THE "PHYSICS AND CHEMISTRY" BEHIND THE "BIOLOGY" OF PULPAL REGENERATION

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Abstract

Root canal therapy for the management of pulpo-periapical diseases involves three phases access to the pulp space, removal of the necrotic content from the canals and obturating the space with synthetic biomaterials. Majority of the failures in root therapy is attributed to the last phase of obturation. Persistence of infection or reinfection is mainly due to the re-establishment of the microbes in the canal space establishing through the interface between the natural root dentin and the artificial sealers and obturating materials. Thus the focus started on filling the space with the natural pulp and dentin structures in the root canal without resorting to synthetic materials. Thus emerged the concept of Pulpal Regeneration. Revascularization of the pulp, use of stem cell engineering are few concepts in this. This article focuses on the current tend and practice of pulpal regeneration and the biomaterials that are used for regeneration and tissue engineerings.

Key words: *Revascularization of pulp, pulpal regeneration, Dental pulp stem cells (DPSC), Stem Cells from Human Exfoliated Deciduous teeth (SHED)*

Introduction

"Restitutio as integrum" is the ultimate goal for all medicinal therapy including pulpal regeneration. Regeneration of pulp can be accepted only when newly formed pulpal tissue has vascularized connective tissue, neural tissue and functional odontoblast lining the dentin wall of the pulp chamber.[1,2] This goal is yet to be achieved in the science and the following review will highlight the path till travelled by the researchers of the past and present.

Techniques for Pulp regeneration

The concept of tissue engineering was coined and explored by Langer and Vacanti in 1993. Regeneration and Repair of pulp are two different biologic processes for replacement of lost pulpal tissue with different outcomes. *Repair* of pulp with biomaterials will lead to formation of new tissue that is devoid of function and structure similar to the original pulp tissue (Scar tissue). *Regeneration* means to restore the lost tissue by new tissue which will not bedifferent from the original tissue in structure and function. *Revascularization* is another terminology that is used in pulpal regeneration, which is ill defined and many times synonymously used with regeneration. Scientific documentation of revascularization dates back to 1960, when Nygaard-Ostby et al reported pulpal healing of infected root canals by mechanical creation of blood clot.[3]

The following are the pulpal regenerative techniques that are being explored for the clinical applications:[4]

1. Root canal revascularization via blood clotting
2. Stem cell based therapies
3. Cell free therapies: scaffold implantation
4. Cell free therapies: injectable scaffold delivery
5. Pulp implantation
6. Three dimensional cell printing
7. Gene therapy

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Revascularization

The present clinical strategy for formation of apical root that has been damaged by trauma or necrotic pulp is the apexification procedure with calcium hydroxide.[5] The disadvantage of this procedure is long treatment plan, unpredictable outcome, weakening of roots structure because of placement of calcium hydroxide.

Difference between the conventional approach for apexification or apexogenesis and the pulp revascularization is that in the former only the root apex is closed, thus leaving the wide canals that can be prone for fracture in long run. Whereas in pulp revascularization, along with the apical closure, there will be continuous root development and increased dentinal walls.[6] This finding has been supported with many case reports and case series.[7,8] However, in current status, the predictability of the outcome is not there.[9] The classical technique and the material that is used for revascularization for irreversible pulpitis with apical periodontitis cases is as follows;[10]

1. Access cavity and disinfection of root canal with sodium hypochlorite and chlorhexidine irrigation.
2. Placement of intracanal medicament with tri-antibiotic paste namely Ciprofloxacin, Metronidazole and Minocycline paste.
3. Creation of blood clot mechanically at cemento-dentinal level
4. Permanent coronal seal with MTA and restoration with resin composite

Disadvantage of tri-antibiotic paste was that, it leads to discoloration of clinical crown as it contains minocycline. To overcome this, the coronal dentinal tubules of access cavity were sealed with bonding agents and flowable composite before placement of tri-antibiotic paste.[11] The four different types of tissue replacement that can be present following revascularization:

1. Complete dentin formation with obliteration of the pulp canal space
2. Cementum and periodontal ligament formation
3. Cementum, periodontal ligament and bone formation from the apical region.
4. Bone and bone marrow formation

Currently, it is not possible to predict or know the type of tissue replacement that occurs in the revascularization cases.

Stem Cell Based Therapies

Stem cells are non specialized cells of either embryonic origin or post natal. The embryonic origin stem cells are totipotent in nature with capability of forming new organ. Post natal stem cells are multipotent in nature with ability to differentiate to other cell types. All stem cells have self renewal property. Stem cell division can either be symmetrical or asymmetrical. The asymmetrical division is responsible for cell with different properties. Two population of dental stem cells are present that give rise to two different tissues. Epithelial stem cells (EpSC) for ameloblasts and Mesenchymal stem cells (MSC) for odontoblasts, cementoblasts, osteoblast and fibroblast.[12]

Stem cell niche for epithelial stem cells have been identified in the cervical loop of rodent incisor and is yet to be isolated in the human. As there is no technology to identify the stem cells directly, indirect assessment are done with markers. Hence its absence cannot be said with certainty in any tissue. The assessments of all physical property of the stem cell are non specific. Only way to prove the function of stem cells is by evaluation of self renewal to show its "Stemness". Some of the markers for identification of Mesenchymal stem cells are STRO-1, CD146 or CD 44.[13]

Treatment option with stem cells can be either cellular approach or acellular approach. Cellular approach requires harvesting and cultivating the stem cells of interest in ex vivo and placing it back in the pulp canal space. For the cellular approach, the stem cells can be cultured either by enzyme-digestion method or by explant outgrowth method.

Enzyme digestion method had higher proliferation rate than the outgrowth method. Stem cell lines are established with one of the culture method. Then it can be differentiated into odontoblast, which under the controlled mineralization-regulatory influence of three non-collagenous proteins namely dentin phosphophoryn(DPP), dentin sialoprotein(DSP) and dentin matrix protein-1(DMP-1) can produce mineralized tissue.[14] However this has major limitations because of the non availability of specific markers. Currently this treatment option is not possible in the clinical practice.[15, 16]

Acellular approach is an in-situ condition, wherein the scaffold and signaling molecules are

placed to attract the stem cells from nearest niche. Currently the research is more focused on the acellular approach. Cell free approaches may be easier to translate from laboratory to clinical setting with satisfactory predication for pulpal regeneration.[17]

Biomaterials For Pulp Regeneration[18]

The Biomaterials required for the pulp regeneration are the Stem cells, Signaling molecules and Scaffolds.[19]The following are sources of the dental stem cells:

- Dental pulp stem cells (DPSC)
- Stem Cells from Human Exfoliated Deciduous teeth(SHED)
- Stem cells from Apical Papilla
- Dental Follicle Progenitor cells
- Periodontal Ligament stem cells

Stem cell will come into play and differentiate to form tissues based on the trigger from the signalling molecules. Signalling molecules are proteins in the form of growth factors and morphogenic factors which bind to the specific membrane receptors and trigger a series of events which eventually leads to formation of new tissue.

The following growth factors signal the reparative process in dentin and pulp

- Transforming growth factors β
- Bone morphogenic protein (BMP)
- Platelet Growth factor
- Fibroblast Growth factor
- Vascular Endothelial Growth factor(VEGF)

Environment plays an important role for formation of tissues. Extra cellularmatrix in the natural tissues have the best physical and chemical properties for formation of new tissues. For iatrogenic pulpal regeneration protocols, scaffolds of natural and synthetic polymers have been tried and tested based on the physical and chemical properties such as degradation rate, pore size and mechanical resistance. The followingare some of biodegradable and biocompatiblematerials used as scaffolds for pulp regeneration:[20,21]

- Collagen
- Hyaluronic acid
- Chitosan
- Poly -(I-lactic acid)(PLLA)
- Poly-(glycolic acid) (PGA)
- Co polymer poly-(lactic-co-glycolic acid) (PLGA)[22]

To translate the pulp regeneration from laboratory to clinical scenario, one of the obstacles is adaptation of the rigid scaffolds to the dentinal walls of the root canal system. Rosa et al., tested and proved the hypothesis that injecting the scaffold of recombinant human collagen containing SHED will lead to differentiation of functional odontoblast on full length of the root canal.[23]

Pulpal regeneration requires sufficiently disinfected dentinal walls and pulp canal space. Current use of non specific disinfection protocol in the root canal treatment is not sufficient for achieving regeneration. Also the disinfection material should not interfere with the regeneration process. Sodium hypochlorite and chlorhexidine may interfere with the regeneration, whereas EDTA, a weak anti microbial may be more important for regeneration. With its chelating property, it will release the bioactive growth factors from the dentin matrix, which may aid in the regeneration of pulp tissue.[24]

The regeneration potential of Dentin Pulp Stem cells (DPSC) from normal and inflamed pulp, formed similar type of mineral deposition in immune-compromised mice. However the osteo/ dentinogenic potential of DPSC of inflamed pulp was less than normal pulp.[25] Vasculogenesis and Angiogenesis are two processes essential for pulp regeneration. Vasculogenesis is defined as formation of new blood vessels. The biomaterial of choice identified as of now is VEGF signalled SHED and DPSC.Angiogenesis, on the contrary is the formation of new blood vessels from pre-existing vasculature. Thus it may come into play during revascularization procedures. In gene therapy and pulp implant, genes and pulp is transferred to the pulp canal space. In cell printing the position of the cells is precisely calculated, suspended in a hydrogel and implanted into the root canal space

Missing Links In Pulpal Regeneration[26]

Some of the important concepts have not been addressed in the pulp regeneration. They are as follows:

1. In all wound healing and regeneration, the size of the defect is important. Beyond a certain size the tissue cannot regenerate without introduction of supportive approaches. The current regeneration protocol as well as the research does not address this issue and still there is no guideline

what is the critical size defect for pulp and dentin.

2. In the cell free approach for pulpal and dentin regeneration, migration of cells from nearby should occur to differentiate into odontoblastic lineages. Thus non-odonblastic lineage differentiating into the odontoblastic lineages and its predictability is not ascertained till now.

3. While it has been proven that the pulp-dentin regeneration does occur in the experimental condition using cell based approach, non-cell based approaches have failed to do the same. Moreover, the quality of the pulp and dentin formed in terms of function and prevention of further bacterial insult, is not being addressed.

4. Good manufacturing practice facilities are not cost effective to initiate stem cell based pulp regeneration; the importance given is not much may be pulpal disease is not a life threatening issues.

Conclusion

The current status of literature has also raised valid questions such as" Is odontoblast mandatory for regenerated tissue?"; "Is complete mineralization or just healthy connective tissue considered as the acceptable outcome of root canal therapy?" [2]

To translate the bench work to clinics, the directions should be clear whether the goal is repair or regeneration. Most importantly, what is the replacement tissue needed as the end result of the regenerative therapies? The research and growth in the pulpal regeneration is rapidlyevolving and in one/ two decades, there will be a paradigm shift in the clinical treatment protocol. Along with this, there may be demand for newer diagnostic tools and new disease classification. This will enable preservation of healthy tooth structure, as more natural and biologic material will be used for replacement for lost pulp-dentin tissue.

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