

Regenerative endodontics: A review

Tolga Han Edebal¹, Gülşen Edebal²

¹ Harran University, Faculty of Dentistry, Department of Endodontics, Şanlıurfa, Türkiye

² Harran University, Faculty of Dentistry, Department of Periodontology, Şanlıurfa, Türkiye

Received: 22 October 2025

Accepted: 30 November 2025

Correspondence:

Dr. Tolga Han EDEBAL

Harran University, Faculty of Dentistry,
Department of Endodontics, Şanlıurfa, Türkiye.
E-mail: edebal@harran.edu.tr



How to cite this article:

Edebal TH, Edebal G. Regenerative endodontics: A review. Int Dent Res 2025;15(3):203-221.
<https://doi.org/10.5577/intdentres.585>

Abstract

Oral health and function are critical components of a patient's overall quality of life. Pulp necrosis in immature permanent teeth, resulting from trauma, caries, or developmental anomalies, presents a significant clinical challenge. Traditionally, apexification has been the routine treatment; however, it fails to promote continued root development, often leaving teeth brittle and susceptible to fractures. This review examines regenerative endodontic procedures (REPs), which represent a paradigm shift toward biologically based treatments designed to replace damaged structures and cells of the pulp-dentin complex. The success of REPs relies on the interaction of three key components: stem cells, bioactive molecules (growth factors), and scaffolds. During these procedures, a blood clot is typically induced to act as a scaffold, facilitating the influx of undifferentiated stem cells, such as stem cells of the apical papilla (SCAPs). The review details current clinical protocols recommended by the American Association of Endodontists (AAE) and the European Society of Endodontology (ESE). Key technical aspects include the use of low-concentration sodium hypochlorite (1.5%) to maintain cell viability and EDTA to promote the release of growth factors from the dentin matrix. Intracanal medicaments, specifically triple antibiotic paste (TAP) and calcium hydroxide, are evaluated for their disinfection efficacy and impact on stem cell survival. While REPs demonstrate high success rates in terms of symptom resolution and apical healing, histological evidence suggests the resulting tissue is often a repair (bone or cementum-like) rather than true pulpal regeneration. Despite these challenges, REPs offer a promising therapeutic pathway for increasing root length and wall thickness, ultimately enhancing the long-term retention of immature permanent teeth.

Keywords: Regenerative endodontics, pulp revitalization, immature permanent teeth, Stem Cells of the Apical Papilla, SCAP, tissue engineering, triple antibiotic paste, root development, scaffolds

1. Introduction

Oral health and function are important for health and quality of life (1). Teeth play an important role in chewing, speech, and aesthetics. Tooth loss leads to loss of these functions and problems such as nutritional and joint disorders (2, 3). These conditions can lead to depression and social isolation due to decreased quality of life (2). Preventing tooth loss is becoming increasingly important in society. Dental caries, pulp necrosis, trauma, and periodontal disease cause dental infections that can have serious consequences affecting both the soft and hard tissues of the oral cavity (3).

The main goal of root canal treatment is to prevent or treat apical periodontitis. The treatment of permanent teeth with incomplete root development aims to restore the natural physiological structures and functions of the pulp-dentine complex. However, the treatment of necrotic and root-incomplete teeth has often been a clinical challenge for various reasons (4).

There are two different options for regenerative approaches in endodontics. One treatment option involves a pulp revitalization approach to stimulate tissue regeneration. In this method, new living tissue is expected to form in the cleaned root canal, thereby increasing root length and thickness. The other option is to actively pursue pulp and dentin regeneration using tissue engineering technology to implant or regrow pulp tissue (5).

The progress in regeneration and tissue engineering has been attributed to three key components: stem cells, bioactive molecules, and biomaterials that serve as scaffolds to promote cell growth and differentiation (6). The interaction of these three components is known to promote the migration of more stem cells to the site of injury and the overall regenerative or repair process while enhancing the repair capacity of resident cells in the tissue (7). To increase the efficacy and feasibility of these treatment options, numerous interventional approaches have been developed, primarily cell-free and cell-based approaches. Cell-free approaches aim to harness resident cells, including stem cells, via bioactive molecules embedded in biomaterials or scaffolds to enhance the regenerative process. This phenomenon is also referred to as cell nesting (8). Cell-based approaches, on the other hand, involve the application of exogenously cultured autologous or allogeneic stem cells to the affected tissue to induce regeneration (9).

1.1. Literature review

The literature search for this article was conducted in two databases (PubMed/Medline and Cochrane Library). The literature search included articles containing the keywords "regenerative endodontics," "regeneration," and "revascularization", and the references of these articles.

1.2. Terminology

The terms regenerative endodontics, revascularization, and revitalization are frequently used in the literature. The term revascularization is used in the endodontic literature to refer to the restoration of pulp vascular structure after traumatic injury to immature teeth in which pulpal circulation has been interrupted. Published case reports of this technique have demonstrated continued root growth in infected immature teeth and defined revascularization as the introduction of a blood clot into the root canal by intentional cannulation. Regenerative endodontics refers more to continued root growth and preservation of the pulp-dentine complex. Many studies suggest that the term "repair" should be used instead of "regeneration" because of the presence of various tissues such as dentin, cementum, periodontal ligament, bone, and pulp in teeth treated with regenerative endodontic protocols (REP). The term revitalization has been proposed because it describes not only blood vessels, as in revascularization but also other non-specific tissues (10).

1.3. Regeneration or repair?

Tissue damage caused by infection or trauma heals through regeneration or repair after appropriate treatment. Regeneration is defined as the replacement of damaged tissue with tissue similar to the original tissue and the restoration of biological functions. Repair is the replacement of damaged tissue with tissue different from the original tissue and the loss of biological functions. Pulp cells have limited regenerative potential (10).

There is considerable controversy over the use of the term regeneration. This is because histological evidence indicates that newly formed tissue in REPs does not resemble the lost pulp-dentin complex. Instead, these procedures enable repair through physiological responses. To date, even in advanced animal models, the tissues formed closely resemble the natural pulp, but true odontoblasts are absent; instead, mineralized cells called odontoblast-like cells are present. Therefore, the definition of regeneration is highly dependent on individual differences in its definition and the methodology used to assess its presence and will remain inadequate until more advanced molecular techniques are used. Perhaps a more accurate term to describe this procedure in its current form would be directed endodontic repair (11).

1.4. A brief history

The beginnings of regenerative endodontic treatment of necrotic teeth were first studied in 1961 by Nygaard-Östby, who investigated the potential for repair when bleeding was induced by flood instrumentation in the periapical region prior to root filling. Forty years later, in 2001, Iwaya et al. reported a case report using a

procedure called revascularization on an infected necrotic immature premolar that showed thickening of the root canal walls with continued root maturation and mineralized tissue. Subsequent case reports have also shown this technique to be effective, especially when triple antibiotic paste or calcium hydroxide is used. Other reports have shown successful results in traumatized central incisors. Several authors have described regenerative endodontic techniques as a paradigm shift in the treatment of immature teeth with necrotic pulp (10).

2. Biological basis and histological evaluation of REPs

2.1. The biological basis of REPs

Over the past few decades, advances in tissue engineering have reshaped the way we think about medical treatment and revitalized regenerative medicine. Pulp tissue engineering research involves three key elements: stem cells, growth factors, and scaffolds (12).

2.2. Stem cells

Several types of mature stem cells have been identified. These cells can be induced to differentiate into odontoblast-like cells under certain conditions, indicating their potential for use in REPs. These include dental pulp stem cells (DPSCs), stem cells of the apical papilla (SCAPs), periodontal ligament stem cells (PDLSCs), inflammatory periapical progenitor cells (iPAPCs), and bone marrow stem cells (BMSCs) (12).

2.3. Growth factors

The dentin matrix has been recognized as a reservoir of growth factors that can be released by demineralization of the dentin matrix with bacterial acid, irrigation with sodium hypochlorite (NaOCl) and ethylenediamine-tetraacetic acid (EDTA), stimulation with calcium hydroxide and silica-calcium biomaterials such as MTA and Biodentine®. Furthermore, the blood clot formed during REPs also contains certain growth factors. Dentin-derived growth factors are believed to play an important role in progenitor cell recruitment, proliferation, differentiation, and promotion of tissue regeneration. Transforming growth factor- β 1 (TGF- β 1) and fibroblast growth factor 2 (FGF2) have been associated with cell migration and proliferation. Vascular endothelial growth factor (VEGF) plays an important role in cell proliferation and regulation of angiogenesis, while bone morphogenetic protein (BMP) and FGF2 mediate the signaling in dentin formation. Non-collagenous proteins

(NCPs), such as dentin matrix protein and dentin phosphoprotein, could play a role in odontogenesis (12).

2.4. Scaffolds

Scaffolds are an essential element in tissue engineering to guide stem cell placement and regulate cell proliferation, differentiation, or metabolism. They also help to promote nutrient and gas exchange. Blood clot, autologous platelet concentrates, and synthetic biomaterials can serve as scaffolds for REPs. Among these, blood clots and autologous platelet concentrates are the most commonly used scaffolds during REPs (12).

2.5. Histological evaluation of REPs

Although most case reports and clinical studies proved that root length increased, the root wall thickened, and the size of the apical foramen decreased in immature teeth after REPs, histologic evaluation of teeth treated with REPs showed that the newly formed tissue was ectopic bone, cementum, and periodontal ligament rather than pulp or pulp-like tissue. Animal studies have also confirmed the above results. Immunohistochemical staining results showed that the expression of osteogenic markers was significantly higher than those in mineralized tissue, suggesting that REPs are a repair rather than regeneration (11, 12).

2.6. Why is revascularization therapy necessary?

Immature permanent teeth can lose their vitality, often as a result of trauma, decay, or congenital abnormalities. Apexification therapy has been a routine procedure for decades to treat and preserve such teeth. Apexification is the process of creating a favorable environment within the root canal and periapical tissue to allow the formation of a calcific barrier across the open apex. Calcium hydroxide [$\text{Ca}(\text{OH})_2$] has been the material of choice for apexification since Frank reported in 1966 its capacity to induce physiological closure of the apices of immature nonvital teeth. However, this technique has several disadvantages, including the unpredictability of apical barrier formation and the long treatment time, often requiring many sessions (13). For immature teeth with necrotic pulp, the use of revascularization techniques to induce apexogenesis and, thus, tissue regeneration rather than tissue replacement using artificial substitutes represents a relatively new form of treatment. Revascularization therapy is based on the theory that tissue repair can occur in devitalized, uninfected teeth in the absence of bacteria and in the presence of a suitable three-dimensional scaffold and root/progenitor cells within the root canal cavity and the creation of a bacteria-proof space. Such biologically based therapeutic approaches are particularly valuable for strengthening the dentin walls to support continued root development and increasing the likelihood of long-term retention of the tooth (13).

3. Regeneration of pulpal tissue

The optimal approach to treat immature permanent teeth with necrotic pulp would be to regenerate functional pulpal tissue. Regenerative endodontics has been defined as biologically based procedures designed to replace damaged structures, including dentin and root structures and cells of the pulp-dentin complex. As recently observed, the goal of tissue regeneration (i.e., the formation of new tissue that reproduces both the anatomy and function of the original tissue) differs from that of tissue repair (i.e., the development of a replacement tissue such as scar tissue without restoration of functionality). The concept of pulpal tissue regeneration was introduced by the classic work of Nygaard-Ostby, who evaluated the bleeding effects caused by over-instrumentation of human or canine root canal systems. Unfortunately, histological analysis revealed tissue repair (e.g., fibroblasts, collagen, and sparse vascularity) without histological evidence of regeneration of the pulp-dentin complex. The work of Nygaard-Ostby et al., together with findings in the trauma literature regarding the negative impact of infection on revascularization, contributed to a fundamental shift in the focus of endodontic research from the 1970s onwards, away from the biological regenerative goal and towards a restorative dental philosophy for endodontic treatment, including disinfection and placement of root fillings. During the period 1993-2007, several important publications encouraged the re-emergence of a biological or regenerative approach to endodontic treatment (14).

4. Biological basis of regeneration

Tissue engineering, simply put, is a discipline that focuses on tissue regeneration rather than tissue repair. Figure 1 illustrates the basic principles of tissue engineering, namely that tissue regeneration requires an appropriate source of stem/progenitor cells, growth factors, and scaffolds to control the development of the targeted tissue. The first element of tissue engineering is a source of cells that can differentiate into the desired tissue component. Figure 2 summarizes the various types of stem cells found in the oral and craniofacial regions according to a review by Egusa et al. Detailed reviews summarizing the characteristics of these orofacial stem cells are available (14).

Stem cells are found in the pulp, apical papilla, and even in inflamed periapical tissue (inflamed periapical progenitor cells) collected during endodontic surgical procedures. These findings suggest that there are opportunities for stem cell collection during clinical procedures. Indeed, stimulated bleeding during endodontic regenerative procedures performed on immature teeth with pulp necrosis reveals a large influx of mesenchymal stem cells into the root canal space. Bleeding from the apical papilla in patients contains 400

to 600 times more mesenchymal stem cell markers (CD73 and CD105) compared to the concentrations of these cells circulating in the patient's systemic blood (14).

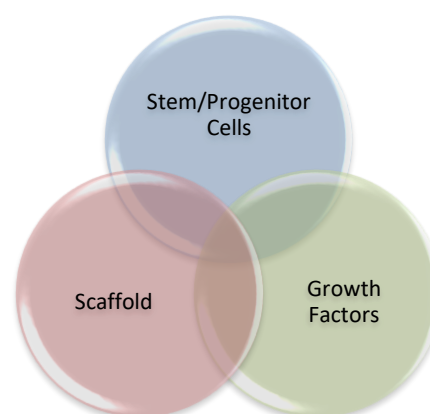


Figure 1. Basic components of tissue engineering.

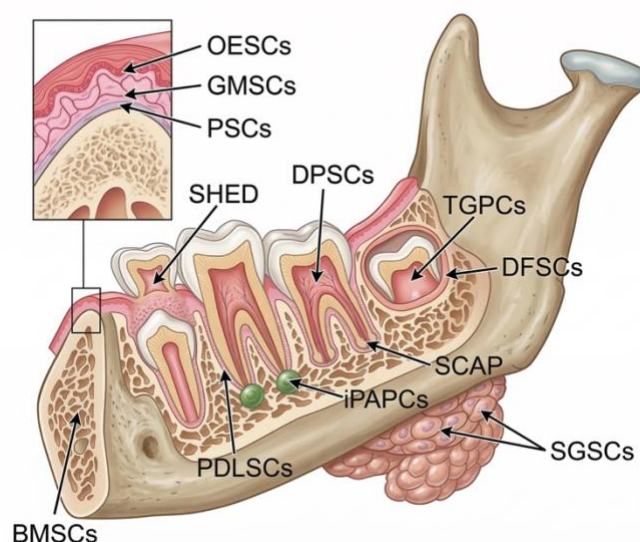


Figure 2. A schematic drawing of potential sources of postnatal stem cells in the oral environment. Cell types include tooth germ progenitor cells (TGPCs), dental follicle stem cells (DFSCs), salivary gland stem cells (SGSCs), stem cells of apical papilla (SCAP), dental pulp stem cells (DPSCs), inflamed periapical progenitor cells (iPAPCs), stem cells from human exfoliated deciduous teeth (SHEDs), periodontal ligament stem cells (PDLSCs), bone marrow stem cells (BMSCs) and oral epithelial stem cells (OESCs), gingival-derived mesenchymal stem cells (GMSCs) and periosteal stem cells (PSCs), as shown in the insert.

5. Clinical problem

Tooth development is a complex and lengthy process of postnatal organogenesis. It takes an additional three years for a tooth to complete its development after eruption, which is seen as the completion of root maturation. Developing teeth are at risk of pulpitis and necrosis due to trauma, caries, and developmental

dental anomalies such as dens evaginatus. In young patients with mixed dentition, the loss of an immature permanent tooth can be devastating. This loss can lead to loss of function, altered development of the maxilla and mandible, phonetic, respiratory and masticatory disorders and, more importantly, can have a severe negative psychosocial impact on young patients. Implants used to compensate for this deficiency are contraindicated in patients with ongoing cranioskeletal development, as their use has been associated with adverse effects on normal orofacial growth (11).

These teeth have been treated in the past with long-term calcium hydroxide therapy or apexification procedures by apical plugging with mineral trioxide aggregate (MTA). Although these treatments usually result in resolution of the signs and symptoms of pathology, they provide little or no benefit for ongoing root development. They can result in thin, brittle dentin walls, exposing the tooth to root fractures and leading to lower survival. A study evaluating root fractures in 885 luxated nonvital incisors after apexification procedures clearly demonstrated that between 28% and 77% of teeth experienced cervical root fractures, depending on the amount of dentin remaining in the tooth. Therefore, clinicians should use all the necessary tools to preserve the natural tooth structure even after the maturation phase during childhood and adolescence. Regenerative therapies should be considered to achieve this goal (11).

6. Etiology of pulp necrosis in immature teeth

The etiologies of necrotic pulp include trauma, caries, and congenital anomalies. Trauma has been recognized as the most common cause of pulp necrosis in immature permanent teeth. Up to 35% of children experience traumatic dental injuries, especially between the ages of 7 and 15 years, when their teeth are in the final stages of root development. Pulp necrosis is likely to be diagnosed in about half of traumatized teeth. A higher incidence of necrosis has been found after severe injuries such as intrusion, avulsion, and combined injuries (11).

The second most common etiology of pulp necrosis in immature teeth is the presence of dens evaginatus or dens evaginatus (approximately 36% of cases treated with REPs). Dens evaginatus is the more common of these two dental anomalies and is the etiology in approximately 33% of cases treated with REPs. On clinical and radiographic examination, it appears as an additional prominence and typically protrudes on the occlusal surface of the lower premolars (most common) or toward the facial or lingual surfaces of the upper anterior teeth (less common). The incidence of dens evaginatus has been reported to affect up to 6% of the population, with a higher incidence in certain ethnic groups. Although the incidence of this dental anomaly is relatively rare, its presence often leads to rapid pulp necrosis when the affected teeth are in functional occlusion, resulting in

direct exposure of the pulp to the oral environment. The subsequent rapid onset of pulpitis and infection-related symptoms forces patients to seek treatment urgently. Early intervention results in good clinical outcomes typically observed in these cases (11).

7. What is regenerative endodontics?

Regenerative endodontic procedures began primarily with revascularization procedures proposed more than fifty years ago (15). Nygaard-Ostby reported that blood clot from root canals through bleeding organized through the gradual transformation of granulation tissue into fibrous connective tissue (15). Ten years later, Nygaard-Ostby and Hjortdal observed cellular cement deposition in cleaned canals when root canals were partially filled with blood through intentional apical bleeding (16).

According to the American Association of Endodontists, the main goal of regenerative treatments is to eliminate symptoms and promote bone healing, as well as to increase dentin thickness and/or root length and achieve a positive response to vitality tests (17).

Regenerative therapies are a valid treatment approach for necrotic immature teeth (open apices) with or without apical periodontitis; this approach continues to achieve high success rates (18-20).

8. Case selection criteria

8.1. Indications

REP is indicated in cases that meet the following criteria:

1. Necrotic permanent teeth with incomplete root formation, regardless of the presence of periradicular lesions;
2. Teeth so destroyed that post-core is required
3. Patients/parents with good compliance;
4. Patients who are not allergic to the drugs or antibiotics used in the procedures.
5. REP is not applicable in certain cases, such as teeth replanted immediately after avulsion, cases with inadequate tooth isolation, teeth with excessive coronal tissue loss requiring post restoration, or teeth with endodontic-periodontal lesions.

8.2. Considerations in case selection

8.2.1. General condition of the patient

General health status should be taken into consideration when making a treatment plan. The American Society of Anesthesiologists (ASA) Health Classification System can be used to assess the physical health status of patients. REPs may be considered for patients classified as ASA 1

and ASA 2. Root canal infection is difficult to control in patients with impaired immune systems, such as uncontrolled diabetes or long-term hormone therapy. REPs are not recommended in patients with uncontrolled hypertension, recent (<3 months) myocardial infarction, cerebrovascular disease or coronary artery disease. Caution should be exercised when performing REP in the following situations: (12)

- 1- Patients with mental health problems, dental phobia or extreme anxiety,
- 2- If patients cannot adapt to multiple visits and longer follow-ups.

8.2.2. Age

More than 90% of patients undergoing REP are under 17 years of age. A clinical trial found that treatment success was higher in the younger patient group (9-13 years) compared to 14-18 years. This suggests that REPs can be administered at any age between 9-18 years, but that younger patients (9-13 years) are better candidates for REPs than older patients (14-18 years). A few studies have reported failure to induce root growth in immature teeth after REPs in patients older than 18 years. This is probably due to the greater healing capacity or regenerative potential of stem cells in younger patients (12).

8.2.3. Root morphology

Research supports the need to consider root morphology in case selection. Recent studies have shown that teeth with larger preoperative apical diameters (≥ 1 mm) have a greater increase in root thickness and length. Remarkably, clinical success was achieved in teeth with apical diameters less than 1 mm after REPs based on clinical and radiographic results of periapical healing and ongoing root development, with the highest clinical success rate of 95.65% in teeth with apical diameters of 0.5-1.0 mm. In general, REPs can be applied to teeth with an apical diameter of 0.24 mm or less within the limitations of the available literature. However, no definitive conclusion has been reached about the correlation between root morphology and the outcome of REPs (12).

8.3. Completion of pre-treatment preparations

8.3.1. Informed consent

For mutual understanding, support, and cooperation, patients, parents, or legal guardians should be provided with detailed information about the current condition of the teeth, regenerative procedures and treatment alternatives.

- The condition of the affected tooth and its effect on treatment.
- Duration, frequency, and follow-up period of treatment.

- Materials and tools used in treatment.
- The possibility of persistent toothache, unhealed or aggravated periapical lesions, and post-treatment stop of root growth
- Treatment alternatives such as apexification or tooth extraction if necessary.
- Possible complications include pain, root canal calcification, and tooth discoloration.
- Treatment costs.

Once the above information has been discussed and fully understood, the Informed Consent Form must be signed before treatment.

8.3.2. General health status

A thorough assessment of a patient's health status, such as the patient's medical condition, anesthesia, and allergic history, should be completed and appropriately documented prior to treatment planning. Ideally, the information should be collected during a consultation appointment before procedures begin, which will help to confirm whether REP is a suitable treatment option.

9. Purpose of regenerative endodontics and clinical approaches

The aim of regenerative endodontics is the regeneration of the pulp-dentin complex in necrotic teeth (21).

Pulp revitalization, which refers to the revascularization of an immature permanent tooth with an infected necrotic pulp that has failed to complete root development and apical periodontitis/abscess, is currently accepted in clinical practice as a regenerative endodontic treatment approach (22). This procedure involves chemical disinfection of the root canal using intracanal medicament and antibiotics, followed by induction of apical hemorrhage to create an environment conducive to regeneration (23). The resulting blood clot acts as a scaffold that facilitates the colonization of stem cells, macrophages, and fibroblasts, while the accumulation of hard tissue continues the development of dentin walls (24).

10. Investigation of agents used in regenerative endodontics

10.1. Platelet Rich Fibrin (PRF)

PRF has been used as an adjunct to biomaterials and natural or synthetic scaffolds to aid the revascularization process. Examples include blood clot and platelet-rich fibrin (PRF) placed in the root canal and agents that promote the slow release of angiogenic cytokines, including fibroblast growth factor (FGF), vascular endothelial growth factor and platelet-derived growth

factor (PDGF) (25, 26). PRF is known to overcome many limitations of Hertwig's epithelial root sheath damage, resulting in impaired or absent root development in immature teeth (27).

10.2. Growth factors and EDTA

Recently, EDTA has been identified as a medicament used to release growth factors favorable for dentin, pulp regeneration. These proteins have been observed in terms of chemotaxis on pulp cells, increased gene expression of odontoblast phenotype, improved mineralization, increased neurogenesis and angiogenesis (28). Researchers have experimented with preparations such as frozen dentin matrix and dentin proteins (29). Based on in vivo studies, Galler et al. proposed a regenerative endodontic approach that can be tested in the clinical setting, which involves root canal preparation and disinfection, irrigation with ethylenediaminetetraacetic acid (EDTA) and EDTA collecting dentin matrix proteins, followed by mixing the collected EDTA with a scaffold material and applying it to the root canal (30).

10.3. Triple antibiotic paste

10.3.1. Why combine antibiotics?

Given the polymicrobial nature of dental infections, antibiotics used alone cannot eliminate all bacteria in the root canal. In addition, the use of non-specific antibiotic therapy can lead to the destruction of the normal bacterial flora and repopulation of the remaining pathogenic microorganisms in the canal. Consequently, it is essential to use a combination of antibiotics against all endodontic pathogens to prevent microbial resistance (31).

10.3.2. Tetracycline

Tetracyclines are a group of broad-spectrum antibiotics, including tetracycline-HCl, minocycline, demeclocycline, and doxycycline, that are effective against a wide range of microorganisms. Tetracycline is in the bacteriostatic subgroup of antibiotics. One of the advantages of this group is that because they do not lysis bacterial cells, they do not generate any antigenic byproducts (such as endotoxins) at the infected site (31). In addition, tetracycline has several unique properties other than its antimicrobial effects, including inhibition of mammalian collagenases that prevent tissue degeneration and inhibition of cells resulting in anti-resorptive activities (32, 33).

In endodontics, tetracyclines have been used to remove the smear layer from instrumented root canal walls, in retrograde cavities during periapical surgical procedures, and as intracanal medication. They are often used in combination with corticosteroids, and these combinations have antibacterial, anti-inflammatory, and also anti-resorptive properties, all of which can help

reduce the inflammatory reaction in the periapical region, including resorption by some cells (32).

10.3.3. Metronidazole

Metronidazole is a nitroimidazole compound with a broad spectrum of activity against protozoa and anaerobic bacteria. It has been widely used in periodontology in both systemic and local forms as it is known for its effective antimicrobial activity against gram-negative and gram-positive bacilli as well as anaerobic cocci. Metronidazole penetrates the cell membranes of bacteria and then binds to their DNA, disrupting the helix structure and causing a very rapid death. Metronidazole has been shown to be effective against anaerobic but not aerobic bacteria and inhibits the growth of all obligate anaerobes tested and is more effective against two strains compared to calcium hydroxide (34, 35).

10.3.4. Ciprofloxacin

Ciprofloxacin is a second-generation fluoroquinolone antibiotic. Its range of action includes *Escherichia coli*, *Legionella pneumophila*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, methicillin-sensitive but not methicillin-resistant *Staphylococcus aureus*. It includes most bacterial pathogens responsible for gastrointestinal, respiratory, urinary tract and abdominal infections, including *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, and *Streptococcus pyogenes*. Ciprofloxacin and other fluoroquinolones are used for this broad spectrum of activity, their availability in both oral and intravenous formulations, and their excellent tissue penetration (36).

10.3.5. Use of triple antibiotic paste in regenerative endodontics

Triple antibiotic paste is used for disinfection of teeth with necrotic infected pulp and creates a favorable environment for tissue regeneration. A retrospective study concluded that regenerative endodontic treatment with triple antibiotic paste and calcium hydroxide had a greater effect on increasing root length than non-surgical root canal treatments or MTA apexification. Compared with calcium hydroxide or formocresol, the percentage of dentin thickness increase was greater when a triple antibiotic paste was applied. In this study, the site of application of calcium hydroxide also affected the result. Calcium hydroxide gave better results when applied to the coronal portion of the root canal than when applied apically (37).

Lovelace et al. demonstrated that the induced bleeding step in regenerative procedures after disinfection with triple antibiotic paste induces the accumulation of undifferentiated stem cells from the periapical region into the root canal (38). These cells participate in the pulp regeneration process after effective disinfection. Therefore, root canals disinfected

with sodium hypochlorite and triple antibiotic paste were significantly less likely to develop periapical lesions and had a higher rate of increase in root length and wall thickness.

10.3.6. Disadvantages of using the triple antibiotic paste

Various methods have been proposed for disinfecting root canals during the revascularization procedure. The American Association of Endodontists recommends using a triple antibiotic paste, a combination of minocycline, ciprofloxacin, and metronidazole, for disinfection (39). The primary disadvantage associated with the triple antibiotic paste is its potential to cause severe discoloration due to minocycline, a tetracycline species (40). Therefore, an alternative known as a dual antibiotic paste containing metronidazole and ciprofloxacin has been introduced. However, there is currently insufficient conclusive evidence to definitively recommend the use of a dual antibiotic patch over a triple antibiotic patch (41, 42).

Several studies and case reports have suggested alternative substances, such as cefaclor to replace the minocycline found in the triple antibiotic patch (43, 44), while others have suggested calcium hydroxide (45) or formocresol as potential alternatives to the triple antibiotic paste (46).

10.4. Calcium hydroxide

Calcium hydroxide forms a strong base (pH 12.6, slightly soluble in water) that is converted to calcium oxide through calcination of calcium carbonate. In contrast, calcium hydroxide is obtained by hydration of calcium oxide, which, when reacted with carbon dioxide, leads to the formation of calcium carbonate (47).

The potential of calcium hydroxide to stimulate the formation of mineralized tissue (hard tissue barrier) by ionic dissociation and its biological mechanism of action was described by Holland (47).

Earlier case reports describing the disinfection protocol in regenerative endodontic treatments included the use of a combination of antibiotics as a root canal medicament (48, 49). However, earlier reports advocated the use of calcium hydroxide alone (50, 51). The vast majority of published cases appear to have used a combination of antibiotics as the preferred in-canal media (52). However, these antibiotic mixtures have been shown to be cytotoxic to SCAP survival in vitro studies at concentrations of 1 mg/mL or higher (53, 54). In contrast, disinfection of the root canal cavity with calcium hydroxide has been shown to promote SCAP proliferation (53, 54). Furthermore, calcium hydroxide has been shown to increase the release of growth factors from dentin, whereas antibiotic mixtures negatively affect growth factor release after the use of EDTA (55, 56).

A retrospective radiographic study using quantitative analysis of teeth treated with regenerative endodontic treatments showed that teeth treated with calcium hydroxide had a significantly greater increase in root length compared to teeth treated with a combined antibiotic mixture (57). More importantly, calcium hydroxide was found to result in a significantly greater increase in the root canal wall thickness of teeth treated with calcium hydroxide compared to teeth treated with the combined antibiotic mixture (57). The administration of calcium hydroxide appears to have positively influenced the outcome of the treatment. As shown radiographically, when calcium hydroxide was confined to the coronal half of the root canal, the median increase in dentin wall thickness was 53.8%. This contrasted with an increase of only 3.3% when calcium hydroxide was also applied to the apical half of the root canal. The percentage change in root length was not affected in any of these different clinical protocols (57). In another study, the results of 12 teeth treated with a triple antibiotic mixture showed apical closure in 66.7% of cases, increased root wall thickness in 41.7%, and increased root length in 41.7%. In contrast, 11 teeth treated with calcium hydroxide showed apical closure in 54.5% of cases, increased root wall thickness in 45.4%, and increased root length in only 27.3% (58).

11. Clinical considerations for regenerative endodontic procedures

In the vast majority of reported cases, REPs were performed in young patients with immature infected teeth in which root maturation had stopped as a result of pulp necrosis. The American Association of Endodontists (AAE) clinical evaluations for regenerative endodontic procedures define success by three criteria (10).

- **Primary goal (essential):** Elimination of symptoms and evidence of bone healing
- **Secondary goal (desirable):** Increased root wall thickness and/or increased root length
- **Tertiary objective:** Positive response to vitality test

Although the primary goal is a goal for all endodontic treatments, in the immature tooth it is the increase in width and/or length that is important. By closing the immature root apex, the incidence of root fracture is reduced. Another assumption in the tertiary goal is that the return of neural capacity may indicate a more organized vital pulp tissue. A 2013 review reported that many cases responded positively to pulp sensitivity testing (10).

11.1. Preparation

During REPs, minimal or no preparation of the canal is advocated. However, in a histologic and histobacteriologic study of a failed REP treatment, it was noted that a bacterial biofilm formed on the canal walls and penetrated the dentinal tubules. Based on the histobacteriologic findings, the authors concluded that some mechanical preparation may also be necessary to disrupt the biofilm on the root canal walls for ongoing root maturation to occur (10).

11.2. Disinfection of the root canal system

Disinfection of the root canal system is considered critical to the success of REPs, as infection inhibits regeneration, repair, and stem cell activity. In the chemical disinfection of the root canal system, it is not only the bactericidal/bacteriostatic properties of the agents that are important; these irrigation solutions must not impair the capacity of the patient's root cells to survive and proliferate. Guidelines recommend copious irrigation with 20 ml of sodium hypochlorite (NaOCl). Closed-tipped and side-perforated needles or EndoVac™ can be used to prevent overflow of irrigation solutions into the periapical space. NaOCl concentrations as low as 1.5% are recommended, followed by irrigation with saline or EDTA (20 mL/channel, 5 min) with the irrigation needle positioned approximately 1 mm from the root apex. Growth factors present in the dentin matrix are released by endodontic irrigating agents. Irrigation of dentin with EDTA promotes the migration and differentiation of pulpal-derived stem cells toward dentin (10).

11.3. Intracanal medicament placement

In 2001, the first report on revascularization of an infected immature tooth used a dual antibiotic paste containing metronidazole and ciprofloxacin. The subsequent report used a combination of metronidazole, ciprofloxacin and minocycline, often referred to as '3mix' or 'triple antibiotic paste (TAP)'. The rationale for the use of TAP was a series of studies of endodontic infections in primary teeth in which a combination of metronidazole, ciprofloxacin, and minocycline at a concentration of 100 µg/mL of each drug completely eliminated culturable bacteria from infected root canals in vitro and in vivo. Triple antibiotic pastes using this combination of antibiotics have been shown to reliably disinfect dentin in infected root canals by diffusion of the drugs through the entire root dentin. The AAE protocol recommends TAP at concentrations no higher than 0.1 mg/mL. At this concentration, TAP is favorable for root cell survival and proliferation and is also effective in eliminating microorganisms in the root canal. The AAE guidelines also recommend calcium hydroxide. The use of calcium hydroxide has been shown to be favorable for SCAP survival and proliferation (10).

11.4. Blood clot formation

After canal disinfection and symptom relief, REPs often involve the use of deliberate over-instrumentation, platelet-rich plasma (PRP) or platelet-rich fibrin (PRF) in periapical tissues to induce bleeding. An important study has shown that the induced bleeding step in regenerative procedures triggers abundant accumulation of undifferentiated stem cells into the canal cavity. Contemporary regenerative endodontics follows bioengineering principles based on the interaction of stem cells, scaffolds, and growth factors (10).

11.5. Effective coronal closure

Once a blood clot or scaffold is placed within the canal, a coronal barrier is placed to prevent coronal leakage. Current protocols recommend carefully placing a pre-prepared Collaplug piece (Zimmer Dental Inc, Warsaw, IN) over the blood clot, which acts as an internal matrix for placement of the MTA after a blood clot is formed, followed by placement of a 3 mm MTA, followed by placement of a 3-4 mm glass ionomer cement (e.g., Fuji IX; GC America, Alsip, IL or other) over the MTA. A composite material (e.g., Z-100; 3M, St Paul, MN, USA, or others) is then placed on top of the glass ionomer. MTA is a biocompatible material with bioactive properties that resist bacterial contamination. Biodentine® (Septodont, Lancaster, PA, USA) can be used as an alternative calcium silicate material (10).

12. Clinical procedures for regeneration/revitalization procedures

12.1. American Association of Endodontists regenerative endodontic procedure (59).

12.1.1. Case selection

- Immature teeth with necrotic pulp.
- Teeth that are not destroyed enough to require post-cores.
- Patient and parent harmony.
- The general systemic condition of the patient is favorable for treatment.
- The patient is ASA-1 or ASA-2.

12.1.2. Informed consent

- Information that the treatment will not end in a single session.
- Knowledge that antibiotics will be used.
- Explaining alternative treatments to the family (Apexification, no treatment, extraction).
- Side effects (coloration of the crown, lack of response to treatment, pain, infection).

12.1.3. First appointment

- Local anesthesia, Rubber Dam isolation, and access cavity.
- Copious, gentle irrigation with 20 ml NaOCl using an irrigation system that minimizes the possibility of irrigants overflowing into the periapical space (e.g., closed-tipped and side-vented needle or EndoVac™).

Lower NaOCl concentrations are recommended; 1.5% NaOCl (20 mL/channel, 5 minutes) followed by saline flushing with the irrigation needle positioned approximately 1 mm from the root tip to minimize cytotoxicity to stem cells in apical tissues. This is followed by irrigation with EDTA (20 mL/canal, 5 minutes).

- The root canals are dried with paper cones.
- Calcium hydroxide or a low-concentration triple antibiotic paste is placed.

If the triple antibiotic paste is used:

1. The pulp chamber must be sealed with a dentin bonding agent (to minimize the risk of staining).
2. Mix 1:1:1 ciprofloxacin: metronidazole: minocycline to a final concentration of 1-5 mg/mL.

Triple antibiotic paste has been associated with tooth discoloration. A double antibiotic paste without minocycline, or using other antibiotics instead of minocycline (e.g., clindamycin, amoxicillin, cefaclor), is a possible alternative to triple antibiotic paste.

- If triple antibiotics are used, keep the paste below the enamel-cement junction (minimize crown discoloration).
- Close the cavity with a 3-4 mm temporary restoration with Cavit™, IRM™, glass ionomer, or other material. The patient is called 1-4 weeks later.

12.1.4. Second appointment

- Assess response to initial treatment. If there are signs/symptoms of infection, an additional session with an antimicrobial or alternative antimicrobial may be performed.
- Anesthesia with 3% mepivacaine without vasoconstrictor, Rubber Dam isolation.
- 20 ml of 17% EDTA with copious, gentle irrigation.
- Dry the canal with paper cones.
- Over instrumentation (root canal file, endodontic explorer) is used to create bleeding in the periapical area (a pre-curved K-type file is used to go beyond the apical foramen by 2 mm, and the entire canal is filled with blood).

An alternative to forming a blood clot is the use of platelet-rich plasma (PRP), platelet-rich fibrin (PRF), or autologous fibrin matrix (AFM).

- Stop the bleeding enough to allow 3-4 mm of restorative material.

- If necessary, place an absorbable matrix such as CollaPlug™, Collacote™, CollaTape™, and white MTA as a closure material on the blood clot.
- 3-4 mm glass ionomer cement (e.g., Fuji IX™, GC America, Alsip, IL) is gently placed over the closure material and light-cured for 40 seconds. The cavity is then closed with a resin-containing material.

12.1.5. Follow-up (6, 12, 24 months)

- Clinical and radiographic examination is performed.
- There should be no pain, soft tissue swelling or sinus tract (most often seen between the first and second appointments).
- Recovery of apical radiolucency (usually observed 6-12 months after treatment).

Increase in the width of the root walls (this is usually observed before the marked increase in root length and often occurs 12-24 months after treatment).

- Increased root length.
- Positive pulp vitality test response.
- Annual follow-up after the first two years is recommended.
- Cone-beam computed tomography (CBCT) is highly recommended for initial assessment and follow-up visits.

12.1.6. Success criteria

The degree of success of Regenerative Endodontic Procedures is largely measured by the extent to which it is possible to achieve the primary, secondary, and tertiary goals:

- **Primary goal:** Elimination of symptoms and evidence of bone healing.
- **Secondary goal:** Increased root wall thickness and/or increased root length (desirable, but perhaps not necessary).
- **Tertiary objective:** Positive response to the viability test (if this is achieved, it may indicate a more organized viable pulp tissue).

12.2. European Association of Endodontics revascularization protocol (59)

12.2.1. Case selection

- A tooth with necrotic pulp and incomplete root formation, with or without periradicular lesions.

- Contraindications:

- Excessive coronal tissue loss (need for post-core).
- Impossibility of adequate dental isolation.
- Immediately after replanting avulsed teeth.

- Patients with ASA III or higher.
- Not recommended in cases of luxation injuries

12.2.2. Informed consent

- Explanation of the pathology present.
- Explanation of procedure advantages and uncertainties.
- Duration of treatment and follow-ups.
- Use of materials and medicines.
Alternatives (MTA apexification, no treatment, extraction, and autotransplantation).
- Possible consequences and risks: (pain, tooth discoloration)

12.2.3. First appointment

- Clinical and radiographic evaluation is performed.
- Detection, local anesthesia (optional), isolation, and disinfection (e.g., with iso-Betadine) are performed.
- The access cavity is opened.
- Necrotic pulp tissue is removed using appropriate endodontic instruments.
- Mechanical instrumentation of the root canal walls is avoided.
- Wash with 1.5-3% sodium hypochlorite (20 mL, 5 minutes),
- Use a side-vented needle, and insert 2 mm above the apical (this can be checked using an operation microscope or by the patient reporting pain). The choice of sodium hypochlorite concentration depends on the balance between adequate disinfection and tissue protection.
- Prolonged irrigation may be required in the presence of bleeding or exudate (control with paper cones).
- Irrigate with sterile physiological saline (5 mL) to minimize the cytotoxic effects of sodium hypochlorite on tissues.
- Dry the root canal with paper cones.
- Irrigate with 20 mL 17% EDTA.
- The calcium hydroxide is placed homogeneously in the root canal.
- Place the minimum amount of filling material, according to the chosen material, directly on the intracanal dressing. The patient is scheduled for an appointment for 2-4 weeks later.

In most published case reports, antibiotics, mainly a triple-antibiotic paste containing ciprofloxacin, metronidazole, and minocycline, have yielded good results. Disadvantages such as discoloration, cytotoxicity, sensitivity, development of resistance, and difficulty in removal from the root canal need to be taken into account. Recent publications advocate the use of calcium hydroxide.

12.2.4. Second appointment (after 2-4 weeks)

- Symptoms are checked.
- Replace calcium hydroxide if signs of inflammation have not subsided. Systemic antibiotics may be considered if the patient reports general health changes such as fever or dysphagia (see the European Academy of Pediatric Dentistry/EAPD recommendations for more information).
- Cleaning of the treatment environment, anesthesia, and rubber dam isolation are performed. The chosen anesthetic should have optimal bone penetration. Current guidelines recommend the use of non-vasoconstrictor anesthetics, but blood clot formation is often prevented by patients' pain sensation. Experience from the first visit regarding patient compliance, anxiety, and pain control should be taken into account.
- The temporary filling is removed.
- Irrigate with 17% EDTA (20 mL, 5 min), use a side-vented needle, and place 2 mm above the apical.
- To reduce the negative effects of irrigation solutions on target cells, irrigation with sterile physiologic saline (5 mL) is performed.
- Excess irrigation solution is dried with paper points.
- Bleeding is induced by mechanically irritating the periapical tissue and rotating a pre-curved file (e.g., Hedström size 40) in the apical direction.
- Allow the canal to fill with blood up to 2 mm below the gum margin and wait 15 minutes for a blood clot to form.
- Cut a collagen matrix e.g., Parasorb Cone (Resorba Medical GmbH, Nuremberg, Germany), Collaplug (Integra LifeSciences Corp., Princeton, NJ, USA) or Hemocollagene (Septodont, Saint-Maur-des-Fossés, France) to a diameter larger than coronal. Place 2-3 mm above the blood clot, ensuring the matrix is wetted with blood to prevent gap formation.
- A thin homogeneous layer of approximately 2 mm of MTA or tricalcium silicate cement is placed on the collagen matrix, and care is taken to ensure that the material does not come into contact with blood.
- A flowable, light-curing glass ionomer or calcium hydroxide cement is applied.
- The cavity walls are cleaned by diamond bur or sandblasting (aluminum oxide).
- The cavity is closed with resin materials.

12.2.5. Follow-up

- Follow-ups should be performed after 6, 12, 18, and 24 months, then annually for five years.

- In cases where long-standing infection and inflammation symptoms cannot be eliminated, a 3-month follow-up is recommended.
- Clinical and radiographic diagnoses should be made according to the checklist.
- In the case of planned orthodontic treatment, it should be taken into account that inflammation and apical root resorption may be more pronounced after revitalization of the teeth. Therefore, bone healing should be awaited, teeth after revitalization should be excluded from orthodontic treatment, or follow-up intervals during orthodontic treatment should be shortened.

12.2.6. Success criteria

- There should be no pain.
- There should be no signs and symptoms of inflammation.
- The pre-existing periapical lesion should heal.
- Increasing root thickness and length.
- Absence of external root resorption.
- There must be a positive response to the vitality test.
- Patient satisfaction must be ensured.
- No tooth coloration should be seen
- Radiographic detection of a new PDL along the root canal

13. Clinical practice procedure

13.1. Case selection

Case selection is very important in regenerative treatments because choosing a patient who is not an appropriate candidate for this treatment is likely to result in failure. Teeth with necrotic pulp and coronal restorations that do not require post-core applications should be selected for this treatment. Patient compliance is very important as multiple sessions are required. Case reports often describe very young healthy patients (6-18 years of age) as having greater

regenerative potential, greater ability to heal, and greater amounts of stem cells (60, 61).

The likelihood of success in teeth with an uncovered root apex may depend on how large the apical opening is: The larger it is, the more likely it is that vessels and stem cells will colonize the root canal (62). It has been recognized that short and open roots are more conducive to tissue ingrowth, and it has also been suggested that the amount of SCAP may be higher in a short and open apex (63). The classification of root apices according to their stage of maturation was proposed by Cvek, as shown in Figure 3 (12).

13.2. Informed consent form

This document should contain all the informative material needed to make an informed decision. It should explain that the treatment will take place over two or more appointments with the need for frequent follow-up, the allergenic potential of the antimicrobials used, and other adverse reactions such as tooth discoloration, non-healing, pain, and infection. Informed consent should also explain other feasible alternative treatments such as apexification, extraction or no treatment at all. If clinicians wish to add a case report to the regenerative database, they should also obtain the necessary parental/guardian consent for underage patients Table 1).

Table 1. Requirements for informed consent according to ESE.	
Explanation of the existing pathology	Explanation of the advantages and uncertainties of the action to be taken
Treatment and follow-up process	Materials and medicines to be used
Alternatives (MTA apexification, no treatment, extraction, and autotransplantation)	Possible consequences and risks (pain, tooth discoloration)

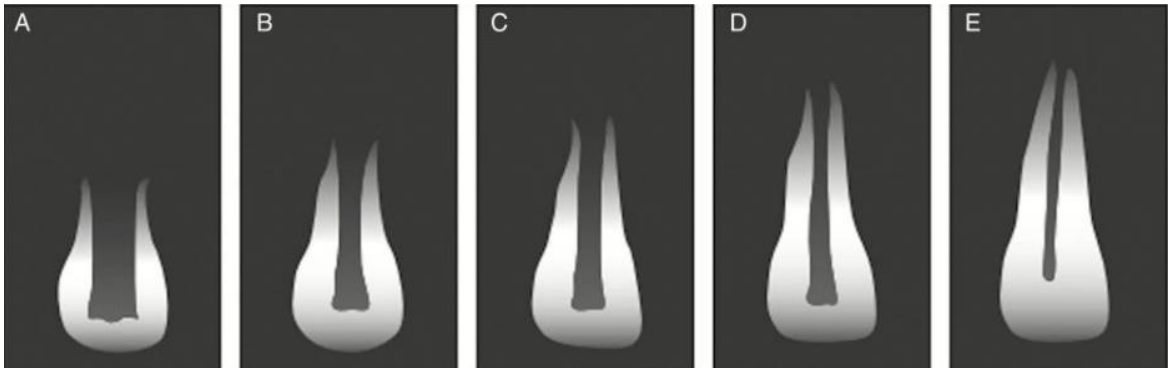


Figure 3. Cvek root tip classification: Stages of root development. A, B, C, D, and E indicate stages 1, 2, 3, 4, and 5, respectively; 1-4 = immature teeth, 5 = mature (12).

13.3. First appointment

In all cases, local anesthesia should be administered, rubber dam isolation should be provided, and the access cavity should be opened under magnification and illumination.

If slight resistance is felt when inserting a file after opening the access cavity, or if the patient reports pain, the presence of remaining vital tissues should be considered, and an apexogenesis procedure should be performed, according to Jung et al (64).

13.4. Irrigation

Irrigation with minimal or no preparation will minimize the possibility of sodium hypochlorite overflow from the apical region. Irrigation should be copious and gentle. Sodium hypochlorite (NaOCl) is the most commonly used irrigation solution, available at concentrations ranging from 1% to 6%. Some authorities have found experimental results suggesting that when lower concentrations of NaOCl are used, the viability of the existing cell population is maintained, and treatment outcome is improved (65), and that a lower concentration will prevent further damage in case of sodium hypochlorite flooding (63).

In another study, Trevino et al. concluded that SCAP survival rate was 74% after irrigation with 6% NaOCl, 17% EDTA, followed by another irrigation with 6% NaOCl (66).

13.5. Use of antimicrobial agents

The most common intra-canal antibiotic application is triple antibiotic paste. Equal amounts of ciprofloxacin, metronidazole, and minocycline should be mixed with saline to obtain a paste-like mixture (67). Some authors have also reported that ciprofloxacin 200 mg, metronidazole 500 mg, minocycline 100 mg, and a carrier such as macrogol ointment or propylene glycol make triple antibiotic paste easier to apply (68). This paste is placed 1-2 mm short of the apex with a lentulo or syringe-type carrier, and then the canal opening is closed with a moist cotton pellet. If a triple antibiotic paste is used, the pulp chamber can be sealed with a dentin bonding agent or flowable composite prior to application to prevent coronal discoloration (69, 70).

Another commonly used medication, calcium hydroxide [$\text{Ca}(\text{OH})_2$], is a root canal disinfectant and hard tissue repair stimulant (71). Given its high pH, this medication can damage cells in the apical papilla and periapical tissues, which are essential for the repair process (49, 72), and can lead to uncontrolled root canal calcification. It may also limit apical bleeding in the second session. To avoid this negative effect, calcium hydroxide should only be applied to the coronal half of the root canal (72, 73). It should not be applied with a lentulo, but a syringe-type carrier should be used instead.

13.6. Temporary coronal restoration

After administration of antimicrobial agents, the access cavity should be closed with a 3-4 mm temporary filling material such as Cavit (63).

13.7. Second appointment

If the patient has symptoms or signs of infection that do not improve, an additional dressing session with the same or a different antimicrobial agent can be performed (64). If there are no symptoms or infection, the next stage of treatment can be started (74).

Anesthetics without vasoconstrictors should be preferred, considering the possibility that vasoconstrictors in local anesthetics may complicate deliberate apical bleeding (75, 76).

13.8. Irrigation

After rubber dam isolation and removal of the temporary restoration, the root canal should be irrigated with saline to remove the dressing material from the root canal.

Current literature recommends the use of EDTA at a concentration of 17% at this stage (77). EDTA releases growth factors in the dentinal tubules (78-80) and, with its chelating properties, removes the smear layer in the root canal, exposing the collagen fibers in the dentin (80).

13.9. Apical bleeding

The periapical area is bled with materials such as a sterile file or explorer to ensure blood flow into the root canal (81). It takes about 15 minutes for the blood to clot and stabilize under the enamel-cement junction, and pressure is applied into the canal with a sterile cotton pellet soaked in sterile saline solution (82). If bleeding does not occur, 17% EDTA may need to be applied to the canal instrument to prevent blood clotting in the apical area (81). If there is still no bleeding, it is possible to draw blood from other canals with a syringe and apply it to the necrotic canal if the procedure is being performed on a tooth with more than one root (83). If the other canals are also necrotic or the tooth is single-rooted, the patient's own blood can be taken from the vein and injected into the canal. (74). At this point, it has been advocated to use collagen as a barrier over the pulp chamber to achieve a tight seal (75).

13.10. Sealing

The literature recommends using a bacteria-proof closure material on top of the barrier material to achieve the desired seal. Silicate cement, white MTA, Biodentin, or gray MTA can be used for this purpose. It is important to note that the closure material should always be applied 2-3 mm below the enamel-cement junction. If MTA is used, adhesive restoration should not be performed on the same day, but if Biodentin is used,

adhesive restoration can be performed after a 12-minute waiting period. (63, 85). The schematic principle of revascularization in immature permanent teeth with

necrotic pulp and its representation are shown in Figures 4 and 5, respectively.

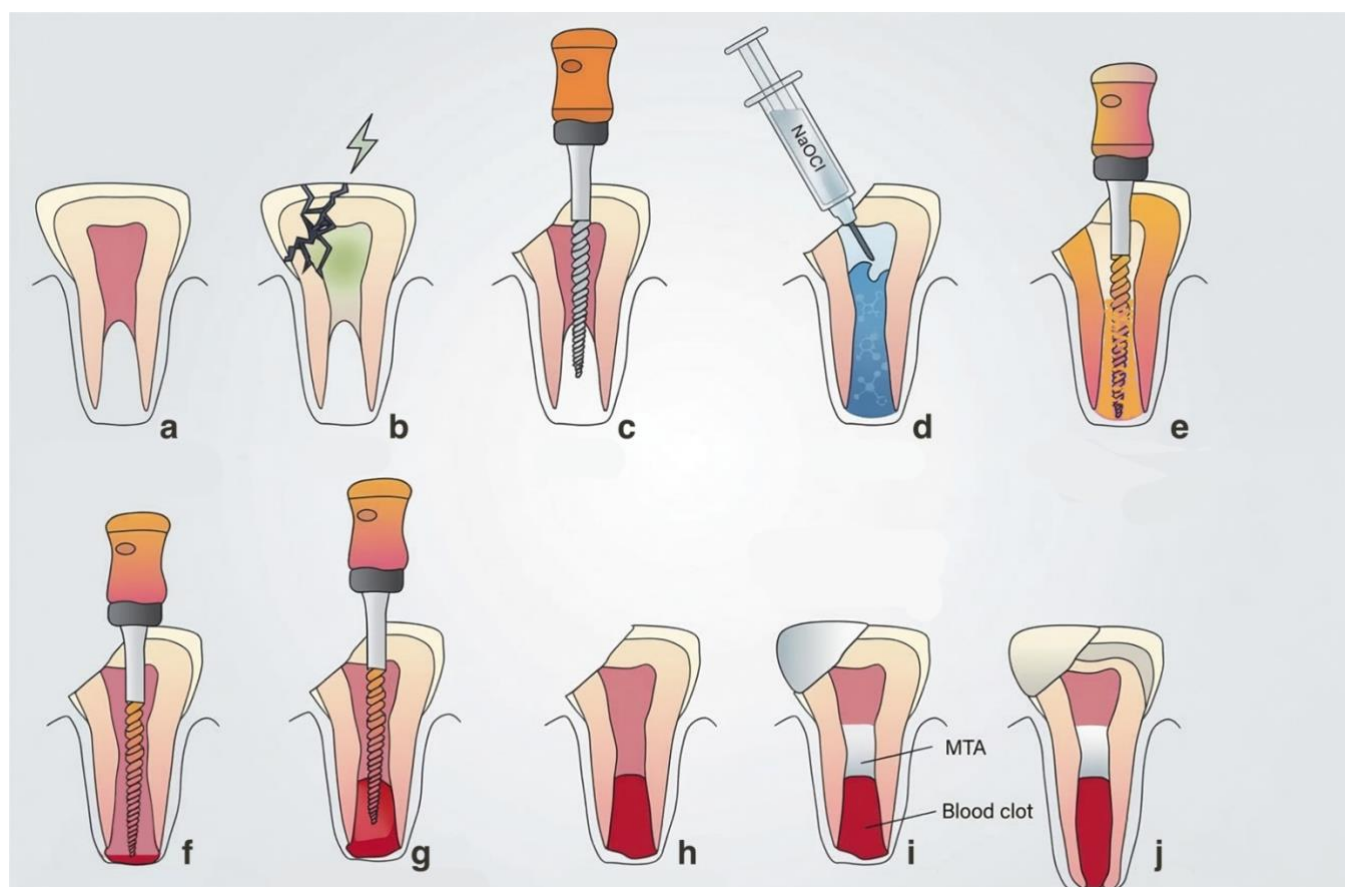


Figure 4. Schematic principle of revascularization of immature permanent teeth with necrotic pulp. (a) Immature permanent tooth, (b) immature permanent tooth with necrotic pulp, (c) pulp removal, (d) disinfection, (e) triple antibiotic paste, (f) apical bleeding by overflow, (g) initial bleeding, (h) blood clot formation, (i) MTA, (j) continued root growth (76).

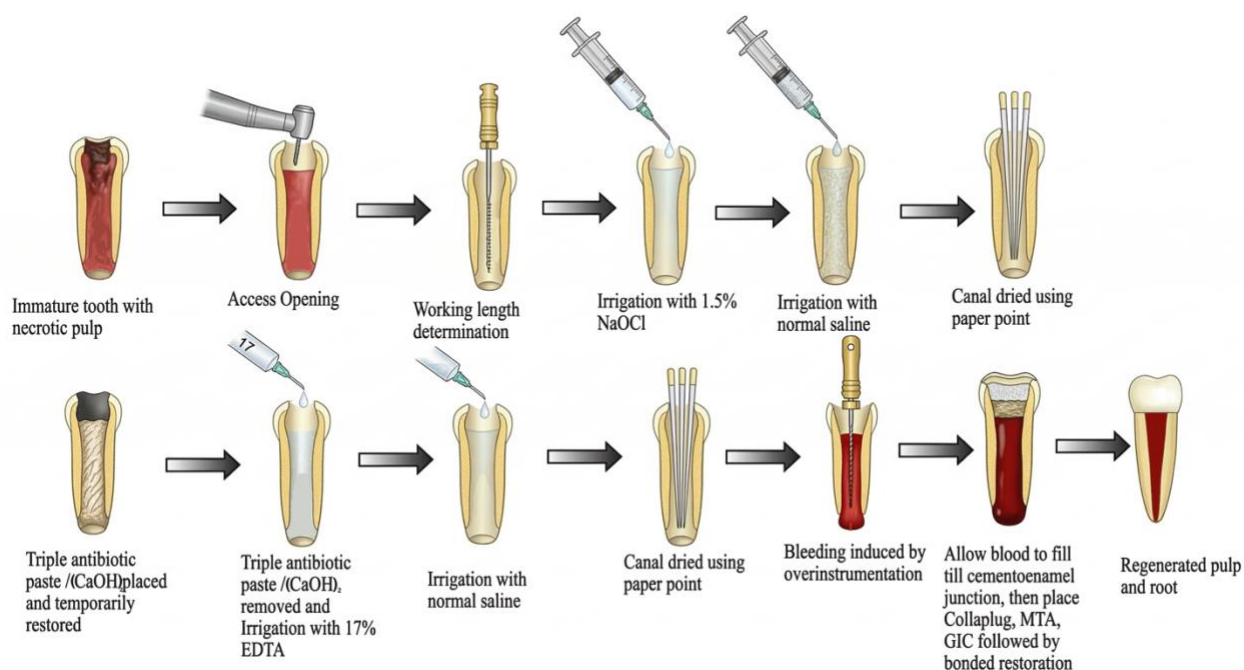


Figure 5. Schematic representation of revascularization (84).

13.11. Follow-up

Regular follow-up sessions should be scheduled for clinical and radiographic evaluation. Some authors have advocated follow-up sessions every 3 months (43), while others have recommended sessions at 3, 6, 12, and 18 months (83).

14. Outcomes of regenerative endodontics

Evaluation of the Outcomes of Regenerative Therapies

There are guidelines from ESE and AAE to assess the effectiveness of REPs.

ESE has defined a set of success criteria for REPs, including absence of inflammation, healing of a pre-existing lesion in the periapical tissue, increased root length and wall thickness, absence of external inflammatory resorption, positive response to vitality tests, radiographic detection of new PDL along the inner wall of the root canal, and absence of tooth discoloration.

The AAE categorizes the success criteria for REPs as primary, secondary, and tertiary objectives.

- The primary goal is symptom relief and bone healing.
- The secondary goal is increased root wall thickness and/or root length, which is desirable but not mandatory.
- The tertiary goal is a positive response to a vitality test, which may indicate more organized vital pulp tissue.

These criteria in both guidelines point to an ideal prognosis that includes the clinical appearance of the teeth and imaging diagnostics, along with the subjective feelings of the patient. However, the aforementioned goals are almost impossible to achieve simultaneously in the clinical scenario today (12).

15. Causes of treatment failures

REPs have been reported to achieve a high treatment success rate of 83.3%–100%. The main reasons for treatment failure are infection and root resorption after treatment. The basic principle of these treatments, minimal mechanical preparation, can retain bacterial biofilm in the dentinal tubules and cause persistent infection in the root canal system. Another cause of recurrent infection is the lack of adequate coronal sealing. This not only allows bacteria from the oral flora to enter the root canal system and cause recurrent infection but also impairs the proliferation and differentiation of root cells, leading to inhibition of

healing of periapical lesions and root development. Other factors, such as inadequate removal of carious tissue, separation of the provisional restoration, or tooth fractures between appointments, also compromise the coronal seal (12).

15.1. Primary treatment goal

The primary goal for a clinician should be to ensure the healing of diseased tissues, the prevention of disease recurrence, and patient comfort. Therefore, the primary therapeutic goal of REPs should be the healing of apical periodontitis while promoting tooth survival and function (11).

15.2. Secondary treatment goal

A secondary therapeutic goal of REPs is continued root development. A retrospective study conducted in 2009 was the first to use an objective, dependent measure of root development to compare radiographic root development between apexification procedures and REPs. These findings were confirmed by Jeeruphan et al. using a similar quantitative technique and reported that teeth treated with revascularization showed a significantly greater increase in root length (14.9%) compared to teeth treated with MTA apexification (6.1%) or calcium hydroxide apexification (0.4%) (11).

15.3. Tertiary treatment goal

Regaining pulp vitality has been reported in approximately 50% of all published REPs. The presence of functional nociceptors following REPs suggests that afferents are directed by specific chemical signals to the canal of apically positioned free nerve endings. Perhaps this axonal routing process is similar to the targeting of axons from trigeminal sensory neurons that accumulate, forming a plexus under the tooth organ and tooth follicle, but do not enter the apical papilla during the late bell stage and the eruption process. During tooth development, undifferentiated cells of the apical papilla or stem cells of the apical papilla are thought to provide axonal transmission to the developing tooth (11).

15.4. Success criteria

The degree of success for this protocol is analyzed under three goals.

- The primary goal is symptom relief and healing of the periapical tissues.
- The secondary goal is the continuation of root development. Realization of the secondary goal is desirable but not essential.
- The tertiary goal is a positive response to vitality tests. This represents the highest level of success (63).

16. Complications

16.1. Pain

Pain can occur during or after REPs. Prevention and management of pain are important to improve the patient's experience of dental treatment. Due to poor control of the working length, pain may occur during treatment due to stimulation of the periapical tissues during irrigation. To avoid this, the working length should be verified at the beginning of treatment and carefully maintained throughout the entire process. During root canal irrigation, gentle procedures should be performed, and the tip of the irrigation needle should be kept at least 1 mm above the apical to minimize the risk of extending beyond the apical foramen and damaging the periapical tissues. Furthermore, given the impaired anesthetic effect using local anesthetic agents without decongestants, drugs with good permeability, such as 3% mepivacaine, can be used to improve pain control during treatment. Residual infection in the root canal or re-infection of the tooth can cause pain between appointments. Therefore, during the entire treatment process, the treated tooth should be well isolated with a rubber dam (12).

If an emergency or post-treatment pain occurs between appointments, appropriate treatment should be given after careful investigation to find the reason. In patients with severe pain, drainage, and root canal disinfection should be performed again to control the infection and relieve associated symptoms. If pain persists or worsens, alternative treatments such as apexification or tooth extraction should be considered according to the individual situation and the patient's demand (12).

16.2. Tooth discoloration

It has been reported in the literature that crown discoloration is a common complication of REPs and can significantly affect patient satisfaction and quality of life. Patients expect both aesthetic effect and successful treatment outcomes, especially in anterior teeth. Therefore, procedures that may cause discoloration should be avoided as much as possible (12).

After the procedures, different stages of treatment can take place, such as tooth discoloration, the use of a triple antibiotic paste, during induction of bleeding, or placement of barrier materials (12).

16.3. Intracanal calcifications

Intracanal calcification is also a common problem in teeth treated with REPs and was defined as revascularization-associated intracanal calcification (RAIC) by Song et al. in 2017. The researchers detected RAIC in 62.1% of revascularization cases and found that calcification was progressive over time. Calcifications in root canals were then divided into two types: calcific

barrier (CB) or canal obliteration (CO). The former is mainly found in the middle third and leaves the rest of the canal cavity visible on radiographs, while the latter implies complete obliteration of the root canal lumen (12).

16.4. Limitations of regenerative endodontic treatments

While regenerative endodontics is a great way to ensure the survival of immature necrotic teeth, it has limitations, such as the risk of crown staining caused by substances such as TAP/minocycline and MTA. Clinicians need to have sufficient knowledge to deal with these technical challenges.

The use of local anesthetics containing epinephrine is a cause of inadequate apical bleeding; this can be addressed by alternatively using mepivacaine (3%) before the procedure.

An important limitation of REP is the lack of SCAP viability. Pre-existing periapical lesions can damage SCAP, and similarly, high concentrations of NaOCl and the use of intracanal medications can also impair SCAP (84).

17. Conclusion

Regenerative endodontics represents a new era in biological and clinical endodontics. Nowadays, this biologically based treatment is considered the first treatment option for immature teeth with pulp necrosis based on the success of many cases published in the literature. REPs have shown a positive effect on the relief of signs/symptoms and the continuation of root growth in immature permanent teeth with pulp necrosis. However, the unpredictable root maturation of REPs is a major concern raised in the current literature, and evidence suggests that true regeneration of the pulp-dentin complex does not occur after REPs.

In recent years, stem cell transplantation and cell homing techniques have received considerable attention for their potential to achieve pulpal regeneration. Conventional regenerative treatments to treat permanent teeth with necrotic pulp and incomplete root development result in the regaining of pulp vitality, but with more root fractures than in natural teeth. For regenerative endodontic treatments, the completion of root formation is a clinically favorable situation for patients. This depends on root canal disinfection during regenerative endodontic treatments, placement of a matrix in the canal for cell migration into the root canal, and bacterial-proof sealing of the pulp chamber. Pulp stem cells artificially introduced into the root canal are far from clinical reality, so we need to rely on cell migration and learn how signaling molecules work.

Disclosures

Peer-review: Externally peer-reviewed.

Author Contributions: Conception - T.H.E.; Design - T.H.E.; Supervision - G.E.; Materials - T.H.E., G.E.; Data Collection and/or Processing - T.H.E.; Analysis and/or Interpretation - T.H.E.; Literature Review - T.H.E., G.E.; Writer - T.H.E.; Critical Review - G.E.

Conflict of Interest: No conflict of interest was declared by the authors.

Funding: The authors declared that this study has received no financial support.

References

- Friedman PK, Lamster IB. Tooth loss as a predictor of shortened longevity: exploring the hypothesis. *Periodontol* 2000. 2016;72:142-152. <https://doi.org/10.1111/prd.12128>
- Friedman PK, Kaufman LB, Karpas SL. Oral health disparity in older adults: dental decay and tooth loss. *Dent Clin North Am*. 2014;58:757-770. <https://doi.org/10.1016/j.cden.2014.06.004>
- Ahmadi H, Ebrahimi A, Ahmadi F. Antibiotic Therapy in Dentistry. *Int. J Dent*. 2021;6667624. <https://doi.org/10.1155/2021/6667624>
- Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. *Dent Traumatol* 2002;18:134e7. <https://doi.org/10.1034/j.1600-9657.2002.00097.x>
- Aksel H, Serper, A. Recent considerations in regenerative endodontic treatment approaches. *J Dent Sci*. 2014;9(3):207-213. <https://doi.org/10.1016/j.jds.2013.12.007>
- Granz CL, Gorji A, Granz CL, et al. Dental stem cells: The role of biomaterials and scaffolds in developing novel therapeutic strategies. *World J Stem Cells*. 2020;12:897-922. <https://doi.org/10.4252/wjsc.v12.i9.897>
- Tollemar V, Collier ZJ, Mohammed MK, et al. ScienceDirect stem cells, growth factors and scaffolds in craniofacial regenerative medicine. *Genes Dis*. 2016;3:56-71. <https://doi.org/10.1016/j.gendis.2015.09.004>
- Tirez E, Pedano MS. Regeneration of the pulp tissue: cell homing versus cell transplantation approach: a systematic review. *Materials (Basel)* 2022;15(23):8603. <https://doi.org/10.3390/ma15238603>
- El-Kadiy AEH, Rafei M, Shammaa R. Cell therapy: types, regulation, and clinical benefits. *Front Med (Lausanne)* 2021; 8:1-24. <https://doi.org/10.3389/fmed.2021.756029>
- Lin LM, Kahler B. A review of regenerative endodontics: current protocols and future directions. *J Istanbul Univ Fac Dent*. 2017;51(3 Suppl 1):S41-S51. <https://doi.org/10.17096/jiufd.53911>
- Diogenes A, Ruparel NB. Regenerative Endodontic Procedures: Clinical Outcomes. *Dent Clin North Am*. 2017; 61(1):111-125. <https://doi.org/10.1016/j.cden.2016.08.004>
- Wei X, Yang M, Yue L, Huang D, Zhou X, Wang X, et al. J. Expert consensus on regenerative endodontic procedures. *Int J Oral Sci*. 2022;14(1):55. <https://doi.org/10.1038/s41368-022-00206-z>
- Bezgin T, Sonmez H. Review of current concepts of revascularization/revitalization. *Dent Traumatol*. 2015; 31(4):267-73. <https://doi.org/10.1111/edt.12177>
- Hargreaves KM, Diogenes A, Teixeira FB. Treatment options: biological basis of regenerative endodontic procedures. *J Endod*. 2013;39(3 Suppl):S30-43. <https://doi.org/10.1016/j.joen.2012.11.025>
- Ostby BN. The role of the blood clot in endodontic therapy. An experimental histologic study. *Acta Odontol Scand* 1961; 19:324-53. <https://doi.org/10.3109/00016356109043395>
- Nygaard-Ostby B, Hjortdal O. Tissue formation in the root canal following pulp removal. *Eur J Oral Sci* 1971;79:333-49. <https://doi.org/10.1111/j.1600-0722.1971.tb02019.x>
- Valsan D, Pulyodan M, Mohan S, Divakar N, Moyin S, Thayyil S. Regenerative endodontics: a paradigm shift in clinical endodontics. *J Pharm Bioall Sci*. 2020;12(5):20. https://doi.org/10.4103/jpbs.JPBS_112_20
- Ding RY, Cheung GS, Chen J, et al. Pulp revascularization of immature teeth with apical periodontitis: a clinical study. *J Endod* 2009;35:745-9. <https://doi.org/10.1016/j.joen.2009.02.009>
- Silujjai J, Linsuwanont P. Treatment outcomes of apexification or revascularization in nonvital immature permanent teeth: a retrospective study. *J Endod*. 2017;43: 238-45. <https://doi.org/10.1016/j.joen.2016.10.030>
- Arsalan H, Ahmed HMA, Şahin Y, Yıldız, ED, Gündoğdu EC, Güven Y, Khalilov R. Regenerative endodontic procedures in necrotic mature teeth with periapical radiolucencies: a preliminary randomized clinical study. *J Endod*. 2019;45(7): 863-872. <https://doi.org/10.1016/j.joen.2019.04.005>
- Wei X, Yang M, Yue L, et al. Expert consensus on regenerative endodontic procedures. *Int J Oral Sci*. 2022;14:55. <https://doi.org/10.1038/s41368-022-00206-z>
- Araújo PR de S, Silva LB, Neto AP dos S, et al. Pulp revascularization: a literature review. *Open Dent J*. 2017;10: 48-56. <https://doi.org/10.2174/1874210601711010048>
- Namour M, Theys S. Pulp revascularization of immature permanent teeth: a review of the literature and a proposal of a new clinical protocol. *Sci. World J*. 2014;2014. <https://doi.org/10.1155/2014/737503>
- Altaii M, Richards L, Rossi-Fedele G. Histological assessment of regenerative endodontic treatment in animal studies with different scaffolds: a systematic review. *Dent Traumatol*. 2017;33:235-244. <https://doi.org/10.1111/edt.12338>
- Wikström A, Brundin M, Lopes MF, et al. What is the best long-term treatment modality for immature permanent teeth with pulp necrosis and apical periodontitis? *Eur Arch Paediatr Dent*. 2021;22:311-340. <https://doi.org/10.1007/s40368-020-00575-1>
- Arshad S, Tehreem F, Rehab Khan M, et al. Platelet-rich fibrin used in regenerative endodontics and dentistry: current uses, limitations, and future recommendations for application. *Int J Dent*. 2021;2021:4514598. <https://doi.org/10.1155/2021/4514598>
- Rizk HM, Salah Al-Deen MS, Emam AA. Pulp revascularization/revitalization of bilateral upper necrotic immature permanent central incisors with blood clot vs platelet-rich fibrin scaffolds-a split-mouth double-blind randomized controlled trial. *Int J Clin Pediatr Dent*. 2020;13: 337-343. <https://doi.org/10.5005/jp-journals-10005-1788>
- Widbiller M, Eidt A, Lindner SR, et al. Dentine matrix proteins: isolation and effects on human pulp cells. *Int Endod J*. 2018;51:e278-e290. <https://doi.org/10.1111/iej.12754>
- Wang F, Xie C, Ren N, et al. Human freeze-dried dentin matrix as a biologically active scaffold for tooth tissue engineering. *J Endod*. 2019;45:1321-1331. <https://doi.org/10.1016/j.joen.2019.08.006>
- Galler KM, Widbiller M. Cell-free approaches for dental pulp tissue engineering. *J Endod*. 2020;46:S143-S149. <https://doi.org/10.1016/j.joen.2020.06.034>
- Abbott PV. Selective and intelligent use of antibiotics in endodontics. *Aust Endod J*. 2000;26(1):30-9. <https://doi.org/10.1111/j.1747-4477.2000.tb00149.x>
- Mohammadi Z, Abbott PV. On the local applications of antibiotics and antibiotic-based agents in endodontics and

- dental traumatology. *Int Endod J.* 2009;42(7):555-67. <https://doi.org/10.1111/j.1365-2591.2009.01564.x>
33. Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, et al. A new solution for the removal of the smear layer. *J Endod.* 2003;29(3):170-5. <https://doi.org/10.1097/00004770-200303000-00002>
 34. Greenstein G. The role of metronidazole in the treatment of periodontal diseases. *J Periodontol.* 1993;64(1):1-15. <https://doi.org/10.1902/jop.1993.64.1.1>
 35. Siqueira JF Jr, de Uzeda M. Intracanal medicaments: evaluation of the antibacterial effects of chlorhexidine, metronidazole, and calcium hydroxide associated with three vehicles. *J Endod.* 1997;23(3):167-9. [https://doi.org/10.1016/S0099-2399\(97\)80268-3](https://doi.org/10.1016/S0099-2399(97)80268-3)
 36. Mohammadi Z, Jafarzadeh H, Shalavi S, Yaripour S, Sharifi F, Kinoshita JI. A Review on Triple Antibiotic Paste as a Suitable Material Used in Regenerative Endodontics. *Iran Endod. J.* 2018;13(1):1-6.
 37. Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B. Regenerative endodontics: a comprehensive review. *Int Endod J.* 2018;51(12):1367-1388. <https://doi.org/10.1111/iej.12954>
 38. 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. *J Endod.* 2011;37(2):133-8. <https://doi.org/10.1016/j.joen.2010.10.009>
 39. Gharechahi M, Kafi MH, Javidi M, Jafari N, Joibary Moghaddam M. The Efficacy of Different Antibiotic Compounds in Regenerative Treatment of Immature Necrotic Teeth. *Front Dent.* 2024;2(21):1. <https://doi.org/10.18502/fid.v2i1i.14638>
 40. Sabrah AHA, Al-Asmar AA, Alsolihat F, Al-Zer H. The discoloration effect of diluted minocycline containing triple antibiotic gel used in revascularization. *J Dent Sci.* 2020;15(2):181-185. <https://doi.org/10.1016/j.jds.2019.06.005>
 41. Sabrah AH, Yassen GH, Gregory RL. Effectiveness of antibiotic medicaments against biofilm formation of *Enterococcus faecalis* and *Porphyromonas gingivalis*. *J Endod.* 2013;39(11):1385-9. <https://doi.org/10.1016/j.joen.2013.05.003>
 42. Devaraj S, Jagannathan N, Neelakantan P. Antibiofilm efficacy of photoactivated curcumin, triple and double antibiotic paste, 2% chlorhexidine and calcium hydroxide against *Enterococcus faecalis* in vitro. *Sci Rep.* 2016;6:2479. <https://doi.org/10.1038/srep24797>
 43. Thibodeau B, Trope M. Pulp revascularization of a necrotic infected immature permanent tooth: case report and review of the literature. *Pediatr Dent.* 2007;29(1):47-50
 44. Bezgin T, Yilmaz AD, Celik BN, Kolsuz ME, Sonmez H. Efficacy of platelet-rich plasma as a scaffold in regenerative endodontic treatment. *J Endod.* 2015;41(1):36-44. <https://doi.org/10.1016/j.joen.2014.10.004>
 45. Chueh LH, Ho YC, Kuo TC, Lai WH, Chen YH, Chiang CP. Regenerative endodontic treatment for necrotic immature permanent teeth. *J Endod.* 2009;35(2):160-4. <https://doi.org/10.1016/j.joen.2008.10.019>
 46. Shah N, Logani A, Bhaskar U, Aggarwal V. Efficacy of revascularization to induce apexification/apexogenesis in infected, nonvital, immature teeth: a pilot clinical study. *J Endod.* 2008;34(8):919. <https://doi.org/10.1016/j.joen.2008.05.001>
 47. Estrela C, Cintra LTA, Duarte MAH, Rossi-Fedele G, Gavin G, Sousa-Neto MD. Mechanism of action of bioactive endodontic materials. *Braz Dent J.* 2023;34(1):1-11. <https://doi.org/10.1590/0103-6440202305278>
 48. Iwaya S, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. *Dent. Traumatol.* 2001;17:185-187. <https://doi.org/10.1034/j.1600-9657.2001.017004185.x>
 49. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: New treatment protocol? *J Endod.* 2004;30:196-200. <https://doi.org/10.1097/00004770-200404000-00003>
 50. Cotti E, Mereu M, Lusso D. Regenerative treatment of an immature, traumatized tooth with apical periodontitis: Report of a case. *J Endod.* 2008;34:611-616. <https://doi.org/10.1016/j.joen.2008.02.029>
 51. Chueh LH, Huang TJ. Immature teeth with periradicular periodontitis or abscess undergoing apexogenesis: A paradigm shift. *J Endod.* 2006;32:1205-1213. <https://doi.org/10.1016/j.joen.2006.07.010>
 52. Kontakiotis EG, Filippatos CG, Tzanetakis GN, Agrafioti A. Regenerative endodontic therapy: A data analysis of clinical protocols. *J Endod.* 2015;41:146-154. <https://doi.org/10.1016/j.joen.2014.08.003>
 53. Ruparel NB, Teixeira FB, Ferraz CC, Diogenes A. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. *J Endod.* 2012;38:1372-1375. <https://doi.org/10.1016/j.joen.2012.06.018>
 54. Althumairy RI, Teixeira FB, Diogenes A. Effect of dentin conditioning with intracanal medicaments on survival of stem cells of apical papilla. *J Endod.* 2014;40:521-525. <https://doi.org/10.1016/j.joen.2013.11.008>
 55. Galler KM, Buchalla W, Hiller KA, Federlin M, Eidt A, Schiefersteiner M, Schmalz G. Influence of root canal disinfectants on growth factor release from dentin. *J Endod.* 2015;41:363-368. <https://doi.org/10.1016/j.joen.2014.11.021>
 56. Galler K, Widbiller M, Buchalla W, Eidt A, Hiller K, Hoffer P, et al. EDTA conditioning of dentine promotes adhesion, migration and differentiation of dental pulp stem cells. *Int. Endod J.* 2015;49(6):581-590. <https://doi.org/10.1111/iej.12492>
 57. Bose R, Nummikosi P, Hargreaves KM. A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. *J Endod.* 2009;35:1343-1349. <https://doi.org/10.1016/j.joen.2009.06.021>
 58. Nagata JY, de Almeida Gomes BPF, Lima TFR, Murakami LS, de Faria DE, Campos GR, de Souza-Filho FJ, de Jesus Soares A. Traumatized immature teeth treated with 2 protocols of pulp revascularization. *J Endod.* 2014;40:606-612. <https://doi.org/10.1016/j.joen.2014.01.032>
 59. Staffoli S, Plotino G, Nunez Torrijos BG, Grande NM, Bossù M, Gambarini G, Polimeni A. Regenerative Endodontic Procedures Using Contemporary Endodontic Materials. *Materials (Basel).* 2019;19:12(6):908. <https://doi.org/10.3390/ma12060908>
 60. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, Huang GT. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: A pilot study. *J Endod.* 2008;34:166-171. <https://doi.org/10.1016/j.joen.2007.11.021>
 61. Murray PE, Stanley HR, Matthews JB, Sloan AJ, Smith AJ. Age-related odontometric changes of human teeth. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 2002;93:474-482. <https://doi.org/10.1067/moe.2002.120974>
 62. Kim JY, Xin X, Moiola EK, Chung J, Lee CH, Chen M, et al. Regeneration of dental-pulp-like tissue by chemotaxis-induced cell homing. *Tissue Eng Part A.* 2010;16:3023-31. <https://doi.org/10.1089/ten.tea.2010.0181>
 63. Staffoli S, Plotino G, Nunez Torrijos BG, Grande NM, Bossù M, Gambarini G, Polimeni A. Regenerative endodontic procedures using contemporary endodontic materials. *Materials.* 2019;12(6):908. <https://doi.org/10.3390/ma12060908>
 64. Jung IY, Lee SJ, Hargreaves KM. Biologically based treatment of immature permanent teeth with pulpal necrosis: A case series. *J Endod.* 2008;34:876-887. <https://doi.org/10.1016/j.joen.2008.03.023>
 65. Essner MD, Javed A, Eleazer PD. Effect of sodium hypochlorite on human pulp cells: An in vitro study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2011;112: 662-666. <https://doi.org/10.1016/j.tripleo.2011.04.030>
 66. Trevino EG, Patwardhan AN, Henry MA, Perry G, Dybdal-Hargreaves N, Hargreaves KM, Diogenes A. Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. *J Endod.* 2011;37:1109-1115. <https://doi.org/10.1016/j.joen.2011.05.013>
 67. Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, Iwaku M. In-vitro antibacterial susceptibility of bacteria

- taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. *Int Endod J*. 1996;29:125-130.
<https://doi.org/10.1111/j.1365-2591.1996.tb01173.x>
68. Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E. Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. *Int Endod J*. 1996;29:118-124.
<https://doi.org/10.1111/j.1365-2591.1996.tb01172.x>
 69. Kim J, Kim Y, Shin S, Park J, Jung I. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: A case report. *J Endod*. 2010;36:1086-1091.
<https://doi.org/10.1016/j.joen.2010.03.031>
 70. Reynolds K, Johnson JD, Cohenca N. Pulp revascularization of necrotic bilateral bicuspid using a modified novel technique to eliminate potential coronal discoloration: A case report. *Int Endod J*. 2009;42:84-92.
<https://doi.org/10.1111/j.1365-2591.2008.01467.x>
 71. Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. *Int Endod J*. 2011;44:697-730.
<https://doi.org/10.1111/j.1365-2591.2011.01886.x>
 72. Jadhav G, Shah N, Logani A. Revascularization with and without platelet-rich plasma in nonvital, immature, anterior teeth: A pilot clinical study. *J Endod*. 2012;38:1581-1587.
<https://doi.org/10.1016/j.joen.2012.09.010>
 73. Huang GT. A paradigm shift in endodontic management of immature teeth: Conservation of stem cells for regeneration. *J Dent*. 2008;36:379-386.
<https://doi.org/10.1016/j.jdent.2008.03.002>
 74. Geisler TM. Clinical considerations for regenerative endodontic procedures. *Dent Clin N Am*. 2012;56:603-626.
<https://doi.org/10.1016/j.cden.2012.05.010>
 75. Petrino JA, Boda KK, Shambarger S, Bowles WR, McClanahan SB. Challenges in regenerative endodontics: A case series. *J Endod*. 2010;36:536-541.
<https://doi.org/10.1016/j.joen.2009.10.006>
 76. Conde MCM, Chisini LA, Sarkis-Onofre R, Schuch HS, Nör JE, Demarco FF. A scoping review of root canal revascularization: relevant aspects for clinical success and tissue formation. *Int Endod J*. 2017;50(9):860-874.
<https://doi.org/10.1111/iej.12711>
 77. Chanda PM, Hegde KS, Bhat SS, Sargod SS, Mantha S, Chattopadhyay S. Tissue engineering in endodontics: Root canal revascularization. *J Clin Pediatr Dent*. 2014;38:291-297.
<https://doi.org/10.17796/jcpd.38.4.j5285857278615r1>
 78. Bègue-Kirn C, Smith AJ, Ruch JV, Wozney JM, Purchio A, Hartmann D, Lesot H. Effects of dentin proteins, transforming growth factor beta 1 (TGF beta 1) and bone morphogenetic protein 2 (BMP2) on the differentiation of odontoblast in vitro. *Int J Dev Biol*. 1992;36:491-503.
 79. Zhao S, Sloan AJ, Murray PE, Lumley PJ, Smith AJ. Ultrastructural localization of TGF beta exposure in dentine by chemical treatment. *Histochem J*. 2000;32:489-494.
<https://doi.org/10.1023/A:1004100518245>
 80. Galler KM, D'Souza RN, Federlin M, Cavender AC, Hartgerink JD, Hecker S, Schmalz G. Dentin conditioning codetermines cell fate in regenerative endodontics. *J Endod*. 2011;37:1536-1541.
<https://doi.org/10.1016/j.joen.2011.08.027>
 81. Law AS. Considerations for regeneration procedures. *Pediatr Dent*. 2013;35:141-152.
<https://doi.org/10.1016/j.joen.2012.11.019>
 82. Wigler R, Kaufman AY, Lin S, Steinbock N, Hazan-Molina H, Torneck CD. Revascularization: A treatment for permanent teeth with necrotic pulp and incomplete root development. *J Endod*. 2013;39:319-326.
<https://doi.org/10.1016/j.joen.2012.11.014>
 83. Nosrat A, Seifi A, Asgary S. Regenerative endodontic treatment (revascularization) for necrotic immature permanent molars: A review and report of two cases with a new biomaterial. *J Endod*. 2011;37:562-567.
<https://doi.org/10.1016/j.joen.2011.01.011>
 84. Hameed MH, Gul M, Ghafoor R, Badar SB. Management of immature necrotic permanent teeth with regenerative endodontic procedures - a review of literature. *J Pak Med Assoc*. 2019;69(10):1514-1520.
 85. Galler KM, Krastl G, Simon S, Van Gorp G, Meschi N, Vahedi B, Lambrechts P. European Society of Endodontology position statement: Revitalization procedures. *Int Endod J*. 2016;49:717-723.
<https://doi.org/10.1111/iej.12629>