphogenic proteins are identified, BMP1 to BMP7. Recombitant human BMP2 stimulates differentiation of adult pulp stem cells into odontoblastoid morphology in culture [20,21]. Recombitant BMP2-4 and 7 induce formation of reparative dentin in-vivo[.22,23.]

Table-I. List of growth factors

Abbreviation	Factor	Usefulness
BMP	Bone morphogenic protein	Differentiation of osteoblasts and odontoblasts,mineralize-tion of bone and dentin
CSF	Colony stimulating factor	Increases stem cell number
EGF	Epidermal growth factor	Proliferation of mesenchymal, glial and epithelial cells
FGF	Fibroblast growth factor	Increases stem cell number
IGF	Insulin like growth factor	Increases stem cell number
IL	Interlukins	Promotes inflammatory cell activity
PDGF	Platelet derived growth factor	Proliferation of connective tissues, glial and smooth muscle cells
TGFα	Transforming growth factor alpha	Epithelial and tissue structure development
TGFβ	Transformatio n growth factor beta	Present in dentin matrix and promotes mineralization of pulp tissues
NGF	Nerve growth factor	Promotes neuron outgrowth and neural cell survivor.

Tissue engineering strategies can be of

1. **Conductive**
2. **Inductive** or
3. **Cell based**

Conductive approach utilizes biomaterials in passive manner to facilitate growth of existing tissue, (example is use of calcium hydroxide in pulp capping).Inductive approach utilises morphogenes alone at the site to activate cells and the third approach involves cell transplantation at the site.

All the above approaches can be used ***In-Vivo or Ex-vivo.***

Regenerative endodontics

The ultimate ain of tissue engineering in endodontics is regeneration of pulp. Various techniques adapted for regenerative endodontics adapted are

1. Root canal revascularization
2. Post natal stem cell therapy
3. Pulp implantation
4. Scaffold implantation
5. Injectable scaffols delivery
6. Three dimensional cell printing
7. Gene therapy

Root canal revascularization

Necrosis of immature tooth with open apex would render the tooth weak and prone to fracture. Ideal treatment would be to induce the root completion followed by restoration. Revascularization method assumes that once root canal space had been disinfected and the formation of blood clot is initiated by over instrumentation results in fibrin matrix formation that traps cells that are capable of initiating new tissue formation[17]. It has been suggested that these cells originates from local tissues adjacent to the apex of root but not from systemic circulation.These undifferentiated mesenchymal cells are derived from SCAP's and differentiates into primary odontoblasts to complete root formation[24], Other possible mechanism of root development can be attributed to stem cells from periodontal ligament [25,26].
It was demonstrated that the regenerated tissue does not histologically resemble to the pulp but it consists of tissues resembling cementum, periodontium and bone.[27]

Post natal stem cell therapy

It involves injection of post natal stem cells into root canal. The best approach would be to use cells from autologous (patient's own) cell sthat has been taken from buccal mucosa or umbilical cord .[17] Advantages includes relative ease of harvesting and delivering with a syringe and the cells have potential to induce new pulp regeneration. Disadvantages of this technique includes low survival rates of cells and secondly the cells might migrate to different areas of body[28] possibly leading to aberrant pattern of mineralization.

Pulp implantation

Cells are grown in vitro on membrane filter and many filters are rolled to form a three dimensional structure and inserted in disinfected pulp space. Advantages is the localization of cells within canals but the disadvantage is the sheets are very fragile to be carried and inserted in desired position.[17]

Scaffold implantation

It involves positioning of cells in scaffold and inserting the assembly in the disinfected pulp space. A scaffold should contain growth factors to aid proliferation and differentiation, leading to improved and faster tissue development.[29]

Injectable scaffold delivery

Root canal have varied three dimensional anatomy and a rigid scaffold may not occupy all the canal space, So cells are injected with liquid scaffold which enables them to reach all the area of canal anatomy. An example of injectable scaffold is hydrogel.

Three dimensional cell printing

One of the major disadvantages of the previously mentioned methods includes aberrant histology of formed pulp. Three dimensional cell printing enables precise spatial arrangement of the cells[30] in predetermined position and the resultant tissue would mimic the natural tissue.

Gene therapy

Gene therapy includes modification or alteration of gene to regulate the cellular processes and responses and to utilize it for therapeutic benefit. Vector is used for this alteration which can be viral or non-viral. The transfected gene can stimulate immune response, modify cellular information, or developmental programme or produce a therapeutic protein with specific function.[31]. One use of gene therapy would be to deliver mineralizing genes into pulp to promote tissue mineralization. However FDA approval for gene therapy was withdrawn, in 2003 when a 9 year old boy receiving gene therapy was found to have developed tumours in different parts of his body.[31].

CONCLUSION

Although many of the above mentioned pulpal regenerative procedures are in initial stages of research and some are only hypothetical. Pulpal revasularisation procedure have shown promising results clinically. A lot of research needs to be done for incorporation of growth factors in capping agents, and introduction of newer materials which would exploit endogenous growth factors.

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Corresponding Author

Dr.Mohammed Ghazanfaruddin
Reader
Department of Conservative Dentistry and Endodontics
GITAM Dental College and Hospital,
Rushikonda, Visakhapatnam-530045,
Andhra Pradesh, India.
Mobile.no:9985243377.
E-mail: m_ghazan@yahoo.com