Review

How to Deal with Pulpitis: An Overview of New Approaches – a Narrative Review

Wojciech Niemczyk , Jakub Fiegler-Rudol * , Katarzyna Janik , Anna Zawilska , Marta Tanasiewicz

Posted Date: 22 October 2024

doi: 10.20944/preprints202410.1657.v1

Keywords: Regenerative Endodontics; Dental Pulp; Platelet-Rich Fibrin; Tissue Scaffolds; Regeneration

Review

How to Deal With Pulpitis: An Overview of New Approaches—A Narrative Review

Wojciech Niemczyk, Jakub Fiegler-Rudol, Katarzyna Janik, Anna Zawilska and Marta Tanasiewicz

Department of Conservative Dentistry with Endodontics, Faculty of Medical Sciences in Zabrze, Medical University of Silesia, Plac Akademicki 17, 41-902 Bytom, Poland

* Correspondence: Jakub.fieglerrudol@gmail.com; Tel. +48 574 004 884

Abstract: Background: Traditional root canal therapy (RCT) has been the primary method for treating necrotic pulp, but it presents limitations such as loss of vitality and postoperative fractures. Recent advancements in regenerative endodontics offer alternative strategies to restore pulp tissue vitality and function. These approaches involve stem cell transplantation, cell homing, and the use of organic scaffolds to promote pulp regeneration. Highlight: This article reviews various regenerative endodontic techniques, including autogenic and allogenic dental pulp transplantation, the use of amniotic membrane scaffolds, platelet-rich fibrin therapy, specialized pro-resolving mediators, nanofibrous and bioceramic-based scaffolds, injectable scaffolds, and dentin matrix proteins. These strategies leverage stem cells, growth factors, and biomaterials to create conducive microenvironments for cell attachment, proliferation, and differentiation, thereby facilitating pulp tissue regeneration. Methods: The literature search was conducted using databases such as PubMed, Scopus, and Google Scholar, employing keywords like "regenerative endodontics," "pulpitis," "pulp regeneration," and "platelet-rich fibrin." Conclusion: The reviewed approaches in regenerative endodontics show promising potential for overcoming the limitations of traditional RCT and promoting pulp tissue regeneration. However, further research is needed to optimize protocols, address challenges, and translate these advancements into routine clinical practice. Ultimately, these innovations have the potential to enhance patient outcomes in endodontic treatment by restoring pulp vitality and function effectively.

Keywords: regenerative endodontics; dental pulp; platelet-rich fibrin; tissue scaffolds; regeneration

1. Introduction

Traditional root canal therapy (RCT) is currently the predominant method for treating necrotic pulp. The treatment involves removing infected dental pulp and replacing it with inorganic materials such as paste and gutta-percha. During RCT, dental pulp loses vitality, which can cause treated teeth to become devitalized, brittle, and prone to postoperative fractures [1]. Recent advances in pulp biology challenge the traditional approach of restoring diseased tooth/pulp tissues with inert materials. Regenerative strategies are emerging to generate new vital tissue. Various strategies, such as stem cell transplantation and cell homing, have been employed for pulp regeneration. Stem cell transplantation involves the isolation, expansion, seeding, and transplantation of stem cells. On the contrary, cell homing involves the recruitment of endogenous cells to the injured tissue via signalling molecules, eliminating the need for in vitro manipulation of stem cells [2]. Organic scaffolds are useful in restoring pulp vitality. Four main categories of scaffolds have been used to engineer pulp-dentine complexes: bioceramic-based, synthetic polymer-based, natural polymer-based, and composite scaffolds. The establishment of a vascular network is essential for the successful regeneration of pulp-dentine complexes. The use of a diverse range of biomaterials and fabrication techniques presents significant potential in meeting the requirements for scaffolds in regenerative endodontics. However, further studies are necessary to develop an ideal scaffold that is suitable for

 doi:10.20944/preprints202410.1657.v1

clinical applications [3]. A scaffold made from a porous decellularized human amniotic membrane (HAM) extracellular matrix (ECM) has been developed to predictably regenerate pulp outcomes in 3D. The HAM was successfully decellularized, and scaffolds were synthesized at different concentrations. The scaffolds were evaluated for various physical properties, including porosity, pore size, PBS absorption, and degradation rate. In vivo assessment revealed a mild to moderate inflammatory response. In root segment models, both cell-free and cell-loaded scaffolds at a concentration of 30 mg/mL facilitated the formation of pulp-like tissue with high revascularization and collagen content. These results demonstrate the potential of the HAM ECM membrane for further exploration in regenerative endodontics [4]. Pulp revascularization is a two-visit therapeutic approach that involves a cell-homing strategy for immature teeth. After disinfection, the root canal system is filled with a blood clot induced by endodontic instruments. Although case reports show some success, histological studies reveal that non-pulp-like tissues, such as cementum and bone-like tissues, are often formed. The success rate is limited due to challenges in achieving efficient disinfection and inducing a blood clot in the canal. Further research is required to improve the formation of pulp-like tissue, increase success rates in immature teeth, and potentially expand this approach to mature teeth [5]. The potential of pulp regeneration strategies is promising for future pulp tissue regeneration. Additionally, the review explores cell-homing strategies for pulp regeneration. This review focuses on current regenerative endodontic approaches, with emphasis on clinical considerations such as root canal disinfection and apical foramen enlargement [6].

2. Materials and Methods

This narrative review was conducted to summarize and analyze the latest advancements in the treatment of pulpitis, with a focus on regenerative approaches. Unlike systematic reviews, this review does not follow strict inclusion and exclusion criteria, offering a broader scope of the literature on the subject. The methodology aimed to explore diverse regenerative endodontic techniques, assess their efficacy, and identify gaps in current knowledge. A comprehensive literature search was performed using electronic databases, including PubMed, Scopus, and Google Scholar. The search strategy employed a combination of MeSH terms and free-text keywords, such as "pulpitis," "regenerative endodontics," "pulp regeneration," "stem cells," "platelet-rich fibrin," "scaffolds," and "nanofibers." The search covered articles published in English between 2000 and 2023. Articles focusing on conventional root canal therapy were excluded, while those related to novel pulp regeneration methods were included. Articles were selected based on their relevance to new and emerging techniques for treating pulpitis. Studies on human, animal, and in vitro models were considered. Preference was given to clinical trials, cohort studies, and in vitro studies demonstrating the effectiveness of regenerative techniques such as stem cell therapies, the use of scaffolds, and specialized biomaterials. Key data was extracted from selected studies, including information on the study design, sample size, methods of pulp regeneration, and clinical outcomes. Additionally, the type of regenerative approach used (e.g., stem cell transplantation, scaffolds, growth factors), and the success rate in terms of pulp vitality restoration were recorded. Data on adverse effects and limitations of each technique were also noted.

3. Autogenic Dental Pulp Transplantation

Autogenic dental pulp transplantation is a regenerative endodontic procedure that involves transplanting dental pulp tissue from one part of a patient's mouth to another within the same individual. This procedure is suitable when a patient has a healthy tooth with intact pulp, and there is a need for pulp regeneration in another tooth. The donor tooth is typically a healthy tooth within the same individual. This may involve extracting a tooth for orthodontic treatment or non-pathological reasons. The dental pulp is carefully removed from the selected donor tooth, as it contains vital cells and growth factors necessary for regeneration. The recipient tooth, which requires pulp regeneration, undergoes endodontic instrumentation and irrigation to create a suitable environment for transplantation. The dental pulp harvested from the donor's tooth is transplanted into the root canal of the recipient tooth. Feitosa et al. conducted a study involving the extraction of

 doi:10.20944/preprints202410.1657.v1

a third molar, removal and preservation of its pulp, and insertion into a premolar's root canal. The teeth were monitored for 12 months, and positive pulp vitality, regression of periapical lesions at 3- and 6-month follow-ups, and revascularization at 9-12 months were observed. Despite the limitations of the study, which included a small sample size, the procedure has shown potential for clinical application in pulp regeneration [7]. Haung et al. conducted a study to investigate pulpal regeneration in a dog model by transplanting deciduous tooth pulp into immature necrotic permanent teeth. Six Beagle dogs were used, with 60 teeth induced with apical periodontitis. Half of the teeth received autologous deciduous pulp transplantation, while the other half received standard treatment. Radiographic analysis at 3 and 6 months showed no significant differences, but the experimental group exhibited a greater reduction in apical diameter. Histological analysis showed the presence of newly formed dentin-like tissue in the experimental group, indicating the potential of autologous transplantation for regenerating dental pulp in necrotic young permanent teeth [8]. Cehreli et al. investigated the use of regenerative endodontic treatment (RET) in young permanent teeth with necrotic pulps, utilizing deciduous tooth pulp as a natural scaffold. RET was performed on five traumatized maxillary incisors in patients aged 8 to 11.5 years. The procedure involved irrigation with 2.5% NaOCl, application of a calcium hydroxide dressing, removal of intracanal medication, and transplantation of pulp tissue from neighbouring deciduous canines. Follow-up revealed complete periapical healing, increased dentinal wall thickness, and ongoing apical closure without clinical symptoms. Positive responses to cold testing indicated a favourable outcome for root-end filling using autotransplantation of deciduous pulp in young permanent incisors [9].

4. Allogenic Dental Pulp Transplantation

Allogenic dental pulp transplantation is a regenerative endodontic procedure that involves transferring dental pulp tissue from a donor to a recipient tooth. The term 'allogenic' refers to the fact that the donor and recipient are from different individuals but within the same species. This procedure is typically suitable for cases where the recipient tooth has irreversible pulp damage, necessitating endodontic intervention. The donor is typically a family member, often a child with deciduous teeth scheduled for extraction. However, they can also be permanent teeth, such as third molars or teeth that require extraction for orthodontic reasons. When using deciduous teeth as a donor, they are extracted, sectioned, and the dental pulp is carefully removed. The harvested pulp contains stem cells and growth factors that are crucial for regeneration. The recipient tooth, typically a single-rooted permanent tooth from a parent, undergoes endodontic instrumentation and irrigation using antibacterial solutions to create a suitable environment for transplantation. The harvested pulp from the donor is then transplanted into the root canal of the recipient tooth with the aim of generating new pulp tissue. After pulp transplantation, the usual procedure involves direct pulp capping, followed by tooth restoration using materials like resin composite [10–12]. Feitosa et al. studied allogenic pulp transplantation as a novel approach to regenerative endodontic therapy. The study involved three patients with single-root teeth, each of whom had a child with deciduous teeth, or third molars scheduled for extraction. The recipient teeth underwent endodontic treatment, and during the transplant procedures, pulp from the child's extracted teeth was carefully transferred to the host tooth's root canal (father/mother). Biodentin was used for direct pulp capping, followed by resin-modified glass ionomer cement and composite for aesthetic restoration. The two-year follow-up included computed tomography, electric pulp vitality tests, and Doppler ultrasound examinations. Two cases showed positive pulp vitality and periapical lesion formation at the 6-month mark, while the third case exhibited significant radiolucency before transplantation but improved after a year. After two years, all teeth showed revascularisation without endodontic or periodontal radiolucency. This suggests that allogenic pulp transplantation could be a potential strategy for pulp revitalisation in specific endodontic cases, despite the limited scope of the study with only three patients and four treated teeth. It is worth noting that no apical bleeding was performed because it was a different protocol than the revascularisation strategy. Additionally, none of the parents received preventive antibiotic coverage [10].

 doi:10.20944/preprints202410.1657.v1

5. Amniotic Membrane

Human amniotic membrane shows potential for regenerating tooth pulp in regenerative endodontics. HAM is derived from the innermost layer of the placenta and undergoes a decellularization process to create a 3-dimensional scaffold with optimal physical properties for pulp regeneration. The scaffold is porous, with controlled variations in concentration to achieve desirable characteristics. Histological analysis confirms successful decellularization, ensuring the absence of cellular remnants. In vitro experiments using human dental pulp stem cells (hDPSCs) have demonstrated the biocompatibility of the HAM scaffold. The scaffold supports cell adhesion, proliferation, and migration. In vivo studies have shown a mild to moderate inflammatory response. HAM scaffolds, whether cell-free or cell-loaded, contribute to the formation of pulp-like tissue in root canal models. This tissue exhibits revascularization and collagen content, which are essential for functional pulp regeneration [4]. A scaffold for pulp regeneration was developed using a decellularized human amniotic membrane (dHAM) extracellular matrix with varying concentrations synthesized and evaluated for physical properties. In vitro studies with hDPSCs showed that the 30 mg/mL ECM scaffold had optimal properties, enhancing hDPSC migration. In vivo assessment revealed a mild to moderate inflammatory response. Both cell-free and cell-loaded 30 mg/mL scaffolds facilitated the formation of pulp-like tissue with high revascularization and collagen content, resulting in positive clinical outcomes. These findings suggest that HAM ECM scaffolds have potential in regenerative endodontics and should be further explored for tooth pulp regeneration due to their biocompatibility and favourable physical characteristics [4]. Johri et al. (2021) used an amniotic membrane to cover pulp stumps after pulpotomy in a tooth with irreversible pulpitis. They placed glass ionomer cement over the pulp. After 18 months of follow-up, the tooth was completely asymptomatic, and vitality tests were similar to control teeth. There were also no changes in X-ray imaging. Further research is needed to determine if the amniotic membrane is a better and more economical material than existing options [13].Saaid et al. (2022) investigated the potential of glycerol and cryopreserved de-epithelialized human amniotic membrane (CAM) to induce odontogenic differentiation of deciduous teeth stem cells (DTSCs) on different sides of the membranes. Both glycerol and CAM supported cell attachment. However, CAM showed higher cell proliferation but lower alkaline phosphatase (ALP) expression compared to glycerol. The ALP expression indicated the potential of glycerol for odontogenic differentiation. When hDPSCs were seeded on glycerol and transplanted into a root canal, they exhibited strong cell attachment[14].Dental trauma frequently results in tooth nonvitality and halted root development. The use of amniotic membranes in transplantation and regeneration procedures has generated significant interest. Joseph et al. (2021) presented an innovative case of a successful regenerative endodontic procedure using an amniotic membrane to treat a traumatized immature right maxillary central incisor in an 8-year-old girl. Follow-up evaluations at 1, 3, 6, 9, and 12 months showed continuous root growth and apical closure, indicating positive clinical and radiographic outcomes [15].

6. Use of PRF for Apical Closure on an Immature Permanent Tooth

The use of advanced platelet-rich fibrin plus (A-PRF+) therapy as a regenerative endodontic treatment has proven effective in treating a 12-year-old patient with necrotic pulp and asymptomatic apical periodontitis. During the 24-month follow-up period after treatment, we observed the resolution of symptoms and complete formation of the root, allowing for healing in the periapical region. The use of regenerative endodontic treatment involving A-PRF+ was successful in addressing apical periodontitis and treating an immature permanent premolar with pulp necrosis [16].

7. Cell Homing Strategy and Stem Cells

Cell homing is a regenerative strategy that utilises the natural migratory and regenerative abilities of endogenous cells to promote tissue repair and regeneration. In the context of regenerative endodontics, the cell homing strategy involves attracting and recruiting the patient's stem cells or progenitor cells to the site of injury within the dental pulp to facilitate tissue regeneration. This

 doi:10.20944/preprints202410.1657.v1

approach aims to create a microenvironment that is conducive to cell migration, proliferation, and differentiation. This will ultimately lead to the restoration of damaged or diseased dental tissues [17–19].The dental pulp contains stem cells, such as dental pulp stem cells (DPSCs) and stem cells from the apical papilla (SCAP), which can differentiate into various cell types, including odontoblasts and endothelial cells. Progenitor cells within the pulp tissue can also contribute to the regenerative process. The application of bioactive molecules, growth factors, and signalling proteins within the root canal can stimulate the migration of endogenous cells from the surrounding tissues towards the injury site. The release of chemotactic signals can guide cells to the areas requiring regeneration. This technique commonly involves inducing the formation of a blood clot within the root canal, which serves as a scaffold and releases signals that attract endogenous cells to the root canal space. The regenerative potential can be enhanced by using biocompatible materials that promote cell homing, such as those containing growth factors or dentin matrix proteins [20]. Cell homing strategies are typically minimally invasive, avoiding the need for complex surgical procedures. Cell homing aims to leverage the body's regenerative mechanisms to create a more natural and physiological environment for tissue repair. The success of cell homing depends on various factors, including the patient's age, overall health, and the presence of systemic conditions that may affect regenerative capacity. Creating an optimal microenvironment within the root canal is crucial to facilitate cell migration, adhesion, and differentiation. It is important to further understand the molecular and cellular mechanisms that underlie cell homing in regenerative endodontics. Additionally, rigorous clinical trials should be conducted to assess the efficacy, safety, and long-term outcomes of cell-homing strategies in diverse patient populations. Cell homing in regenerative endodontics is a promising approach that utilises the patient's cells to tap into their natural regenerative potential. As research progresses, refining techniques to enhance cell homing and optimising treatment protocols will contribute to advancing regenerative therapies for dental pulp and root canal regeneration [21].Previous experiments have demonstrated the therapeutic potential of mobilized dental pulp stem cells (MDPSCs) in achieving full pulp regeneration. In a study conducted by Nakashima et al. on five patients with irreversible pulpitis, their progress was monitored for up to 24 weeks after MDPSC transplantation. MDPSCs were obtained from discarded teeth and were expanded following good manufacturing practice (GMP) standards. The quality of MDPSCs at passages 9 or 10 was verified through karyotype analyses. Transplantation of MDPSCs, along with granulocyte colony-stimulating factor (G-CSF) in atelocollagen, was performed in teeth following pulpectomy. No adverse events or toxicity were observed during clinical and laboratory assessments. After 4 weeks, the electric pulp test (EPT) showed a robust positive response, and at 24 weeks, magnetic resonance imaging (MRI) indicated regenerated tissue with a signal intensity in the root canal comparable to normal dental pulp in the untreated control. Furthermore, cone beam computed tomography revealed functional dentin formation in three of the five patients. This pilot clinical study indicates that human MDPSCs are both safe and effective for achieving complete pulp regeneration in humans [22].

8. Nanofibrous Scaffolds

Nanofibrous scaffolds are essential in regenerative endodontics, providing a promising approach for tissue regeneration in the dental pulp. These scaffolds are characterized by their nanoscale fibre structure, which mimics the natural extracellular matrix. This structure offers a high surface area and promotes cell adhesion, proliferation, and differentiation. Nanofibrous scaffolds can be fabricated from various biocompatible materials, such as polymers or natural substances. Common materials used for nanofibrous scaffolds include synthetic polymers such as polycaprolactone (PCL) and natural polymers like collagen. These scaffolds exhibit a porous architecture that enables the diffusion of nutrients, oxygen, and waste products, which is essential for supporting cell growth and tissue regeneration [23]. The scaffolds' nanoscale fibres create an optimal environment for cell attachment, proliferation, and differentiation into various cell types required for pulp regeneration. Nanofibrous scaffolds can also be engineered to release growth factors in a controlled manner, promoting specific cellular activities such as angiogenesis or

 doi:10.20944/preprints202410.1657.v1

odontogenesis, which are crucial for dental pulp regeneration. Many nanofibrous scaffold materials are biodegradable, which allows them to gradually degrade as new tissue forms. This property is important for maintaining the structural integrity of the scaffold during tissue regeneration [24]. Nanofibrous scaffolds can be used as effective drug delivery systems, allowing for controlled release of bioactive molecules, antimicrobial agents, or other therapeutic substances to create an optimal environment for tissue regeneration. The electrospinning technique is commonly used to produce nanofibrous scaffolds, involving the creation of nanofibers using an electric field to draw and stretch polymer solutions into thin fibres. Extensive in vitro and in vivo studies have been conducted on nanofibrous scaffolds to evaluate their biocompatibility, effectiveness in supporting cell growth, and potential for promoting tissue regeneration within the dental pulp. Nanofibrous scaffolds can be combined with other regenerative endodontic techniques, such as stem cells, growth factors, or bioactive molecules, to enhance their overall effectiveness in promoting pulp regeneration [25]. Nanofibrous scaffolds are a cutting-edge technology in regenerative endodontics, providing a versatile and effective platform for pulp tissue engineering and regeneration. Their unique properties make them valuable tools in advancing the field towards successful clinical applications [26]. Palasuk et al. conducted a study on the synthesis and evaluation of polydioxanone (PDS) based polymer scaffolds containing a combination of antibiotics (metronidazole and ciprofloxacin) for use in regenerative endodontics. The researchers used electrospinning to create nanoscale fibres and confirmed their morphology and chemical characteristics through various analyses. The scaffolds exhibited greater tensile strength compared to pure PDS. Antimicrobial testing demonstrated inhibition of bacterial growth. There were slight reductions in viability for dental pulp stem cells, indicating a potential for a non-cytotoxic drug delivery system in regenerative endodontics. The study suggests that the integration of multiple antibiotics in a nanofibrous scaffold holds promise for enhancing antimicrobial effectiveness in root canal treatments [27]. The study conducted by Lovelance et al. examines the effectiveness of regenerative endodontic procedures in treating immature teeth with open apices. These teeth have traditionally had a poor prognosis due to the fracture risk and susceptibility to recontamination. The regenerative approach involves introducing a blood clot, growth factors, and stem cells into the root canal space. The study aims to determine the efficacy of these procedures in delivering stem cells into the canal space and identifying the tissue origin of these cells. The study shows a significant accumulation of transcripts for stem cell markers (CD73 and CD105) in the root canal samples compared to systemic blood. This selective effect indicates the presence of undifferentiated stem cells in the canal space, potentially contributing to the regeneration of pulpal tissues after antibiotic paste therapy. The results suggest that the evoked-bleeding step in regenerative procedures plays a crucial role in recruiting stem cells for effective treatment [28].

9. Bioceramic-Based Scaffolds

Bioceramics are inorganic non-metallic materials that are biocompatible. They can be classified into three main groups: bioinert, bioactive, and biodegradable. Bioinert materials, such as alumina and zirconia, do not interfere with biological systems. Bioactive materials, such as bioactive glass ceramics, hydroxyapatite (HA), and calcium silicates, interact with biological systems. Biodegradable materials, such as tricalcium phosphate (TCP), are eventually replaced or incorporated into tissue [29]. Scaffolds made from these materials are extensively used in the field of Regenerative Endodontics due to their suitable porous structure [30] and ability to enhance osteo- and odontogenic differentiation and biomineralization. This is because they can absorb osteoinductive substances from the surrounding regenerating tissues [31,32]. Additionally, they release many ions, such as Si4+, PO43-, Zn2+, Mg2+ or Ca2+, which play a significant role in several signalling pathways and intracellular interactions[33,34]. These scaffolds can form a hermetic seal and chemical bond with tooth tissues. They also exhibit evident radiopacity, which is crucial in the clinical management of patients and during the follow-up period [35,36].Calcium phosphate compounds (CPCs), particularly HA and TCP, are the most usedbioceramics in clinical settings. They are available in various forms, such as powder, granules, blocks, or excipients [3]. When used for pulp-capping, they promote the

 doi:10.20944/preprints202410.1657.v1

regeneration of hard dentin-like tissue surrounding odontoblast-like cells. However, the low resorption rate of the material has implications for the quality of the restored tissues, which can result in a disorganised structure [37–39].

10. Injectable Scaffolds and Stem Cells

Biodegradable hydrogels are a novel category of medical materials. They consist of homopolymers, such as polylactic acid (PLA), polyglycolic acid (PGA), and polycaprolactone (PCL), and co-polymers, such as poly(lactide-co-glycolide)-polyethene glycol (PEG-PLGA) and L- and DL-lactide (PLDLA). Hydrogels are injectable scaffolds that can be delivered by syringe, making them potentially noninvasive and easy to administer into root canal systems. The hydrogel can facilitate pulp regeneration by providing a substrate for cell proliferation and differentiation into an organized tissue structure. The lesions can biodegrade within a short period of weeks or months, converting to carbon dioxide and water. This process allows natural tissue to fill the space previously occupied by the lesions. However, these polymers lose strength before they lose mass. Early ingrowth of natural tissue is inhibited, and subsequent rapid mass loss can cause inflammation due to the production of acidic degradation products [40,41]. Puramatrix™ is a liquid that can be poured into a pulp chamber and self-polymerizes under physiological conditions to form a solid gel that can support cell growth. This application is appealing from an endodontic perspective because a liquid can conform more easily to the variable shape of a pulp chamber than a solid or moldable scaffold. According to Cavalcanti et al. (2013), dental pulp stem cells can survive and proliferate in a 3D Puramatrix™ scaffold [42].

11. Dentin Matrix Proteins

Dentin matrix proteins (DMPs) play a significant role in regenerative endodontics by facilitating the formation and regeneration of dental tissues, particularly dentin. These proteins are extracted from the extracellular matrix of dentin and possess bioactive properties that can stimulate and guide tissue repair and regeneration. Collagen, the primary protein in the dentin matrix, provides structural integrity and acts as a scaffold for tissue regeneration. Non-collagenous Proteins include dentin sialoprotein (DSP), dentin phosphoprotein, and matrix extracellular phosphoglycoprotein (MEPE). These proteins contribute to dentin mineralization and regulate mineral crystal growth. Dentin matrix proteins can induce odontogenic differentiation of dental pulp stem cells and progenitor cells. The bioactive motifs contained within can initiate signalling pathways involved in cell adhesion, proliferation, and differentiation. Dentin matrix proteins are essential for dentin mineralization and significantly impact the formation of a dentin-like matrix during regeneration [43].Dentin matrix proteins are used in direct pulp capping procedures to stimulate reparative dentin formation when treating exposed pulp. In vital pulp therapy, dentin matrix proteins may be used to encourage the regeneration of pulp tissue. The study investigated the effect of using a combination of treated dentin matrix (TDM) proteins and small extracellular vesicles (sEVs) from dental pulp cells (DPCs) on repairing the pulp-dentin complex for vital pulp therapy. These proteins and vesicles contribute to the formation of a blood clot within the root canal, providing a scaffold for tissue regeneration in revascularization procedures. TDM was produced by chemically demineralising and mechanically disrupting teeth, while sEVs were extracted from DPC culture supernatants. In vitro evaluations showed that sEVs improved DPC proliferation and migration. Furthermore, the combination of TDM proteins and sEVs synergistically enhanced DPC migration while suppressing proliferation. In vivo studies using a minipig model demonstrated that TDM and sEV-TDM promoted the formation of continuous reparative dentin. Odontoblast-like high columnar cells were observed on the pulp side of the dentin bridge. The sEV-TDM complex demonstrates intrinsic biological activities, suggesting its potential use as a bioactive pulp-capping material in vital pulp therapy [44].The research explored the role of dentin-derived Bone Morphogenetic Proteins (BMP) in inducing the differentiation of stem cells from exfoliated deciduous teeth (SHED) into odontoblasts. The hypothesis suggests that BMP from dentin is crucial for this differentiation process. The study observed that when SHED were cultured in human tooth slices/scaffolds or implanted in immunodeficient mice, they expressed

 doi:10.20944/preprints202410.1657.v1

markers indicative of odontoblastic differentiation (DSPP, DMP-1, MEPE). However, the study found that when SHED were cultured in deproteinized tooth slices/scaffolds or scaffolds without a tooth slice, they did not express these markers. The research also established that SHED expresses BMP receptors (BMPR-IA, BMPR-IB, BMPR-II), and inhibiting BMP-2 signalling blocked the expression of odontoblastic markers in SHED cultured in tooth slice/scaffolds. In summary, the study concludes that dentin-derived BMP-2 is essential for inducing the differentiation of SHED into odontoblasts [45]. Combining dentin matrix proteins with bioactive scaffolds and stem cells can enhance the overall regenerative potential by providing a comprehensive microenvironment for tissue repair. Dentin matrix proteins can guide the differentiation of stem cells toward odontogenic lineages, promoting the regeneration of dentin and pulp tissues [46]. It is important to further understand the molecular mechanisms by which dentin matrix proteins influence cellular responses and tissue regeneration. Conducting additional clinical trials to evaluate the safety and effectiveness of regenerative approaches based on dentin matrix proteins in diverse patient populations is recommended. Dentin matrix proteins act as bioactive components that can have a positive impact on tissue regeneration in regenerative endodontics. Their inclusion in treatment protocols, along with other regenerative strategies, holds great promise for advancing the field and enhancing outcomes in dental pulp and root canal regeneration [47].

12. Resolvin e1

Endogenous specialised pro-resolving mediators (SPMs) such as resolvins, lipoxins, protectins and maresins control inflammation resolution. Resolvin E1 (RvE1), a major dietary omega-3 polyunsaturated fatty acid metabolite, is involved in resolving inflammation and promoting wound healing. Liu et al. (2014) evaluated the effect of the combination of RvE1 and lipoxin A4 (LXA4) on lipopolysaccharide (LPS)-induced dental pulp fibroblasts. The authors revealed that the combination of RvE1 and LXA4 effectively inhibited NF-κB activation in DPFs, reduced the production of pro-inflammatory factors, promoted complete resolution, and prevented ectopic mineralisation beyond the pulp exposure site. Based on these findings, they proposed using the combination of RvE1 and LXA4 for pulpitis resolution. Similarly, Chen et al. investigated the effects of RvE1 on LPS-induced DPSCs and a pulp injury model using Sprague-Dawley rats. The study found that the application of RvE1 on DPSCs, with or without LPS, enhanced proliferation, chemotaxis, and odontoblastic differentiation. In vivo, RvE1 induced the resolution of pulp inflammation and the formation of reparative dentin [48].

13. Conclusions

The field of endodontic therapy has significantly evolved from traditional root canal therapy towards more biologically driven approaches aimed at restoring pulp vitality and function. Autogenic and allogenic dental pulp transplantation have emerged as promising avenues for pulp regeneration, demonstrating positive outcomes in both preclinical and clinical settings. Amniotic membrane scaffolds have shown great potential in creating a conducive environment for the development of pulp-like tissue. Additionally, platelet-rich fibrin therapy and specialized pro-resolving mediators, such as resolvin E1, are highly effective in resolving inflammation and promoting wound healing in dental pulp. Nanofibrous and bioceramic-based scaffolds have significantly contributed to creating microenvironments that are conducive to cell attachment, proliferation, and differentiation, which are necessary for pulp tissue regeneration. Injectable scaffolds also enhance the regenerative potential of endodontic procedures, providing a minimally invasive approach for delivering regenerative agents into the root canal system. Incorporating dentin matrix proteins into regenerative procedures has shown great potential in directing stem cell differentiation and facilitating the development of dentin and pulp-like tissues. This is a vital step in repairing and regenerating tissues within the dental pulp. The exploration of diverse regenerative strategies highlights the potential for successful pulp regeneration and functional restoration in endodontic therapy. Further research and clinical studies are necessary to optimize these approaches, address existing challenges, and translate these promising findings into routine clinical practice.

 doi:10.20944/preprints202410.1657.v1

Regenerative endodontics advancements promise to improve treatment outcomes and enhance patient care in endodontic therapy.

References

1. Niazi SA, Bakhsh A. Association between Endodontic Infection, Its Treatment and Systemic Health: A Narrative Review. Medicina 2022;58:931. https://doi.org/10.3390/medicina58070931.
2. Kharchi AS, Tagiyeva-Milne N, Kanagasingam S. Regenerative Endodontic Procedures, Disinfectants and Outcomes: A Systematic Review. Prim Dent J 2020;9:65–84. https://doi.org/10.1177/2050168420963302.
3. Noohi P, Abdekhodaie MJ, Nekoofar MH, Galler KM, Dummer PMH. Advances in scaffolds used for PULP–DENTINE complex tissue engineering: A narrative review. Int Endodontic J 2022;55:1277–316. https://doi.org/10.1111/iej.13826.
4. Bakhtiar H, Ashoori A, Rajabi S, Pezeshki-Modaress M, Ayati A, Mousavi MR, et al. Human amniotic membrane extracellular matrix scaffold for dental pulp regeneration *in vitro* and *in vivo*. Int Endodontic J 2022;55:374–90. https://doi.org/10.1111/iej.13675.
5. Wang Y, Zhu X, Zhang C. Pulp Revascularization on Permanent Teeth with Open Apices in a Middle-aged Patient. Journal of Endodontics 2015;41:1571–5. https://doi.org/10.1016/j.joen.2015.04.022.
6. Yang J, Yuan G, Chen Z. Pulp Regeneration: Current Approaches and Future Challenges. Front Physiol 2016;7. https://doi.org/10.3389/fphys.2016.00058.
7. Feitosa VP, Mota MNG, Vieira LV, De Paula DM, Gomes LLR, Solheiro LKR, et al. Dental Pulp Autotransplantation: A New Modality of Endodontic Regenerative Therapy—Follow-Up of 3 Clinical Cases. Journal of Endodontics 2021;47:1402–8. https://doi.org/10.1016/j.joen.2021.06.014.
8. Huang Y, Tang X, Cehreli ZC, Dai X, Xu J, Zhu H. Autologous transplantation of deciduous tooth pulp into necrotic young permanent teeth for pulp regeneration in a dog model. J Int Med Res 2019;47:5094–105. https://doi.org/10.1177/0300060519862094.
9. Cehreli ZC, Unverdi GE, Ballikaya E. Deciduous Tooth Pulp Autotransplantation for the Regenerative Endodontic Treatment of Permanent Teeth With Pulp Necrosis: A Case Series. Journal of Endodontics 2022;48:669–74. https://doi.org/10.1016/j.joen.2022.01.015.
10. Feitosa VP, Mota MN, Savoldi R, Rifane T, De Paula D, Borges L, et al. The Allogenic Dental Pulp Transplantation from Son/Daughter to Mother/Father: A Follow-Up of Three Clinical Cases. Bioengineering 2022;9:699. https://doi.org/10.3390/bioengineering9110699.
11. Gomez-Sosa JF, Diaz-Solano D, Wittig O, Cardier JE. Dental Pulp Regeneration Induced by Allogenic Mesenchymal Stromal Cell Transplantation in a Mature Tooth: A Case Report. Journal of Endodontics 2022;48:736–40. https://doi.org/10.1016/j.joen.2022.03.002.
12. Hargreaves K, Law A. Regenerative Endodontics. Cohen's Pathways of the Pulp. 10th ed., 2011, p. 602–19.
13. Johri S, Verma P, Bains R, Tikku AP. Human amniotic membrane as therapeutic agent in pulpotomy of permanent molars. BMJ Case Rep 2021;14:e243414. https://doi.org/10.1136/bcr-2021-243414.
14. Saaid AS, Ensanya AAN, Suzina SAH, Nurul AA, Samsudin AR, Azlina A. Odontogenic induction of human amniotic membrane scaffold for dental pulp regeneration. Materials Chemistry and Physics 2022;280:125780. https://doi.org/10.1016/j.matchemphys.2022.125780.
15. Joseph E, Karuna M, Rao A, Rao A, Nayak A. A novel regenerative endodontic procedure in a traumatized immature tooth using amniotic membrane. Dent Res J 2021;18:28. https://doi.org/10.4103/1735-3327.313123.
16. Hosseini S, Chitsaz N, Hamrah MH, Maleki D, Taghizadeh E. Regenerative Endodontic Management of an Immature Necrotic Premolar Using Advanced Platelet-Rich Fibrin. Case Reports in Dentistry 2023;2023:1–6. https://doi.org/10.1155/2023/1135413.
17. Morotomi T, Washio A, Kitamura C. Current and future options for dental pulp therapy. Japanese Dental Science Review 2019;55:5–11. https://doi.org/10.1016/j.jdsr.2018.09.001.
18. Tsutsui T. Dental Pulp Stem Cells: Advances to Applications. SCCAA 2020;Volume 13:33–42. https://doi.org/10.2147/SCCAA.S166759.
19. Ferro F, Spelat R, Baheney CS. Dental Pulp Stem Cell (DPSC) Isolation, Characterization, and Differentiation. In: Kioussi C, editor. Stem Cells and Tissue Repair, vol. 1210, New York, NY: Springer New York; 2014, p. 91–115. https://doi.org/10.1007/978-1-4939-1435-7_8.
20. Kobayashi Y, Shimizu E. Current and Future Views on Cell-Homing-Based Strategies for Regenerative Endodontics. In: Duncan HF, Cooper PR, editors. Clinical Approaches in Endodontic Regeneration, Cham: Springer International Publishing; 2019, p. 139–59. https://doi.org/10.1007/978-3-319-96848-3_8.

21. Ahmed GM, Abouauf EA, AbuBakr N, Fouad AM, Dörfer CE, Fawzy El-Sayed KM. Cell-Based Transplantation versus Cell Homing Approaches for Pulp-Dentin Complex Regeneration. Stem Cells International 2021;2021:1–23. https://doi.org/10.1155/2021/8483668.
22. Nakashima M, Iohara K, Murakami M, Nakamura H, Sato Y, Ariji Y, et al. Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study. Stem Cell Res Ther 2017;8:61. https://doi.org/10.1186/s13287-017-0506-5.
23. Diedkova K, Pogrebnjak AD, Kyrylenko S, Smyrnova K, Buranich VV, Horodek P, et al. Polycaprolactone–MXene Nanofibrous Scaffolds for Tissue Engineering. ACS Appl Mater Interfaces 2023:acsami.2c22780. https://doi.org/10.1021/acsami.2c22780.
24. Ahmadi S, Shafiei SS, Sabouni F. Electrospun Nanofibrous Scaffolds of Polycaprolactone/Gelatin Reinforced with Layered Double Hydroxide Nanoclay for Nerve Tissue Engineering Applications. ACS Omega 2022;7:28351–60. https://doi.org/10.1021/acsomega.2c02863.
25. Gupta KC, Haider A, Choi Y, Kang I. Nanofibrous scaffolds in biomedical applications. Biomater Res 2014;18:5. https://doi.org/10.1186/2055-7124-18-5.
26. Chen H, Truckenmüller R, Van Blitterswijk C, Moroni L. Fabrication of nanofibrous scaffolds for tissue engineering applications. Nanomaterials in Tissue Engineering, Elsevier; 2013, p. 158–83. https://doi.org/10.1533/9780857097231.1.158.
27. Palasuk J, Kamocki K, Hippenmeyer L, Platt JA, Spolnik KJ, Gregory RL, et al. Bimix Antimicrobial Scaffolds for Regenerative Endodontics. Journal of Endodontics 2014;40:1879–84. https://doi.org/10.1016/j.joen.2014.07.017.
28. Lovelace TW, Henry MA, Hargreaves KM, Diogenes A. Evaluation of the Delivery of Mesenchymal Stem Cells into the Root Canal Space of Necrotic Immature Teeth after Clinical Regenerative Endodontic Procedure. Journal of Endodontics 2011;37:133–8. https://doi.org/10.1016/j.joen.2010.10.009.
29. Best SM, Porter AE, Thian ES, Huang J. Bioceramics: Past, present and for the future. Journal of the European Ceramic Society 2008;28:1319–27. https://doi.org/10.1016/j.jeurceramsoc.2007.12.001.
30. Goudouri OM, Theodosoglou E, Kontonasaki E, Will J, Chrissafis K, Koidis P, et al. Development of highly porous scaffolds based on bioactive silicates for dental tissue engineering. Materials Research Bulletin 2014;49:399–404. https://doi.org/10.1016/j.materresbull.2013.09.027.
31. Surya Raghavendra S, Jadhav GR, Gathani KM, Kotadia P. BIOCERAMICS IN ENDODONTICS – A REVIEW. J Istanbul Univ Fac Dent 2017;51. https://doi.org/10.17096/jiufd.63659.
32. AbdulQader ST, Kannan TP, Rahman IA, Ismail H, Mahmood Z. Effect of different calcium phosphate scaffold ratios on odontogenic differentiation of human dental pulp cells. Materials Science and Engineering: C 2015;49:225–33. https://doi.org/10.1016/j.msec.2014.12.070.
33. Swarup S, Rao A, Boaz K, Srikant N, Shenoy R. Pulpal Response to Nano Hydroxyapatite, Mineral Trioxide Aggregate and Calcium Hydroxide when Used as a Direct Pulp Capping Agent: An in Vivo study. Journal of Clinical Pediatric Dentistry 2014;38:201–6. https://doi.org/10.17796/jcpd.38.3.83121661121g6773.
34. Hoppe A, Güldal NS, Boccaccini AR. A review of the biological response to ionic dissolution products from bioactive glasses and glass-ceramics. Biomaterials 2011;32:2757–74. https://doi.org/10.1016/j.biomaterials.2011.01.004.
35. Utneja S, Nawal RR, Talwar S, Verma M. Current perspectives of bio-ceramic technology in endodontics: calcium enriched mixture cement - review of its composition, properties and applications. Restor Dent Endod 2015;40:1. https://doi.org/10.5395/rde.2015.40.1.1.
36. Prati C, Gandolfi MG. Calcium silicate bioactive cements: Biological perspectives and clinical applications. Dental Materials 2015;31:351–70. https://doi.org/10.1016/j.dental.2015.01.004.
37. Zhu X, Liu J, Yu Z, Chen C-A, Aksel H, Azim AA, et al. A Miniature Swine Model for Stem Cell-Based *De Novo* Regeneration of Dental Pulp and Dentin-Like Tissue. Tissue Engineering Part C: Methods 2018;24:108–20. https://doi.org/10.1089/ten.tec.2017.0342.
38. Matsui M, Kobayashi T, Tsutsui TW. CD146 positive human dental pulp stem cells promote regeneration of dentin/pulp-like structures. Human Cell 2018;31:127–38. https://doi.org/10.1007/s13577-017-0198-2.
39. Imai M, Hayashi Y. Ultrastructure of wound healing following direct pulp capping with calcium-β-glycerophosphate (Ca-BGP). J Oral Pathology Medicine 1993;22:411–7. https://doi.org/10.1111/j.1600-0714.1993.tb00132.x.

40. Shiehzadeh V, Aghmasheh F, Shiehzadeh F, Joulae M, Kosarieh E, Shiehzadeh F. Healing of large periapical lesions following delivery of dental stem cells with an injectable scaffold: New method and three case reports. Indian J Dent Res 2014;25:248. https://doi.org/10.4103/0970-9290.135937.
41. Jones TD, Kefi A, Sun S, Cho M, Alapati SB. An Optimized Injectable Hydrogel Scaffold Supports Human Dental Pulp Stem Cell Viability and Spreading. Advances in Medicine 2016;2016:1–8. https://doi.org/10.1155/2016/7363579.
42. Cavalcanti BN, Zeitlin BD, Nör JE. A hydrogel scaffold that maintains viability and supports differentiation of dental pulp stem cells. Dental Materials 2013;29:97–102. https://doi.org/10.1016/j.dental.2012.08.002.
43. Bi F, Zhang Z, Guo W. Treated Dentin Matrix in Tissue Regeneration: Recent Advances. Pharmaceutics 2022;15:91. https://doi.org/10.3390/pharmaceutics15010091.
44. Wen B, Huang Y, Qiu T, Huo F, Xie L, Liao L, et al. Reparative Dentin Formation by Dentin Matrix Proteins and Small Extracellular Vesicles. Journal of Endodontics 2021;47:253–62. https://doi.org/10.1016/j.joen.2020.11.017.
45. Casagrande L, Demarco FF, Zhang Z, Araujo FB, Shi S, Nör JE. Dentin-derived BMP-2 and Odontoblast Differentiation 2010;89:603–8. https://doi.org/10.1177/0022034510364487.
46. Luo S, Pei F, Zhang W, Guo W, Li R, He W, et al. Bone marrow mesenchymal stem cells combine with Treated dentin matrix to build biological root. Sci Rep 2017;7:44635. https://doi.org/10.1038/srep44635.
47. Jung C, Kim S, Sun T, Cho Y-B, Song M. Pulp-dentin regeneration: current approaches and challenges. J Tissue Eng 2019;10:204173141881926. https://doi.org/10.1177/2041731418819263.
48. Ballikaya E, Çelebi-Saltik B. Approaches to vital pulp therapies. Aust Endodontic J 2023;49:735–49. https://doi.org/10.1111/aej.12772.