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Review Article

Regenerative Endodontics- Potential Approaches in Revitalizing the Tooth Pulp - A Review Article

Aayushi Aggarwal[1], Vinisha Pandey[2], Neetu Bansal[3]

[1]BDS Student, [3]Senior Lecturer, Department of Conservative Dentistry and Endodontics, Institute Of Dental Studies and Technologies, Kadrabad, Modinagar, U.P., India;
[2]Associate Professor, Department Of Conservative Dentistry And Endodontics, Maharana Pratap Dental College, Kanpur, U.P., India

ABSTRACT:
When the maintenance of tooth in vital condition is no longer possible, the conventional treatment philosophy advocates the endodontic treatment to retain it in a functional state. A paradigm shift of this thinking manifested with revascularization/regenerative procedures. Regenerative endodontics is the creation and delivery of tissues to replace diseased, missing, and traumatized pulp. This review provides an overview of regenerative endodontics and possible techniques that will allow regenerative endodontics to become a reality. These potential techniques include root- canal revascularization, postnatal (adult) stem cell therapy, pulp implant, scaffold implant, three-dimensional cell printing, injectable scaffolds, and gene therapy. These regenerative endodontic techniques will possibly involve some combination of disinfection of infected root canal systems with apical enlargement to permit revascularization and use of stem cells, scaffolds, and growth factors. Although the challenges of introducing endodontic tissue engineering therapies are substantial, the potential advantages to patients and the profession are equally ground breaking. Patient demand is staggering both in scope and expense, because tissue engineering therapy offers the chance of restoring natural function instead of surgical placement of an artificial prosthesis.
Key words: Regenerative endodontics, revascularization, pulp stem cells, tissue engineering, scaffolds, growth factors.

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Corresponding author: Dr. Vinisha Pandey, Associate Professor, Department Of Conservative Dentistry And Endodontics, Maharana Pratap Dental College, Kanpur, U.P., India

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INTRODUCTION

"Regenerative endodontics" is a branch of regenerative medicine and has been defined as biological procedures designed to replace damaged, diseased, or missing dental structures, including dentine and root as well as cells of the pulp-dentin complex, with living, viable tissues, preferably of the same origin, that restore the normal physiological functions of the pulp-dentin complex.[1] Regenerative dental procedures have a long history, originating around 1952, when Dr. B. W. Hermann reported on the application of $Ca(OH)_2$ in a case report of vital pulp amputation.[2]
The goals of regenerative endodontics procedures are:

Primary goal: Elimination of symptoms & evidences of bony healing.

Secondary goal: Elimination root wall thickness &/or increased root length.

Tertiary goal: Positive response to vitality testing.[3]

The concept of the endodontic therapy has started with the idea of retrieving the vitality of dental pulp as a potential treatment option. It focuses on substituting injured and diseased pulp with functional pulp tissue with the use of biologically based procedures which restores the normal function of pulp-dentin structure. Though the outcomes are difficult to predict, deposition of hard tissue and continued root development are expected to occur under ideal conditions after this treatment. By providing an overview

of the methodological issues required to develop potential regenerative endodontic therapies, we hope to present a call for action to develop these approaches for clinical use.

Counterarguments

The counter argument to the development of regenerative endodontic procedures is that the pulp in a fully developed tooth plays no major role in form, function, or esthetics, and thus its replacement by a filling material in conventional root canal therapy is the most practical treatment but in terms of esthetics, there is a potential risk that endodontic filling materials and sealers may discolor the tooth crown. In addition, it has been retrospected that endodontically treated human teeth found the long-term intracanal placement of calcium hydroxide may reduce the fracture resistance of root dentin. It has also been noted that although root canal therapy prolonged tooth survival, the removal of pulp in a compromised tooth may still lead to tooth loss in comparison with teeth with normal tissues. On the other hand, although the replacement pulp has the potential to revitalize teeth, it may also become susceptible to further pulp disease and may require retreatment. The implantation of engineered tissues also requires enhanced microbiological control methods required for adequate tissue regeneration.[4]

Tissue engineering

The concept of tissue engineering was introduced by Charles Vacanti & Robert Langer[5] that applies principles of engineering and life sciences towards development of biological substitutes that restore, maintain or improve tissue function. MacArthur and Oreffo[6] defined tissue engineering as "understanding the principles of tissue growth, and applying this to produce functional replacement tissue for clinical use." The principles of regenerative medicine can be applied to endodontic tissue engineering. Regenerative endodontics comprises research in adult stem cells, growth factors, organ- tissue culture, and tissue engineering materials.[4] [**Fig.1**]

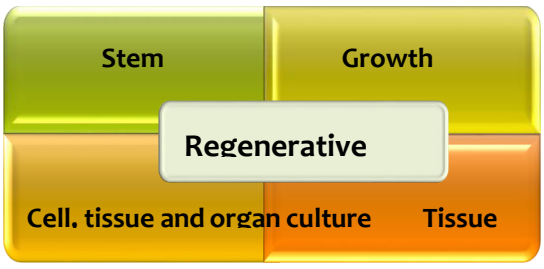


Figure 1. Regenerative endodontic procedures comprises research in stem cells, growth factors, organ, tissue culture and tissue engineering materials.

Stem cells

Stem cells are the ones that continuously divide and produce progeny cells and can differentiate into various other types of cells or tissues.[7] They can be pluripotent or multipotent in nature and are located in specialized regions within tissue termed as stem cell niches.[3] Niches are variable, containing different cell types depending on the need of its environment. The niche may be thought of as an anchor for a particular stem cell that generates extrinsic factors that control stem cell numbers and their fate.[1]

Oral stem cells come under the post-natal stem cell category & includes: Dental pulp stem cells (DPSCs), Stem cells of apical papilla SCAP), Inflammatory periapical progenitor cells (iPAPCs), Periodontal ligament stem cells (PDLSCs), Stem cells from human exfoliated deciduous teeth (SHED), Dental follicle stem cells (DFSCs)[7] These cells have the capability of differentiating into odontoblast-like cells facilitating a progressive repopulation of the radicular pulp space, promoting organized tissue repair, angiogenesis & reinnervation.[3]

Growth factors:

Growth factors are extracellularly secreted proteins/signals that bind two receptors on the cell signals morphogenesis/organogenesis during epithelial-mesenchymal interactions. They regulate the specialization of stem cells to the desirable cell type & mediates key cellular events in tissue regeneration including cell proliferation, differentiation chemotaxis & matrix synthesis[7] Currently, a variety of growth factors have been identified with specific functions that can be used as a part of stem cell & tissue engineering therapies.[4] Common growth factors are bone morphogenic proteins (BMP), fibroblast growth factor I or II(FGF), insulin like growth factor I or II, colony stimulating growth factor (CSF), epidermal growth factor (EGF), interleukin IL-1, IL- 13, transforming growth factor α, β(TGF α & β), vascular endothelial growth factor (VEGF), platelet-derived growth factor (TGDF) & nerve growth factor (NGF).[7]

SCAFFOLD:

Scaffold is a three dimensional structure that contains growth factors. It perform functions like it supports cell organization & vascularization, aids stem cell proliferation & differentiation, leads to improved & faster tissue development, contains nutrients to promote cell survival & growth, may contains antibiotics to prevent bacterial in growth in the canal systems, also show some mechanical & biological functions.[8]

Classification of Scaffolds

(I) Natural: collagen, platelet-rich plasma, fibrin and GAGs

(II) Synthetic: polylactic acid, polyglcolic acid(PGA), and poly (lactic-co-glycolic) acid (PLGA).

Natural polymers offer good biocompatibility and bioactivity while synthetic polyers elaborate physiochemical features such as dehydration rate,

microscopic and mechanical strength.[3] The choice of a scaffold is critical in tissue regeneration. Most scaffolds are organic in nature and used to provide surfaces on which cells may adhere, grow, and organize. Scaffolds chosen for laboratory studies are diverse, including natural or synthetic polymers, extracellular matrices (EMCs), self-assembling systems, hydrogels, and bioactive ceramics. Recently, a synthetic polymer polycaprolactone was successful in growing increasing numbers of stem cells from apical papilla stem cells with apparent identification of NOTCH signaling expression. The use of a self-assembling peptide system that allows a "bottom-up" approach of generating EMC materials, offering high control at the molecular level, will be a major step forward in constructing future scaffolds.[1] Scaffold-based approaches have the potential for rapid formation of a functional tooth of the correct shape and in the desired location but it has to overcome challenges associated with attachment to the jaw, infection, repetitive movement, and ability to withstand load during maturation.[8]

Regenerative procedural approach in Endodontics

1. Root canal revascularization via blood clotting:

Iwaya and associates[9] were the first to coin the term "revascularization" in their endodontic treatment of an immature permanent tooth with apical periodontitis and a sinus tract.[10] Revascularization of pulp can be defined as growth of undifferentiated stems cells of periapical region into the empty sterile/disinfected root canal space.[7]

The possible mechanisms by which revascularization takes place are as follows:

A few vial pulp cells remaining the apical end of root canal might proliferate into the newly formed matrix and differentiate into odontoblasts. This may happen under the influence of cells of HERS which are quite resistant to destruction, even in the presence of inflammation. Atubular dentin is laid down by odontoblasts at the apical end of canal and on the lateral aspects of dentinal walls of root canal. This leads to apexogenesis and reinforcing and strengthening of root respectively.

Continues root development could be due to multipotent dental stem cells, which are present abundantly in the immature permanent teeth. These cells from apical end might be seeded on to the existing dentinal walls and might differentiate into odontoblasts and deposit tertiary or atubular dentin. Stem cells in the periodontal ligament can proliferate and grow into apical end and within the root canal. They may deposit hard tissue both at the apical end and on the lateral root walls. The evidence in support of this hypothesis is presented by documentation of cementum and sharpey's fibers in the newly formed tissues. The fourth possible mechanism of root development could be attributed to SCAP or to the bone marrow. Instrumentation beyond the confines of the root canal to induce bleeding can also transplant mesenchymal stem cells from the bone into the canal lumen. These cells have extensive proliferating capacity.[3] An important aspect of these cases is the use of intracanal irrigants (NaOCl and chlorhexdine) with placement of antibiotics (e.g. a mixture of ciprofloxacin, metronidazole, and minocycline paste) for several weeks. This particular combination of antibiotics effectively disinfects root canal systems and increases revascularization of avulsed and necrotic teeth, suggesting that this is a critical step in revascularization.[4]

Size of apical foramen:

The size of apical foramen appears to be a major concern in the regenerative endodontic therapy. It was suggested that an apical foramen of atleast 1.1 mm in diameter was necessary to promote vascularization and to maintain initial cell viability via nutrient diffusion.[10]

Advantages:

Foremost, this approach is technically simple and can be completed using currently available instruments and medicaments without expensive biotechnology. Subsequently, the regeneration of tissue in root canal systems by a patient's own blood cells avoids the possibility of immune rejection and pathogen transmission from replacing the pulp with a tissue engineered construct. Plasma-derived fibrin clots are being used for development as scaffolds as a useful aspect of this approach.

Limitations:

However, there is a critical limitation to a blood clot revascularization approach, because tissue engineering is found on the delivery of effective concentrations and compositions of cells to restore function; it is possible that variations in cell concentration and composition, particularly in older patients (where circulating stem cell concentrations may be lower) may lead to variations in treatment outcome.[4]

2. Postnatal stem cell therapy

This method involves placement of postnatal stem cells into the disinfected root canal for regeneration of lost dental tissue. The first postnatal stem cell therapy in medicine was done for the treatment of severe combined immune deficiency. Postnatal stem cells may be sourced from umbilical cord blood, peripheral blood, bone marrow, body fat, and other body tissues, like the pulp tissue of teeth. The drawbacks of postnatal stem cell therapy include low survival rates of injected cells, minimal chance of cells to migrate to different parts within the body, which may lead to aberrant patterns of mineralization and difficulty in isolating the cells.[7]

3. Pulp Implantation

This concept seeks to renovate the two dimensional cell cultures to a three-dimensional structure by growing the cells in biodegradable membrane filters. Bohl et al.,[11] reported the formation of tissue with high cell density

similar to the natural pulp when pulp cells are cultured in vitro on polyglycolic acid (PGA). Buurma et al.,[12] observed extracellular matrix to be expressed in immunecompromised mice when PGA was seeded with pulp cells and implanted into subcutaneous spaces. Angiogenesis occurred through PGA implants after three weeks of implantation. The pulp stem cells must be organized in such a way that it should support cell organization and vascularization. Growing dental pulp cells on collagen matrix was not successful and matrices like vitronectin and laminin require further investigation. The advantage of this philosophy is the ease of growing cells on filters within the laboratory and their existence as collective sheets which are a more stable than unconnected cells and can be easily injected into empty disinfected root canal systems. However, the drawback of this technique is that there is a need for specialized procedures to ensure proper adherence of cells to root canal walls and handling fragile filters.[7]

4. Scaffold Implantation

Pulp stem cells may be ordered into a three dimensional arrangement that can sustain cell organization and vascularization. This can be consummate using a porous polymer scaffold which is seeded with pulp stem cells. A scaffold should contain growth factors that aid proliferation and differentiation of stem cells, leading to enhanced and more rapid tissue development. The scaffolds that are used for this procedure are either synthetic or natural. Synthetic scaffolds include polyester materials like polycaprolactone (PCL), polyglycolic acid (PGA) and polylactic acid (PLA). Natural scaffolds may be collagen, chitosan, fibrin or glycosamino glycans. Dentin chips have been reported to stimulate reparative dentin bridge formation in teeth with exposed pulp. Dentin chips will be a reservoir of growth factors thereby acting as a matrix for pulp stem cell attachment.[7]

5. Injectable Scaffold Delivery

Rigid tissue engineered scaffold structures provide excellent support for cells used in bone and other body areas where the engineered tissue is required to provide physical support. However, in root canal systems a tissue engineered pulp is not required to provide structural support of the tooth. This will allow tissue engineered pulp tissue to be administered in a soft three-dimensional scaffold matrix, such as a polymer hydrogel. Hydrogels are injectable scaffolds that can be delivered by syringe. Hydrogels have the potential to be noninvasive and easy to deliver into root canal systems. In theory, the hydrogel may promote pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure.[4]

6. Three-Dimensional Cell Printing

In theory, an ink-jet-like device is used to dispense layers of cells suspended in a hydrogel to recreate the structure of the tooth pulp tissue. The three-dimensional cell printing technique can be used to precisely position cells, and this method has the potential to create tissue constructs that mimic the natural tooth pulp tissue structure. The ideal positioning of cells in a tissue engineering construct would include placing into cleaned odontoblastoid cells around the periphery to maintain and repair dentin, with fibroblasts in the pulp core supporting a network of vascular and nerve cells. However there is a disadvantage of using the three-dimensional cell printing technique theoretically that careful orientation of the pulp tissue construct according to its apical and coronal asymmetry would be required during placement and shaped root canal systems. However, early research has yet to show that three-dimensional cell printing can create functional tissue *in vivo*.[4]

Gene therapy

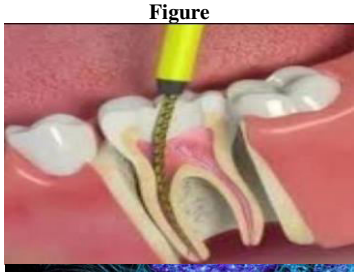
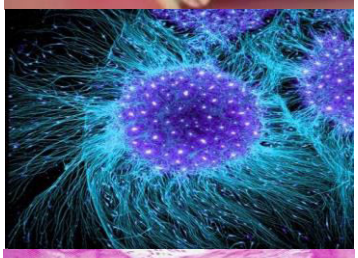
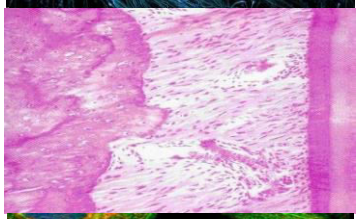
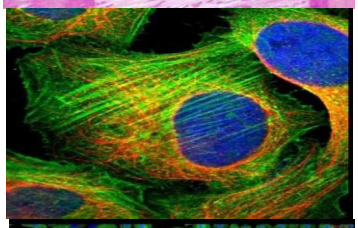
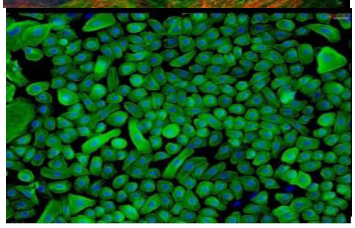
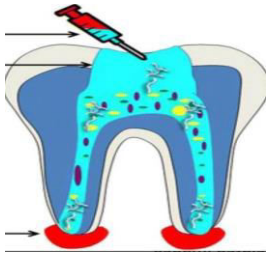
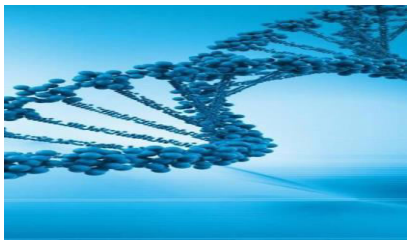
Gene therapy is a relatively new field in regenerative endodontics where preliminary attempts focuses on delivering genes that are capable of matrix formation and mineralization into pulp space to promote tissue repair using vectors. Its practicality, potential health hazards and ethical concerns are much demanding, which accounts to the limited exploration of the technique in endodontics. Once successful, this technique could be a landmark development in the field of regenerative endodontics.[4] [**Table-1**]

Challenges in regenerative endodontics

A. Disinfection of root canal and discolouration of crown:

One of the biggest challenges encountered in endodontics is inadequate disinfection of infected root canals. NaOCl is the most widely recommended irrigant (concentration ≤ 1.5%) . 17 % EDTA is another chemical agent that is recommended in regeneration procedure as it helps in the dissolution and release of impregnated growth factors in root dentin during dentinogenesis. These growth factors enhance the stem cell survival and its proliferation. EDTA is also useful in reversing the detrimental effect of high concentration of NaOCl. Regarding intracanal medicaments, Triple Antibiotic Paste (TAP) is difficult to remove from the root canal and causes discoloration of the crown portion over a period of time due to presence of minocycline in it that binds with calcium of dentin forming insoluble complexes.[7] To avoid staining while using TAP, the pulp chamber should be sealed with dentin bonding agent and ensure that TAP remains below CEJ. Besides this, Calcium hydroxide also has deleterious effect like weakening the root dentin, with a potential for fracture.[4]

Table1: Summarized table for regenerative procedural approaches in endodontics

Technique name	Procedure	Figure
Root canal revascularization	Open up tooth apex to 1mm to allow bleeding into root canals	
Stem cell therapy	Autologous or allogenic stem cells are delivered to tooth via injectable matrix	
Pulp implantation	Pulp tissue is grown n the laboratory in sheets and implanted surgically	
Scaffold implantation	Pulp cells are seeded onto a 3-D scaffold made of polymers and surgically implanted	
3-D cell printing	Ink-jet like device dispenses layers of cells in a hydrogel which is surgically implanted.	
Injectable scaffolds	Polymerizable hydrogels, alone or containing cell suspension are delivered by injection	
Gene therapy	Mineralizing genes are transfected into vital pulp cells of necrotic and symptomatic teeth.	

A. Removal of smear layer

Even though there is minimal instrumentation of the root canal in regenerative procedure, there is limited smear layer formation. This prevents stem cells from adhering on to root canal walls, contributing to failure of treatment. A 17% solution of EDTA as final rinse in the root canal will aid in removal of smear layer as well as enhance the attachment of the stem cells by providing a better surface on the root dentin for their survival. Other chemicals used for this purpose are doxycycline, citric acid and MTAD.[7]

B. Developing an ordered functional pulp tissue

Developing a functional tissue is still a problem in regeneration. Despite best efforts to recreate lost tissue, the type of tissue is still unknown. The development of the normal harmony and spatial arrangement of dentin pulp complex is still in research. The 3D cell printing will be a possible solution in the near future, but the problem with this technology is the difficulty in exact placement and positioning inside root canal. Only by achieving such a balanced spatial oriented cell lineage in the root canal through regeneration can this method be deemed successful.[7]

C. Appropriate coronal seal

A leakage-free coronal restoration is mandatory in regenerative endodontics as well as in subsequent restorative procedures. The material that provides such seal should be biocompatible, have to maintain cell viability of the regenerated pulp, should minimize microleakage and provide adequate adhesion to the overlaying restorative material. The materials under consideration are calcium silicate based cements like mineral trioxide aggregate (MTA) and biodentine.[7]

Future prospective

Regenerative endodontic strategies are continuously being updated and improved to benefit dentistry in every possible way. American Association of Endodontists Foundation has recently awarded a grant of $1.7 million[13] to evaluate the effectiveness of two regenerative approaches (REGENDO and REVASC) compared with the conventional MTA apexification. The trial will be carried out in collaboration with Loma Linda University, University of Texas Health Science Center at San Antonio and the University of Maryland School Of Dentistry and is estimated to complete in December 2019. Iohara *et al*.[14] aims to use pulp stem cells with granulocyte-colony stimulating factor (G-CSF) for pulp/dentin regeneration to fully restore the tooth instead of filling, capping or extracting it. Misako Nakashima (Japan) said that a clinical trial of pulp regeneration has already been initiated with the permission of the Japanese Ministry of Health, Labor and Welfare. Recently, PRF box has been announced[15] to produce homogenously thickened hydrated exudate rich in platelets, vitronectin, leukocytes, and fibronectin expressed from the fibrin clots that have improved the issues regarding the handling of the platelet-rich fibrin (PRF) clot. It is likely that the next advance in regenerative dentistry is the availability of regenerative dental kits, which will enable the dentists the ability to deliver regenerative therapies locally as part of routine dental practice.[8]

Conclusion

Regenerative endodontics holds promise of restoring pulp-dentin complex in teeth with immature roots and necrotic pulps. This procedure has potential advantages versus traditional treatment procedures of increasing root wall thickness and root length while maintaining immune competency. Still significant scientific hurdles need to be overcome with continued growth in knowledge and armamentarium.[3]

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