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Review Article

Regenerative Endodontics: A Comprehensive Review

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ABSTRACT:

Regenerative endodontics represents a significant advancement in the field of dental medicine, focusing on the repair and restoration of the pulp-dentin complex in necrotic or injured teeth. This comprehensive review explores the historical development, biological principles, and clinical applications of regenerative endodontics, highlighting key techniques such as revascularization and revitalization. We analyse various protocols that employ scaffold materials, growth factors, and stem cells to facilitate tissue regeneration, emphasizing the importance of a sterile environment and proper sealing techniques in ensuring successful outcomes. Furthermore, this review discusses the challenges and future directions in regenerative endodontics, aiming to provide insightful guidance for practitioners seeking to incorporate these innovative approaches into their clinical practice.

Keywords: *Regenerative endodontics, Dental Pulp, Stem Cells*

Introduction

Regenerative endodontics is an emerging field that bridges traditional endodontic treatments with advances in tissue engineering and regenerative medicine. This approach aims to restore the vitality of the dental pulp and promote the regeneration of the pulp-dentin complex in teeth that have suffered from necrosis due to caries, trauma, or other pathological conditions. The foundation of regenerative endodontics was laid with significant research and clinical innovations in the mid-20th century, gradually leading to the development of protocols that enhance healing mechanisms in the dental pulp.[1]

Historically, treatments primarily focused on root canal therapy aimed at eliminating infection; however, the evolving understanding of pulp biology has paved the way for regenerative techniques that prioritize the preservation and repair of natural tissues. Key milestones, such as the introduction of various antibiotic paste formulations and the formalization of terms like revascularization and revitalization, have shaped the current landscape of these techniques.

This review aims to provide an exhaustive overview of the principles underlying regenerative endodontics, elucidating the biological processes involved in pulp regeneration and the clinical methodologies employed. By synthesizing existing literature, we offer insights into effective treatment protocols, as well as the challenges faced in clinical practice and the potential future developments in this dynamic field.[1,2]

Historical Evolution

The evolution of regenerative endodontics has seen significant milestones since the early 1960s. Dr. B. W. Hermann pioneered the application of Calcium Hydroxide for vital pulp therapy, laying the groundwork for future advancements. In the early 1960s, Nygaard-Ostby conducted experimental research that introduced the concept of regenerative endodontics by demonstrating the possibility of inducing bleeding from periapical tissues into partially filled root canals after chemomechanical debridement. In 1966, Rule DC introduced double antibiotic paste, followed by Hoshino's introduction of triple antibiotic paste in 1993, which further advanced infection management in endodontics. The term “revascularization” was introduced by Iwaya in 2001, who explored methods to treat immature permanent teeth with sinus tract infection and apical periodontitis by inducing intracanal hemorrhage.

In 2004, Banchs and Trope provided a modified revascularization protocol that involved creating a blood clot in the canals, serving as a matrix for new tissue growth, coupled with a bacterial-tight coronal seal to prevent bacterial invasion into the pulp space. The concept of the triad of tissue engineering was presented by Nakashima and Akamine in 2005, contributing to the understanding of regenerative processes. The term "regenerative endodontics" was formalized by Murray et al. in 2007 and subsequently approved by the American Association of Endodontists (AAE). In 2008, Huang and Lin introduced the term "revitalization," which was later endorsed by the European Society of Endodontology (ESE) in its position statement in 2016, solidifying revitalization as a key concept in the field.[1-5]

Goals of Revascularization:[5,6]

According to Banchs and Trope and AAE described the following goals:

1. Eradication of the apical periodontitis, infection, and clinical symptoms.
2. The thickening of root dentinal walls and ongoing root completion.
3. Improvement in pulp vitality.

Triad of tissue engineering:[2-4,7]

Triad of regeneration involves stem cells, scaffold, and growth factors.

1. Stem cells

"Distinct subpopulation of undifferentiated cells with self-renewal and differentiation potential" is called stem cells. They are characterized as undifferentiated cells that are exposed to and respond to the right signals, and they are kept in this state by their surroundings and/or the cell populations that surround them. The capacity

for extended self-replication. Hold onto their capacity for multiple differentiation for the duration of the organism's existence.

Types of stem cells

a. According to their source:

- Autologous cells: Derived from the same person that the transplant will be placed in.
- Allogeneic cells: Derived from the same-species donor's body.
- Xenogeneic cells: Separated from members of a different species. For instance, heart implant construction uses animal cells.
- Syngeneic/isogenic cells: Isolated from genetically identical organisms. For instance, clones and twins.

b. According to their potency:

- Totipotent: Differentiable cells that can become new creatures. For instance, early embryonic cells.
- Pluripotent: Cells that can differentiate into almost any type of cell, but not an entire creature. For instance, blastocyst.
- Multipotent: Cells having a restricted variety of cell types during differentiation. For instance, dental pulp stem cells (DPSCs), cord blood, and fetal tissues

c. Adult stem cells in the oral region:

- SCAP: Stem cells of apical papilla
- iPACs: Inflammatory periapical progenitor cells
- DFSCs: Dental follicle stem cells
- DPSCs: Dental pulp stem cells
- PDLSCs: Periodontal ligament stem cells
- BMSCs: Bone marrow stem cells
- TGPCs: Tooth germ progenitor cells
- SGSCs: Salivary gland stem cells
- SHED: Stem cells from human exfoliated deciduous teeth
- OESCs: Oral epithelium derived stem cells

- GMSCs: Gingival derived mesenchymal stem cells
- PSCs: Periosteal derived stem cells

2. Growth factors / Morphogens / Signaling Molecules

Certain mesenchymal stem cells are stimulated by these stimuli to transform into cells resembling odontoblasts. Patients on long-term corticosteroids exhibit a sharp decrease in the pulp chamber's radiographic size and a five-fold rise in the predentin layer's thickness. Dentin is thought to be a growth factor and cytokine reserve. They serve to promote neighbouring cell division, specific cells to differentiate along a predetermined route, revascularization. Following biomineralization, these growth factors and cytokines that the odontoblast produced during initial dentinogenesis are sequestered and petrified into the dentin.

Different growth factors are growth hormone (Paracrine/ Autocrine role), insulin like growth factor (IGF-1, IGF-2), transforming growth factor β (TGF β-1, TGF β-2, TGF β-3), bone morphogenic proteins (BMP-2, BMP-4, BMP-6), fibroblast growth factors (FGFs), tumour necrotic factors (TNFs), colony stimulating factors, interlukins, platelet derived growth factors (PDGF), nerve growth factors (NGF)

Morphogens includes second level of regulation by transcription factor MSX-1 and MSX-2 and toll like receptors (TLR-4 activated by lipopolysaccharides).

3. Scaffold / Matrix Scaffold

It creates a 3-Dimentional biologic and physiochemical atmosphere that supports cell adhesion and migration throughout cell development and transformation. It is a tool that helps cells develop and differentiate by organizing, guiding, and supplying chemical or physical cues to provide a spatially accurate location for the cell and to control breakdown, transformation, or multiplication while encouraging gaseous and nutrition transfer. Ideal scaffold must be permeable to enable the implantation of growth agents and cells, enable efficient movement of waste, oxygen, and nutrients, compostable and produce no contaminants, reconstructive cell needs to take position while maintaining the final tissue structure's shape and form, biocompatible and mechanically and physically strong enough.

Types of scaffolds

a. **Biological/Natural:** Blood clot, platelet rich plasma (PRP), platelet rich fibrin (PRF), collagen, chitosan, glycosaminoglycans, demineralized dentine matrix.
b. **Synthetic/Artificial:** Polymers-Polylactic acid, polyglycolic acid, bioceramics like calcium, phosphate, bioactive glass, glass ceramics.

Techniques of tissue engineering:[8-11]

1. Root canal vascularization via blood clotting: Apical section of the root canal might be challenging to clean and shape when the roots are blunderbuss. Presence of thin brittle dental walls may prone to fracture during preparation or filling of root canals which possess risk of extruding materials into peri-radicular tissues. American association of endodontists clinical consideration for a regenerative procedure:

Case Selection:

- Nonvital and immature tooth.
- Final restoration does not require post/core.
- Cooperative patient/parent.
- Not allergic to medicaments and antibiotics (American Society of Anesthesiologists (ASA) 1 or 2).

Informed Consent: Informed consent should include that this procedure required more than 2 visits, antibiotics are used, tooth discolouration can be seen, deficiency of response to therapy, discomfort/contamination, other treatment modalities like apexification, no treatment, extraction, and permission to procedure.

First Appointment: Rubber dam isolation following local anaesthesia and access opening was done. Use a side-vented needle and 20ml of sodium hypochlorite (NaOCl) for extensive irrigation. Lower dosage of 1.5% sodium hypochlorite (20ml / canal for 5 mins) is indicated followed by irrigation with saline / EDTA (20ml / canal for 5 mins) with irrigating needle inserted around 1 mm from root end to decrease cytotoxicity to stem cells in apical tissues. Higher NaOCl concentrations reduce SCAP survival. While 1.5% NaOCl has little detrimental effect on SCAP, 17% EDTA improves SCAP survival. Because chlorhexidine is harmful to stem cells, it should not be used for irrigation. Paper points are used to dry the canals. Triple antibiotic paste (TAP) should be used in accordance with AAE protocol, with a maximum dose of 0.5 mg/ml for beneficial to stem cell viability. Metronidazole, Ciprofloxacine, and Minocycline are included in TAP in a 1:1:1 ratio at a concentration of 0.1 mg/ml via syringe. Pulp chamber can be cured with a dentin bonding agent or persist underneath CEJ to minimize risk of staining. Apply a 3–4 mm layer of a transient material, such as glass-ionomer, Cavit, IRM, or similar transient material. Dismiss patient for one to four weeks.

Second Appointment: It should be after one to four weeks from first visit. Evaluate the first treatment's response. If there are indications or symptoms of a recurrent conditions, think about using an alternate or additional antibiotic for therapy. Dental dam isolation and 3% mepivacaine anesthesia without vasoconstrictor

are used. Thoroughly yet gently irrigate with 20 milliliters of 17% EDTA. Use of paper points to dry the canals and excessive instrumentation (endo file, endo explore) to stimulate hemorrhage into the canal space. The objective is to fill the canal with blood all the way to the cement-enamel junction by rotating a pre-curved K-file two millimeters past the apical foramen. After the blood clot forms, an effective coronal seal is crucial. A piece of premeasured Collaplug was placed on top of the blood clot to act as an internal matrix for the installation of about 3 mm of MTA and a layer of 3-4 mm GIC that was reinforced composite-bonded over.

Follow-up: At interval of 6-, 12-, 24-months, after the first two years, a yearly follow-up is advised to evaluate clinical and radiographic examination, no signs of discomfort, swelling or sinus tract also seen in 1st and 2nd appointments, apical radiolucency eradication (typically seen 6–12 months following therapy), wider root walls (usually seen 12–24 months after therapy; usually seen prior to apparent increase in root length), extended root length, positive pulp vitality test. after the first two years, a yearly follow-up is advised.

Outcome assessment: Restoring pulp vitality and fostering ongoing root development are the objectives of REP. The radiographic root area increased by 31.6 percent after REP. For teeth treated with REPs, Chen et al. (2012) documented five different types of responses:

1. Deeper root canal walls and ongoing root development.
2. There is no discernible continuation of root growth, and the tips of the roots become tight and blunt.
3. Persistent root growth with an open apical foramen.
4. Severe canal space calcification.
5. A hard tissue barrier developed in the canal between the root apex and coronal MTA plus.

Potential means by which REPs could continue to develop their roots that there are not many essential pulp cells at the apical end. These cells can divide and develop into odontoblasts under the guidance of intact HERs or stem cells from the periodontal ligament is viable if the apical papillary tissues and HERs are destroyed or transplanting SCAP into the tissue lumen is the result of instrumentation that extends beyond the canal's apical limit or blood clot can store growth factors like TGF and PDGF. It promotes the undifferentiated precursors of fibroblast, odontoblast, and cementoblast to develop, grow, and mature.

2. Post natal stem cell therapy: In postnatal stem cell therapy, an infected root canal is injected with cells. Stem cells are divided into embryonic (pluripotent) cells that are isolated from blastocyst is one of the kinds

of stem cells in which all three germ layer derivatives can arise from them and postnatal cells that is taken from cord blood or bone marrow having less differentiation potential and are less malleable.

Stem cells of dental origin are:

- DPSCs: Dental repair is handled by DPSCs. able to rebuild the pulp-dentin complex. SHED: Has the ability to develop into cells resembling odontonblasts, which create dentin-like structures.
- PDLSCs: Found in PDL that has been processed by enzymes. can create constructions resembling PDL or cementum.
- SCAP: Found at apices of growing teeth at junction of apical papilla and dental pulp. They can differentiate into odontoblastic, osteogenic, and neurogenic forms.

3. Pulp implantation: Pulp tissue produced in library is transferred to sterilized root canal. Sheets of biodegradable polymer nanofibers are used to create pulp tissue. (In vitro). To create 3-D pulp tissue, sheets are rolled together. This method's inability to guarantee whether cells are correctly affixed to the wall is a drawback.

4. Scaffold implantation: An optimal scaffold should guarantee enough neurovascular supply to the newly formed pulp tissue during dental pulp regeneration. For instance, DPSCs are cultivated in vitro, implanted surgically, and seeded on a three-dimensional polyglycolic acid matrix.

5. Injectable scaffold delivery: Injections are used to provide polymerizable hydrogel either alone or in combination with cell cultures. By acting as an extracellular matrix substitute, it might promote regeneration. Low cell survival rates and little control over tissue creation are disadvantages.

6. 3-D cell printing: By carefully positioning the cells, the created tissue resembles the structure of the dental pulp in its original state. Also help to replicate dental pulp tissue, layers of cells floating in hydrogel are dispersed using an inkjet equipment. Accurate three-dimensional models are needed for each pulp cavity and efficient delivery method.

7. Delivery of genes: It is a method of introducing genes encoding growth factors, morphogens, and extracellular matrix components into a person's somatic cells, which has a therapeutic impact.

Complications

- Discolouration: Minocycline is to blame.

- MTA material collapsing into the canal. Collaplug can be used to control this by maintaining it above the blood clot and waiting for at least fifteen minutes following the onset of bleeding.
- REPs cannot be used on teeth that require post retention in the canal space.
- Rely on a blood clot.
- Sometimes a canal develops total calcification.
- Achieving the entire root length is challenging.
- Development of bacterial strains that are resistant (caused by prolonged usage of antimicrobial drugs).
- Hypersensitivity response to intracanal medication.
- Necrosis could occur if the tissue becomes infected again.
- There are not many cases reports available yet.

Conclusion

Regenerative endodontics represents a paradigm shift in the management of necrotic teeth and offers a promising avenue for restoring dental pulp vitality and function. This comprehensive review has highlighted the significant advancements in the field, detailing the pivotal studies and clinical protocols that have emerged over the past few decades. The integration of biological principles, including the use of stem cells, growth factors, and scaffold materials, has proven essential for successful pulp regeneration and revitalization.

Despite the encouraging outcomes reported in various studies, challenges remain in the standardization of treatment protocols, as well as ensuring consistent and predictable healing responses across different patient populations and clinical scenarios. Ongoing research is critical to address these challenges, refine techniques, and establish clear guidelines that practitioners can follow to optimize treatment outcomes.

As the field of regenerative endodontics continues to evolve, it holds the potential to significantly enhance the quality of care provided to patients with compromised dental pulp. By embracing innovative approaches and fostering interdisciplinary collaboration among dental professionals and researchers, regenerative endodontics can pave the way for more effective and biologically-based therapies in dental practice, ultimately improving patient outcomes and preserving tooth vitality. Future developments in biocompatible materials, advanced diagnostic techniques, and personalized treatment approaches will likely further enhance the efficacy of regenerative strategies, solidifying their role in contemporary endodontic therapy.

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