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Innovative approaches in regenerative endodontics: A review of current literature

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***Abstract*---**Background: Biomimicry or biomimetics refers to developing materials and techniques inspired by natural systems. In dentistry, this approach aims to replicate natural tooth structures and

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functions, addressing limitations of conventional materials and techniques. Regenerative endodontics, including cell homing and revascularization, represents significant advancements in this field, focusing on pulp-dentin regeneration and tooth vitality restoration. Aim: This review explores innovative biomimetic approaches in regenerative endodontics, including the latest techniques and their clinical implications. It aims to assess the effectiveness and future prospects of these methods in enhancing tooth repair and regeneration. Methods: The review synthesizes current literature on regenerative endodontic procedures, including cell homing, revascularization, scaffold implantation, and gene therapy. It examines experimental studies, clinical trials, and advancements in biomimetic materials and techniques, highlighting their applications and outcomes. Results: The review finds that regenerative endodontics has evolved from traditional apexification to advanced techniques such as cell homing and revascularization. Cell homing, which leverages the body's natural healing processes without cell transplantation, shows promise in generating pulp-dentin tissue. Revascularization, involving the induction of a blood clot and stem cell recruitment, has demonstrated potential in restoring vitality to necrotic teeth. Scaffold implantation and gene therapy also offer novel approaches, though they require further research for optimization. Conclusion: Innovative biomimetic approaches in regenerative endodontics represent a promising frontier in dental restoration. While techniques like cell homing and revascularization offer significant potential for pulp and dentin regeneration, ongoing research is essential to refine these methods and translate them into broader clinical practice. Future studies should focus on optimizing protocols, understanding underlying mechanisms, and improving material efficacy to achieve consistent and reliable outcomes.

***Keywords*---**regenerative endodontics, biomimicry, cell homing, revascularization, pulp-dentin regeneration, scaffolds, gene therapy.

Introduction

The fundamental principle of “biomimicry” or “biomimetics” involves the development of engineered designs inspired by natural systems [1]. The term “biomimicry” derives from Greek, where "bios" means life and "mimesis" refers to imitation, and it is envisioned as a phenomenon that is either fully or partially biologically induced [2]. In medical, dental, biotechnological, and pharmaceutical domains, the shortcomings of conventional materials often arise from their inability to integrate seamlessly with biological systems by following cellular pathways [3]. In the 1950s, Otto Schmitt, a biomedical engineer, introduced the concept of “biomimetic” [4,5]. The Greek term “bio” signifies life, while “mimetic” pertains to the simulation or mirroring of nature. The objective of biomimetics is to create biological materials and procedures that replicate natural phenomena [4,6]. Central to novel biomimetic approaches is the integration of inorganic ions with organic protein molecules [7,8]. Consequently, biomimetic methodologies encompass diverse fields including bioengineering, biology, chemistry, and materials science. Additionally, nanotechnology plays a crucial role in the fabrication of various biomimetic materials [5,7]. Clinically, biomimetics pertains to replicating the characteristics of natural teeth in restorative dental procedures through biomimetic techniques and materials [7,9]. For instance, biomimetic coatings of calcium phosphate (CaP) and hydroxyapatite (HA) have been investigated and utilized to enhance the osseointegration of dental implants [10,11]. Similarly, biomimetic techniques are applied in adhesive restorative materials to achieve aesthetic outcomes that mimic natural teeth and tooth morphology. Over recent decades, the field of restorative dentistry has advanced from mechanical retention to sophisticated adhesion techniques. Composite-resin materials and adhesive dentistry have become essential tools in this evolution. Biomimetic dentistry principles advocate for the incorporation of advanced composite-restorative materials into clinical practice that align with the nature and integrity of dental tissues [12,13]. Tissue engineering has shown promising results in regenerating oral tissues [14,15,16]. Furthermore, various endodontic procedures—such as forming dentin barriers through pulp-capping, root formation during apexogenesis or apexification, apical healing with root-end fillings, and pulp regeneration using cell-homing techniques [17,18]—employ biomimetic strategies in endodontology.

Biomimetic dentistry encompasses the art and science of restoring or repairing damaged teeth using methods that replicate natural dentition in both aesthetics and function. These methods involve minimally invasive dental management with bioinspired materials to achieve remineralization [5]. Emerging fields like regenerative endodontics and tissue engineering offer potential solutions for repairing damaged or partially developed teeth with functional pulp-dentin tissue [19]. This approach involves providing a natural extracellular matrix (ECM) environment, signaling molecules, stem cells, and scaffolds. The absence of pathology, pain, and formation of root dentine are indicators of clinical success [20]. Contemporary endodontic regeneration often includes revascularization, wherein the root-canal system is disinfected with intracanal medicaments, and a blood clot is formed to stimulate the root apex tissues. The presence of a blood clot acts as a natural scaffold within the root canal, facilitating the proliferation and differentiation of pulp-dentin stem cells [20,21]. The current concept of cell-homing supports the recruitment of pulp-apex tissue by endogenous mesenchymal stem cells [22,23]. Additionally, various macromolecules are being explored to recruit endogenous pulp cells through different methods, including chemo-attractants, platelet-rich plasma, and ECM molecules [24]. Ideally, dental biomimetic materials should replicate the properties and functions of different tooth components [25], aiming to recreate biological tissues and utilize materials that emulate the biological effects of oral structures [26]. Commonly utilized biomaterials for dental pulp tissue engineering include collagen or poly(lactic) acid and hydrogel scaffolds. However, administering collagen into narrow pulp spaces is challenging. Thus, numerous bioactive biomaterials, including synthetic and natural hydrogels, have been investigated for their suitability in dental pulp engineering [27], which will be discussed further in subsequent sections.

The ultimate goal of regenerative endodontics is to improve patient management through various strategies that translate the biological aspects of pulp regeneration into clinical practice. These clinical protocols range from leveraging the natural healing ability of the pulp to regenerating the affected pulp-dentin complex or achieving revascularization of an empty root canal [20]. This paper aims to provide a comprehensive review and discussion of various biomimetic approaches for pulp regeneration and endodontic applications, as well as current trends and future research prospects for translating these biomimetic approaches into clinical endodontic practice.

Development of Regenerative Endodontic Procedures (REP)

In 1961, Nygaard-Østby pioneered the concept of treating necrotic pulp through regenerative endodontics [28]. Murray and Gracia define regenerative endodontic procedures as "biological events designed to replace missing, diseased, underdeveloped, or damaged components of tooth structures, including root and dentin, to restore the physiological functions of the pulp-dentin complex" [29,30]. The key components involved in regenerative endodontic procedures are stem cells, signaling molecules, and scaffolds associated with the extracellular matrix (ECM) [20,21,31,32]. The primary objective of REP is to foster pulp tissue regeneration, root development, and the proliferation of progenitor-stem cells from the bone or tooth region [33]. Osteo/odonto-progenitor-stem cells in the apical papilla protect the root from infection and necrosis due to their proximity to the periodontal blood supply [34]. Additionally, REP can influence angiogenesis, cell survival, differentiation, migration, and proliferation. Utilizing markers for mesenchymal stem cells, regenerative endodontics has demonstrated diverse potential [35,36]. The immunostaining technique has been employed to detect the abundance of CD31/collagen-IV and vascular endothelial growth factor (VEGF) during the differentiation of endothelial progenitor cells and the revascularization process [10]. Despite the limited number of clinical trials, regenerative endodontics is widely acknowledged and appreciated by clinicians globally.

In dental pulp regeneration, cells can be introduced either through cell transplantation or cell homing [37,38]. Research by Torabinejad et al. [39] revealed that a 1–4 mm layer of uninflamed tissue is advantageous for successful pulp regeneration following a revascularization procedure. This study focused on immature animal teeth [39]. Complete pulp tissue regeneration, including capillaries and neuronal cells, was observed in canine pulp regeneration within 14 days in 2009. Iohara et al. [40] utilized scaffolds loaded with collagen fibers (types I and III) and dental pulp stem cells. However, transplantation of the scaffold alone did not achieve engraftment at the pulpotomy site [40]. Souron et al. [41] investigated the transplantation of rat pulp cells into scaffolds made of type-I rat collagen. After one month, living, mitotically active fibroblasts, new vessels, and nerve fibers were found where the pulp was seeded with cells, whereas a lack of cell colonization was noted where lysed cells were used [41].

In another study, Jia et al. [42] injected simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme-A reductase. The scaffold used was a gelatin sponge combined with dental-pulpal-stem cells on extirpated pulps. Simvastatin accelerated the mineralization process and pulp regeneration after 10 weeks [42]. A study by Ito et al. [43] involved the use of a poly(l-lactic acid)/Matrigel scaffold combined with bone-marrow-mesenchymal-stem cells in immunosuppressed rats.

Following 14 days of implantation, complete pulp regeneration, including nestin-expressing odontoblast-like cells beneath the dentin, was observed. Nestin is a type VI intermediate-filament protein found in neural stem cells [43]. The American Association of Endodontics (AAE) considers regenerative endodontics as one of the most exciting advancements in dentistry [35,44,45].

Revascularization or Revitalization

In 1971, revascularization techniques were attempted for teeth with apical periodontitis and immature root apices [46]. Initial attempts were unsuccessful due to limitations in materials, instrumentation, and techniques. However, with advancements in techniques, materials, and instruments, this approach is now successfully incorporated into clinical practice [47,48]. Revascularization differs from both apexification and apexogenesis [47,48]. Apexification is defined as "the creation of an apical barrier to prevent toxins and bacteria from entering periapical tissues from the root canal" [5,49]. In cases of pulp disease and apical periodontitis, calcium hydroxide is commonly used. Its success rate, availability, and affordability make it a valuable medicament [50,51]. Before 2004, traditional apexification was the primary method for treating pulpal necrosis in immature teeth, but it required multiple visits and had a higher incidence of cervical fractures [19]. ProRoot Mineral Trioxide Aggregate (MTA) is now used in artificial apical barrier techniques to facilitate root canal obturation [49].

Apexogenesis is performed when the pulp is inflamed in an incompletely developed tooth. It addresses the limitations of capping inflamed dental pulp and aims to conserve vital pulp tissue to promote continued root development and apical closure. Calcium hydroxide paste is used as a wound dressing after removing most or all of the coronal pulp [53]. Recent advancements in the treatment of necrotic immature teeth have shifted focus from apexification and artificial barrier procedures to revascularization, which involves proliferating tissues in the pulp space of the affected tooth [33]. Bleeding induced in the canal space allows for significant accumulation of undifferentiated mesenchymal stem cells [54]. Thibodeau et al. and Wang et al. conducted animal studies that showed cementum and bone formation when using triple antibiotic paste and the blood clot technique [55,56].

A retrospective study comparing conventional apexification and apexogenesis with regenerative endodontics found that revascularization-treated teeth had the highest survival rates [57]. However, some studies noted that weak root structures in many cases led to lower reliability and success rates [58]. Kahler et al. [59] compared conventional disinfection approaches with regenerative blood-clot induction in 16 clinical cases and reported continued root maturogenesis in only two cases upon radiographic examination. Gomes-Filho et al. [60] incorporated bone marrow aspirate, platelet-rich plasma, and artificial hydrogel scaffolds with basic fibroblast growth factor in their study. They found that adding PRP and bone marrow aspirates to debrided root canals did not significantly improve tissue ingrowth. The study concluded that revascularization procedures in humans did not benefit from the addition of an artificial hydrogel scaffold combined with basic fibroblast growth factor [61]. Iwaya et al. [48,62] demonstrated improved results with revascularization techniques compared to apexification in a case of a permanently immature tooth with apical periodontitis and a sinus tract. "Revitalization" refers to the endodontic procedure performed to restore tooth vitality in cases of necrotic pulp, while "regeneration" involves replacing lost or damaged pulp-dentin tissue complex [29]. The underlying mechanisms of dentin-pulp complex regeneration remain poorly understood, with root-canal therapy often resulting in repair or healing [63].

Advantages of the Revascularization Approach

- Technically straightforward.
- Avoids the use of costly biotechnological tools due to the availability of current instruments and techniques.
- Minimal risk of immune rejection as the approach relies on the patient's own blood.
- Bacterial microleakage can be mitigated by introducing stem cells into the root canal space, followed by an intra-canal barrier and blood clot induction.
- Addresses concerns regarding restoration retention.
- Reinforces root walls in immature teeth.
- For avulsed immature teeth with necrotic pulp and an open apex, newly formed tissue can easily reach the coronal pulp horn due to the short distance required for proliferation. The approach aims to balance pulp-space infection and new tissue proliferation.
- Promotes additional growth of open-apex roots through minimal instrumentation, preserving viable pulp tissue.
- Recognizes the potential for increased stem cell regeneration and rapid tissue healing in young patients.

Disadvantages of the Revascularization Approach

The origins of regenerated tissue are not yet fully understood. Researchers emphasize that effective tissue engineering requires precise cell composition and concentration. However, cells are often encased in fibrin clots, which complicates their use for tissue engineering. Consequently, blood-clot formation is not relied upon for the function of tissue engineering. Treatment outcomes are inconsistent due to variations in cell composition and concentration [64,65,66,67].

Prerequisites for Revascularization Approach

Studies have identified the following prerequisites for successful revascularization:

- Open apices and necrotic pulp: Trauma-induced necrotic pulp with an open apex of less than 1.5 mm is necessary.
- Microorganism removal: Agents like antibiotic paste, calcium hydroxide [68], or formocresol [69] can be used to disinfect the canal.
- Effective coronal seal: An effective seal is essential.
- Matrix for tissue growth: A matrix or means for new tissue growth is required.

- Bleeding induction: Anaesthesia should be administered without a vasoconstrictor [70].
- Non-instrumentation: Canals should not be instrumented.
- Irrigation: Sodium hypochlorite should be used.
- Blood-clot formation: Blood-clot formation is crucial.

Postnatal Stem Cell Therapy

Postnatal stem cells from sources such as bone, buccal mucosa, fat, and skin can be injected into a disinfected root-canal system after opening the apex. This technique is considered straightforward [72]. Benefits include ease of cell harvesting and delivery, and its use in regenerative medicine for applications like bone-marrow replacement and endodontic treatments [73]. However, challenges include low survival rates, potential for migration leading to unusual mineralization patterns, and the need for bioactive signaling molecules, growth factors, and scaffolds to support tissue regeneration [74,75]. This technique is still not approved.

Pulp Implantation

In pulp implantation, purified pulp stem cells or lab-grown pulp tissue is transplanted into the root canal. Tissues are cultured using biodegradable polymer nanofibers or extracellular matrix proteins like collagen I or fibronectin [76]. Collagens I and III have not been effective for growing pulpal cells [77]. The technique requires a scaffold for cellular proliferation and a blood supply within 200 µm to prevent anoxia and necrosis. Further research and controlled clinical trials are necessary to assess the success rates, immune responses, and health risks [78].

Scaffold Implantation

Scaffold implantation involves seeding pulp stem cells onto a porous polymer scaffold to organize cells in a three-dimensional structure [79]. Nano scaffolds aid in distributing therapeutic medicines and provide necessary biological and mechanical properties [80,81]. Dentin chips can accelerate dentin-bridge formation by providing growth factors and a matrix for stem cell attachment [82,83,84]. Hydrogels, including photo-polymerizable hydrogels, offer a non-invasive delivery method and support tissue structure development, though they require further research [86,88,89].

Three-Dimensional Cell Printing

Three-dimensional cell printing is a technique to precisely position cells and mimic natural pulp-tissue structure [90,91]. This method requires expertise to address apical and coronal asymmetry during pulp placement in cleaned root canals. Currently, this technique is not clinically available, and there is limited literature on its functionality [92].

Gene Therapy

Gene therapy in regenerative endodontics involves delivering mineralizing genes to promote tissue mineralization [73,93]. Though initial research, such as Rutherford's work, faced challenges and the FDA withdrew approval in 2003 due to adverse effects, there is potential for gene therapy with bone morphogenetic proteins (BMPs) like BMP2 to enhance tooth development and dentin formation [94,95,96]. Safe, cell-specific gene therapy remains a goal.

Nitric Oxide

Nitric oxide (NO) is a potent vasodilator and key regulator of angiogenesis through vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1 (HIF-1) [97]. NO promotes VEGF expression and can be used in conjunction with antibiotics in biomimetic nanomatrix gels for enhanced antibacterial effects and pulp-dentin regeneration [98,100]. Further exploration of NO's effects and concentrations is needed.

Platelet-Rich Plasma (PRP)

PRP, derived from autologous whole blood, can act as a scaffold and promote regeneration by providing a fibrin matrix with high levels of growth factors and cytokines [33,102,103,104]. Studies have shown PRP's efficacy in treating necrotic pulp and immature teeth, leading to apex closure and healing of periapical lesions [25,33,108]. PRP can be utilized when minimal bleeding occurs, but further exploration of stem cell sources, interactions, and inflammatory responses is needed for optimal results [104,112].

Cell Homing

Cell homing represents a novel approach in tissue regeneration that focuses on leveraging the body's natural healing processes without the need for cell transplantation. Initially developed for articular cartilage regeneration, this concept has been adapted for dental-tissue regeneration. Here’s an overview of how cell homing is applied to regenerative endodontics:

1. Concept and Process:
   - Origin: The idea of cell homing was first introduced in 2010, with its application to dental-tissue regeneration emerging shortly thereafter.
   - Procedure: The process involves shaping and cleaning the root canal of extracted human teeth, followed by the delivery of growth factors, scaffolds, and stem cells. Initial steps include deactivating residual proteins in the root canal through sterilization. Collagen gel, potentially combined with growth factors such as basic fibroblast growth factors (bFGFs), vascular endothelial growth factors (VEGFs), platelet-derived growth factors (PDGFs), nerve growth factors (NGFs), or bone morphogenetic proteins (BMPs), is then infused into the prepared root canal.
   - Animal Models: Experiments, often involving Sprague-Dawley rats, have demonstrated the potential for dental pulp-like tissue formation with the

presence of blood vessels. Techniques such as enzyme-linked immunosorbent assay (ELISA) are used to quantify biomolecules and assess tissue regeneration.

2. Advantages:
   - Non-invasive: Unlike cell transplantation, cell homing does not require ex-vivo cultivation or in-vivo transplantation of cells.
   - Efficiency: This method utilizes the body's natural ability to recruit and differentiate cells at the site of injury or defect, potentially leading to more effective and less invasive regeneration.
3. Comparison with Cell Transplantation:
   - Cell Transplantation: Involves the direct transplantation of isolated stem/progenitor cells into the root canal. This method relies on the viability and differentiation of transplanted cells to regenerate pulp tissue.
   - Cell Homing: Focuses on delivering growth factors and scaffolds to stimulate the body's own cells to migrate, proliferate, and differentiate into the necessary tissue types, such as odontoblasts and pulp fibroblasts.
4. Cell Processes Involved:
   - Recruitment: The process of cells migrating to the site of injury or defect.
   - Differentiation: The transformation of stem/progenitor cells into mature cells that form specific tissues such as pulp and dentine. This process is crucial for the development of odontoblasts and pulp fibroblasts.
5. Research Findings:
   - Studies have shown that the combination of growth factors such as bFGF, BMP7, PDGF, VEGF, and NGF can lead to the development of vascularized and cellularized tissues within the root canal.
   - Microscopic analysis has revealed new pulp tissue containing erythrocyte-filled blood vessels and endothelial-like cell linings.
6. Current Limitations and Future Directions:
   - Endothelial Cells Formation: More research is needed to confirm whether endothelial cells are directly formed from dental pulp stem/progenitor cells.
   - Optimization: Further studies are required to refine the delivery methods and concentrations of growth factors to maximize tissue regeneration and ensure consistent results.

The cell homing approach offers a promising alternative to traditional cell transplantation methods by harnessing the body's own regenerative capabilities and reducing the need for ex-vivo cell manipulation. Continued research and development are essential to advance this technique and enhance its clinical application in regenerative endodontics (113).

Conclusion

The review of innovative approaches in regenerative endodontics highlights a transformative shift in dental treatment paradigms, moving beyond traditional methods to embrace biomimetic techniques. Central to these advancements is the concept of biomimicry, which involves designing dental materials and procedures inspired by natural biological systems. The evolution of regenerative endodontics reflects a growing understanding of biological processes and an increasing emphasis on minimally invasive techniques. Cell homing has emerged as a promising approach, leveraging the body's natural ability to regenerate tissue without the need for ex-vivo cell cultivation or transplantation. This technique involves infusing growth factors and scaffolds into prepared root canals, stimulating endogenous stem cells to form new pulp-dentin tissue. Research has demonstrated the potential of this method in creating vascularized and functional tissues, though further studies are needed to optimize growth factor delivery and confirm endothelial cell formation. Revascularization remains a cornerstone of regenerative endodontics, offering a technique to restore vitality to necrotic teeth by inducing a blood clot and recruiting stem cells from the pulp apex. While effective in many cases, the success of revascularization can be influenced by factors such as root structure and the consistency of tissue regeneration. Comparisons with traditional apexification techniques reveal that revascularization may provide better outcomes, particularly in immature teeth. Other innovative approaches, including scaffold implantation, three-dimensional cell printing, gene therapy, and the use of platelet-rich plasma, offer additional avenues for enhancing dental pulp regeneration. Each technique has its own set of advantages and limitations, emphasizing the need for continued research and development. In conclusion, the field of regenerative endodontics is rapidly advancing, with new biomimetic techniques offering promising solutions for tooth repair and regeneration. Ongoing research is crucial to refine these methods, ensure their clinical efficacy, and address existing challenges. The integration of these innovative approaches into clinical practice holds the potential to revolutionize endodontic treatments and improve patient outcomes significantly.

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