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Pulp Revascularization /Regeneration

This research is submitted as part of the requirements for the degree of Bachelor of Dental Surgery (BDS), College of Dentistry, AL Mustaqbal University

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Quotation

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﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ﴾

صدق الله العلي العظيم

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Dedication

This research is dedicated to my family, friends, and teachers for their continuous support and encouragement throughout my academic journey.

Acknowledgement

I would like to express my sincere gratitude to my supervisor Dr Alaa Hashim, lecturers, and all staff members who guided and supported me throughout this research. Special thanks to my family and friends for their encouragement and understanding during the course of my studies.

abstract

Background

Pulp revascularization and regenerative endodontic procedures (REPs) have emerged as innovative biological approaches for the management of immature permanent teeth with necrotic pulp. Traditional treatment modalities, such as apexification, primarily aim to achieve apical closure without promoting continued root development, often resulting in structurally compromised teeth with thin dentinal walls and increased fracture risk. In contrast, regenerative endodontics focuses on restoring the vitality of the pulp–dentin complex and enabling physiological root maturation. These procedures are based on tissue engineering principles involving the interaction of stem cells, scaffolds, and signaling molecules, with stem cells from the apical papilla playing a key role due to their regenerative potential.

Discussion

Pulp revascularization relies on the induction of intracanal bleeding to form a blood clot that acts as a natural scaffold, facilitating cell migration and the release of growth factors necessary for tissue regeneration. The success of this approach depends on effective yet biologically compatible disinfection strategies, including minimal mechanical instrumentation and the use of low-concentration irrigants to preserve stem cell viability. Intracanal medicaments such as calcium hydroxide or antibiotic pastes are used to control infection while maintaining a favorable regenerative environment. Clinical outcomes have shown promising results, including healing of periapical lesions, continued root development, and increased dentinal wall thickness. However, limitations persist, such as variability in treatment protocols, possible tooth discoloration, and the unpredictable nature of the regenerated tissue, which may not fully replicate the original pulp histologically.

Conclusion

Pulp revascularization represents a significant advancement in regenerative endodontics, providing a biologically based alternative to conventional apexification. Although clinical outcomes are encouraging, further research is required to standardize treatment protocols and to better understand the long-term behavior and histological nature of regenerated tissues.

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Chapter one

Introduction

Introduction

Traumatic dental injuries (TDIs) represent a significant global health concern and account for approximately 5% of all injuries for which individuals seek medical or dental treatment. These injuries occur most frequently among young children, particularly preschool and school-aged children, as well as young adults, due to increased physical activity and exposure to accidents during these development stages(Petersson, Andersson and Sörensen, 1997; Andreasen, Andreasen and Andersson, 2018)**.** Trauma affecting permanent teeth during their developmental stages can have serious consequences, particularly when it involves immature teeth with incomplete root formation. Traumatic injury to an immature permanent tooth may result in pulpal necrosis and interruption of normal root development. When root development is arrested, several unfavorable anatomical and structural outcomes may occur, including an inadequate crown-to-root ratio, thin and fragile root canal walls, an increased susceptibility to root fracture, and the presence of an open or blunderbuss apex (Alrahabi and Ali, 2014)**.** An open apex may also develop as a result of pulpal necrosis caused by extensive dental caries before the completion of root formation (Jyothi, 2012)**.** In addition, open apices may occur in fully developed teeth due to pathological conditions such as extensive apical resorption following orthodontic treatment, inflammatory root resorption, or periradicular surgical procedures. The management of permanent anterior teeth with necrotic pulp, with or without apical closure, presents a considerable clinical challenge for endodontists. Conventional endodontic treatment relies on chemomechanical preparation of the root canal system to eliminate infection; however, this approach becomes particularly complex in immature teeth with open apices. The thin dentinal walls of such teeth increase the risk of root fracture during instrumentation, while the wide apical opening complicates adequate canal

obturation and raises the risk of extrusion of filling materials into the periapical tissues (Narayan *et al.*, 2018)**.** Incomplete root development is commonly attributed to trauma or severe infection capable of disrupting pulpal blood supply and destroying the cells responsible for continued dentin deposition. As a result, root maturation is halted, leaving the tooth structurally compromised and functionally vulnerable (Friedlander, Cullinan and Love, 2009)**.** Traditionally, apexification has been the primary treatment modality for immature teeth with necrotic pulp. Apexification aims to induce apical closure by forming a calcified barrier at the root apex, either through long-term application of calcium hydroxide or the placement of a mineral trioxide aggregate (MTA) apical barrier, thereby allowing subsequent obturation of the root canal system (Rafter, 2005)**.** In contrast, apexogenesis is a biologically based treatment strategy aimed at preserving vital pulp tissue in immature teeth to enable continued physiological root development and apical closure. This approach emphasizes the importance of maintaining pulpal vitality to allow ongoing dentin deposition and strengthening of root canal walls (Moleri, Moreira and Rabello, 2011)**.** Although apexogenesis is traditionally indicated for teeth with vital pulp tissue, emerging evidence suggests that regenerative approaches may also be applicable in non-vital teeth. Pulp revascularization, also referred to as regenerative endodontic treatment, represents a paradigm shift in the management of immature teeth with necrotic pulp. This biologically driven procedure focuses on thorough disinfection of the root canal system followed by the induction of bleeding into the canal space to facilitate the formation of new tissue. Standard disinfection protocols typically include irrigation with sodium hypochlorite (NaOCl) and the use of an intracanal medicament composed of a combination of antibiotics, such as ciprofloxacin, metronidazole, and minocycline, commonly known as triple antibiotic paste (Nosrat, Seifi and Asgary, 2011a)**.**

Aim of this study

To review and evaluate the concept, biological basis, and clinical outcomes of pulp revascularization as a regenerative endodontic approach for the management of immature permanent teeth with necrotic pulp.

Chapter two

Literature Review

Literature review

2.1 The Revascularization / Regeneration Concept

Two possible approaches are described to explain the pulpal tissue regeneration. The first being revascularization that is defined as the invagination of undifferentiated periodontal cells from the apical region in immature teeth. Hence, a new pulp tissue is expected to grow into the root canals from the remaining tissues that exist apically in the root canal (Banchs and Trope, 2004)**.**The second concept explains the replacement of the diseased pulp with a healthy tissue that is able to revitalize the tooth and thereby, restore formation of dentin. The stem cell therapy, gene therapy, three-dimensional (3D) cell printing, scaffold implantation, and pulp implantation are suggested for this approach. (Murray, Garcia-Godoy and Hargreaves, 2007; Pannu, 2017) In the procedure of pulp revascularization of a necrotic tooth induction of blood clot which consists of cross-linked fibrin act as a scaffold. It serves as a pathway for the migration of regenerative cells into the root canal space. These cells differentiate into odontoblasts and deposit hard tissue both at the apical end and lateral root walls. (Sonoyama *et al.*, 2008a)**.** Pulp revascularization and regeneration represent a paradigm shift in endodontic therapy, moving beyond traditional approaches that rely on mechanical debridement and inert filling materials to treat necrotic or non-vital teeth. Rather than merely eliminating infection and sealing the root canal system, regenerative endodontic procedures (REPs) aim to biologically restore lost or damaged pulp-dentin tissues, preserve vitality, and enable continued functional development of the tooth structure (Kyaw *et al.*, 2025)**.** This treatment modality is grounded in the principles of tissue engineering, incorporating stem cells, scaffold materials, and signaling molecules to facilitate the reconstruction of a living, vascularized tissue within the root canal space (Abdellatif *et al.*, 2024)**.** The conceptual

foundation of revascularization originated from early experiments demonstrating that inducing bleeding into a disinfected root canal could introduce living cells and blood components capable of promoting tissue healing (Ariwala and Calcuttawala, 2025). Building on this, revascularization protocols expanded to include strategies that encourage the recruitment of endogenous stem cells from apical tissues, such as the apical papilla and dental pulp stem cells, to migrate into the canal and differentiate into cells capable of forming vascularized tissue (Kyaw *et al.*, 2025). This approach seeks not only to control infection and induce repair but to recreate a biologically functional pulp-dentin complex that restores sensory, immune, and reparative capabilities to the treated tooth(Kim and Kim, 2025; Srinivasan, Suresh and Cadambi, 2025).

2.1.1 How does revascularization happen?

There are a number of theories that explain the revascularization mechanism. The periapical region of immature teeth presents multipotent periodontal cells with great potential for differentiating into new fibroblasts and cementoblasts(Saad, 1988). So, it has been suggested that differentiated cementoblasts and fibroblasts are responsible for increasing dentinal walls and apical closure(Shah *et al.*, 2008). Another hypothesis suggests that residual multipotent stem cells from pulp tissue may be abundant in young, immature teeth, adhering to dentinal walls to generate *odontoblast-like* cells for root-end development(Saad, 1988). A third possibility involves the ingrowth of stem cells from apical papilla that could proliferate inside root canals through the blood induction of periapical tissues, since these cells have high proliferative capacity, probably being transported inside root canals in association with bleeding induced from the periapical tissue(Gronthos *et al.*, 2000a).

In addition to the abovementioned hypothesis, various growth factors incorporated in the blood clot and/ or dentin may play an important role in the cell proliferation inside the root canal space(Lieberman and Trowbridge, 1983). Finally, the root anatomy of immature teeth (e.g. presenting open apex, wide root canal and thin radicular dentin walls) may favor the communication of canal space and periodontal tissue to achieve apical healing with periodontal tissue. With regard to the apical opening, revascularization seems to be more predictable when the apical diameter is greater than 1 mm and is unlikely to occur in apical openings narrower than 0.3 mm25. This type of repair was previously described in classic studies with dogs that induced the formation of blood clots in immature teeth filled beyond the apex foramen, resulting in the ingrowth of periodontal tissue repair in the region(BN, 1961). However, in immature teeth, the mechanical removal of microorganisms is not recommended due to the fragility of the thin root walls, requiring a decontamination restricted to the use of irrigant solutions and intracanal medication(Lovelace *et al.*, 2011).

2.2 Indications

Pulp revascularization and regeneration are advanced endodontic procedures primarily indicated for treating immature necrotic teeth. These techniques are used when traditional methods such as apexification are not sufficient. Below are the primary indications:

2.2.1 Immature Necrotic Teeth:

Pulp revascularization/regeneration is essential for immature teeth with incomplete root development, especially in adolescents and young adults. Traditional treatments like apexification only form an apical barrier but do not promote continued root development or thickening of the dentinal walls. Unlike apexification, regenerative procedures allow for further root lengthening and

dentinal wall thickening, while also restoring pulp vitality and strengthening the tooth structure (Kyaw *et al.*, 2025)**.**

2.2.2 Traumatic Dental Injuries:

Traumatic injuries leading to pulp necrosis, particularly in teeth with open apices, are another common indication. Injuries such as falls or impacts can disrupt the pulp's blood supply, causing necrosis. Regenerative endodontics aims to re-establish vascularity within the root canal, helping to reform functional pulp tissue and contribute to the long-term resilience and viability of the tooth (Ariwala and Calcuttawala, 2025; Srinivasan, Suresh and Cadambi, 2025)**.**

2.2.3 Persistent Apical Periodontitis:

Persistent apical periodontitis in necrotic teeth that does not respond to conventional treatments may also require regenerative procedures. When bacterial infection persists, regenerative techniques can help eliminate pathogens and promote tissue regeneration. This approach becomes particularly necessary when conventional retreatment is not viable or fails to resolve symptoms(Dos Reis-Prado *et al.*, 2024)**.**

2.2.4 Root Maturation in Patients with Large Periapical Lesions:

Regenerative treatments are indicated for root maturation in patients with large periapical lesions associated with immature infected teeth. These lesions create challenges due to the instability of the tooth structure. Regenerative techniques encourage continued root development and dentinal wall thickening, improving the mechanical strength of the tooth and reducing the risk of fractures (Kim *et al.*, 2018; Kyaw *et al.*, 2025)**.**

2.2.5Tooth Preservation:

Regeneration is particularly valuable for tooth preservation, especially when significant pulp tissue has been lost but the apical region remains intact. In such cases, regenerative treatments can restore pulp functionality and prevent the need for tooth extraction. This is especially crucial for young patients, where maintaining tooth structure is important for both functional and aesthetic reasons (Ariwala and Calcuttawala, 2025)**.**

2.3. Stem cells dental origin

2.3.1. Dental pulp stem cells *(DPSCs).*

Gronthos et al. first isolated DPSCs from adult human dental pulp (Fig. 1A) (Gronthos *et al.*, 2000b). DPSCs are found to generate tissue resembling dental pulp, surrounding it with odontoblast-like cells and a dentin-like structure in vivo. DPSCs have self-renewal capability, multi-lineage differentiation potential, and clonogenic efficiency (Gronthos *et al.*, 2002). Classical MSC makers have been identified in DPSCs, such as CD105, CD73, CD90(Svandova *et al.*, 2023). They are further marked by neurovascular-associated makers, such as CD31, p75, Snail-1 and -2, and SOX-1 (Mattei *et al.*, 2015; Hu *et al.*, 2020). Moreover, various subpopulations of DPSCs may have diverse and complementary functions in facilitating repair and regeneration within the dental pulp. Ishizaka et al. (Ishizaka *et al.*, 2013) transplanted dental pulp CD31(□) side population (SP) cells with tooth root subcutaneously into SCID mice and found that CD31□ SP cells expressed higher levels of angiogenic and neurotrophic factors such as RECA1 and PGP9.5, displayed enhanced migration activity, and produced neurite outgrowth, making them a promising choice for cell therapy and regenerative applications. Additionally, the combination of DPSCs with growth factors enhances tissue regeneration more effectively. A study isolated highly angiogenic, neurogenic, and

regenerative modified DPSCs (MDPSCs) subpopulation using granulocyte-colony stimulating factor (G-CSF). In vivo experiments have demonstrated that MDPSCs could promote the formation of blood vessels and dental pulp tissue (Murakami *et al.*, 2013). The formation of regenerative dentin is crucial in the process of dental pulp regeneration. A pilot clinical study transplanted MDPSCs with G-CSF into teeth with irreversible pulpitis. Through electric pulp test and magnetic resonance imaging, the regenerated pulp exhibited good function and structure. At the same time, cone beam computed tomography (CBCT) demonstrated the formation of functional dentin (Nakashima *et al.*, 2017). These studies suggested that human MDPSCs hold promise for complete pulp regeneration in humans. In addition, DPSCs are considered more suitable for dental tissue regeneration and neurodegenerative disease applications (Ellis *et al.*, 2014).

2.3.2 Stem cells from human exfoliated deciduous teeth (SHEDs)

SHEDs are extracted from the pulp of human deciduous primary teeth (Fig. 1A). Miura et al. [(Miura *et al.*, 2003)] first discovered SHEDs and found that they were highly proliferative and capable of differentiating into various cell types, including neural cells, adipocytes, and odontoblasts [(Wu *et al.*, 2021)]. Both SHED and DPSCs share a phenotypic profile of MSCs and express multiple conventional MSC markers, like CD44, CD73, CD90, CD166 (Govindasamy *et al.*, 2010). In dentistry, when SHEDs are injected with Puramatrix™ or rhCollagen type I into human root canals and subsequently implanted into immunodeficient mice, SHEDs developed pulp-like tissues with functional odontoblasts capable of producing new dentin within the root canals [(Rosa *et al.*, 2013)]. This suggests that SHEDs, combined with appropriate scaffolds, have the potential to promote pulp-like tissue regeneration and root formation. Likewise, exosomes derived from SHED aggregates (SA-Exo), promoted angiogenesis for dental pulp regeneration in vivo

[(Wu *et al.*, 2021)], and in vitro experiments demonstrated that it enhanced SHEDs endothelial differentiation and angiogenic abilities by regulating the TGF-β/SMAD2/3 signaling pathway. In addition, Xuan et al. (Xuan *et al.*, 2018) implanted autologous pulp stem cells from exfoliated deciduous teeth into necrotic immature permanent teeth and successfully regenerated dental pulp. In a clinical trial, implanting SHEDs led to the regeneration of three-dimensional pulp tissue with improved root length and reduced apical foramen width compared to traditional treatment methods. Importantly, SHEDs implantation was found to be safe during a 24-month follow-up period, suggesting its potential for effectively treating tooth injuries resulting from trauma.

2.3.3 Bone Marrow Mesenchymal Stem Cells (BMMSCs)

Friedenstein et al. (Friedenstein *et al.*, 1968) first identified BMMSCs as fibroblast-like cells capable of differentiating into multiple tissues, including bone, cartilage, adipose tissue, tendon, and muscle. They express markers such as Stro-1, CD271, CD73, CD105, and CD90, while lacking hematopoietic and endothelial markers like CD31, CD34, and CD45 (Bernardo, Locatelli and Fibbe, 2009). Under specific in vitro conditions, BMMSCs can differentiate into osteogenic, chondrogenic, adipogenic, and neurogenic lineages (Baksh, Song and Tuan, 2004). Recent studies combining rat BMMSCs (rBMSCs) with bioceramic materials demonstrated successful formation of vascularized pulp-like tissue, with increased blood vessel formation after implantation for 3 months (Wen *et al.*, 2020).

2.3.4 Adipose-Derived Stem Cells (ADSCs)

Zuk et al. (Zuk *et al.*, 2001) identified adipose-derived stem cells (ADSCs), also known as processed lipoaspirate (PLA) cells, from human adipose tissue. These cells exhibit mesenchymal characteristics and can differentiate into adipogenic,

chondrogenic, myogenic, and osteogenic lineages, making them a promising source for tissue regeneration (Fig. 1). ADSCs express markers such as CD34, CD44, CD106, CD146, and CD166, but not STRO-1 (Gronthos *et al.*, 2001). Comparative studies showed that both ADSCs and dental pulp stem cells (DPSCs) can generate tooth-like structures with similar differentiation potential. However, ADSCs demonstrate higher proliferation rates and greater resistance to cellular aging, highlighting their value in regenerative dentistry (Hung *et al.*, 2011). Additionally, ADSCs have shown superior outcomes compared to BMMSCs in pulp regeneration, producing larger amounts of pulp-like tissue and increased matrix formation, indicating stronger regenerative potential (Ishizaka *et al.*, 2012).

2.3.5 Stem Cells from the Apical Papilla (SCAPs)

Sonoyama et al. (Sonoyama *et al.*, 2006) identified Stem Cells from the Apical Papilla (SCAPs) from the root apical papilla of human teeth. These cells express markers such as STRO-1, CD24, CD29, CD73, CD90, CD105, CD106, CD146, and CD166, with CD24 considered a specific marker. They also express neurogenic markers including nestin, βIII tubulin, GAD, and NeuN (Sonoyama *et al.*, 2008b). SCAPs exhibit higher proliferation and mineralization potential compared to dental pulp stem cells (DPSCs) (Bakopoulou *et al.*, 2011). Recent studies showed that VEGF and nerve growth factor (NGF) enhance gene expression related to dentin and neural development in SCAPs, especially under bacterial conditions, indicating their role in improving pulp regeneration (Shen *et al.*, 2021). Furthermore, Sequeira et al.(Sequeira *et al.*, 2021)demonstrated that combining SCAPs with platelet-rich plasma and bioactive materials promotes dentin–pulp complex formation, dentin bridge formation, and increased mineralized tissue deposition, highlighting their strong potential in dental tissue regeneration.

3.4.6 Induced Pluripotent Stem Cells (iPSCs)

Takahashi et al. (Takahashi and Yamanaka, 2006) first identified induced pluripotent stem cells (iPSCs), which share characteristics with embryonic stem cells and can differentiate into tissues from all three germ layers. iPSCs have been used to generate dental epithelial-like stem cell lines (EPI-iPSCs) through co-culture with Hertwig's epithelial root sheath/epithelial rests of Malassez (HERS/ERM), promoting epithelial differentiation. These cells enhance odontogenic gene expression and support mineralized nodule formation (Kim *et al.*, 2020). Recent studies have also used iPSC-derived cranial neural crest-like cells (CNCLCs) to mimic dental pulp tissue. When combined with peptide hydrogels in animal models, they successfully reproduced pulp-like structures (Kobayashi *et al.*, 2022).

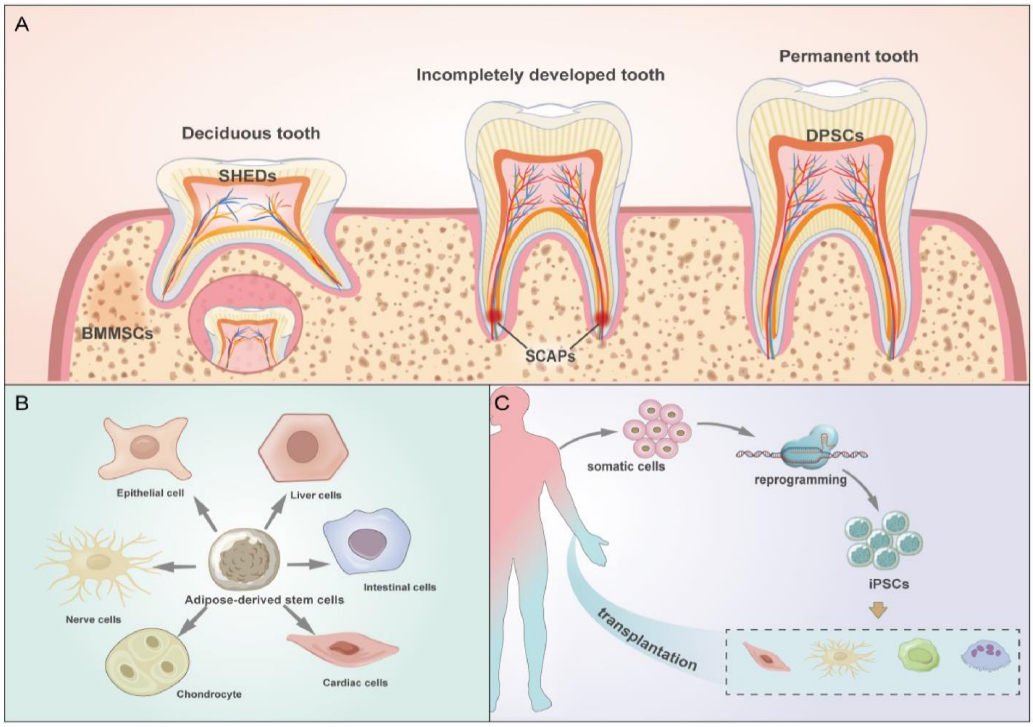

Fig. 1. Cells used for pulp regeneration.

2.4 Scaffolds for regeneration pulp tissue

2.4.1 Natural Scaffolds (Scaffolds of biological origin)

2.4.1.1 Blood clot

Ostby was the first to employ the use of a blood clot to regenerate dental pulp tissues, which led to the development of granulation tissue, fibrous tissue, or cementum-like tissue inside the root canals. Later, Myers and Fountain (1974) successfully used blood clots to form approximately 0.1–1.0 mm of soft connective tissue within the root canal space.

The fibrin matrix of the blood clot acts as a natural scaffold that traps and confines cells required for tissue repair (Bansal and Bansal, 2011). It also provides a favorable pathway for fibroblasts and macrophages from the periapical region to migrate into the root canal and promote new tissue formation (Harrison and Jurosky, 1991).

In addition, blood clots contain a high concentration of growth factors, which play an important role in cell differentiation and tissue regeneration (Rady *et al.*, 2024)).

2.4.1.2 Dentin

Fully enclosing the root canal space is an acellular dentin matrix that is rich in growth factors such as IGF-1, IGF-2, BMP-2, BMP-4, BMP-6, and TGF-β1, β2, and β3 (Guo *et al.*, 2009).

These growth factors are essential in regulating inflammatory responses, promoting tissue repair and regeneration, and inducing odontoblast differentiation (Begue-Kirn *et al.*, 1994). It has been demonstrated that dentin tissues can be

regenerated in vivo when treated with EDTA, which facilitates the release of these bioactive molecules (Guo *et al.*, 2009).

2.4.1.3 PRP and PRF

Platelet-rich plasma (PRP) was introduced to dentistry by Whitman in 1997 and has been shown to attract stem cells from surrounding periapical tissues (Freymiller and Aghaloo, 2004). Increased tissue regeneration has been observed in root canals treated with PRP combined with dental pulp cells (Zheng *et al.*, 2014).

PRP promotes faster formation of vital tissue compared to conventional approaches (Torabinejad *et al.*, 2003)). Platelet-rich fibrin (PRF), introduced by Choukroun in 2001, is considered a second-generation platelet concentrate and provides a slower and more sustained release of growth factors over 7–14 days. In contrast, PRP releases growth factors more rapidly within 7–14 hours (Guo *et al.*, 2009).

2.4.2 Synthetic Scaffolds

2.4.2.1 Synthetic polymers

Vacanti et al. (1988) first proposed the use of biodegradable synthetic polymers such as polyglycolic acid (PGA), polylactic acid (PLA), and poly-lactic-co-glycolic acid as scaffolds for cell transplantation.

PGA combined with human pulpal fibroblasts was used in early in vitro pulp tissue engineering, producing tissue-like constructs resembling normal pulp in cellularity (Mooney *et al.*, 1996). Similarly, poly-L-lactic acid (PLLA) scaffolds supported the formation of pulp-like tissue when combined with human dermal microvascular endothelial cells or stem cells from human exfoliated deciduous teeth (SHED) (Rosa *et al.*, 2013)

In vivo studies showed that tissue engineered using PGA/PLLA scaffolds and tooth bud cells could regenerate complex tooth structures, including enamel, dentin, and a defined pulp chamber in animal models (Young et al., 2002). Additionally, open-cell PLA scaffolds have demonstrated promising results in supporting SHED attachment and pulp-like tissue formation in root canals (Gotlieb *et al.*, 2008).

2.4.2.2 Bioactive ceramics

Bioactive ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP) have shown strong potential for dentin and pulp-dentin complex regeneration. Cells grown on porous ceramic scaffolds can express dentin-specific markers such as dentin sialophosphoprotein and demonstrate proliferation and attachment ability (Sonoyama *et al.*, 2006).

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] has been widely proposed as an effective scaffold for mineralized tissue regeneration. Although HA is non-biodegradable and tricalcium phosphate [$Ca_3(PO_4)_2$] is biodegradable, both materials have been shown to support the formation of bone- and dentin-like tissues when combined with pulp-derived cells and transplanted in vivo (Mastrangelo *et al.*, 2008).

2.5 Materials Used for Pulp Regeneration

Regenerative endodontic procedures (REPs), particularly pulp revascularization/regeneration, aim to restore the biological function of the pulp–dentin complex rather than merely replacing infected tissue with inert filling materials. Successful regeneration depends largely on three essential components of tissue engineering: stem cells, signaling molecules, and scaffolds. Among these

elements, biomaterials play a critical role in providing a suitable microenvironment that supports cell migration, proliferation, differentiation, and vascularization. Two of the most widely investigated and clinically applied materials in pulp regeneration are Mineral Trioxide Aggregate (MTA) and bioceramic-based scaffolds, both of which exhibit high bioactivity and excellent biocompatibility.

2.5.1. Mineral Trioxide Aggregate (MTA)

Mineral Trioxide Aggregate (MTA) is considered one of the most important biomaterials used in regenerative endodontics and is often regarded as the gold standard coronal sealing material in pulp revascularization procedures. MTA is a calcium silicate–based cement primarily composed of tricalcium silicate, dicalcium silicate, tricalcium aluminate, and bismuth oxide as a radiopacifier. Upon hydration, it forms calcium silicate hydrate gel and releases calcium hydroxide, resulting in an alkaline environment favorable for tissue healing and regeneration.

One of the major advantages of MTA is its exceptional biocompatibility. Numerous studies have demonstrated that MTA promotes survival and differentiation of human dental pulp stem cells (hDPSCs), stimulating odontoblastic activity and hard tissue formation. The material induces the release of bioactive ions such as calcium ions, which enhance mineralization and dentin bridge formation. These biological properties make MTA particularly suitable for regenerative procedures where tissue regeneration rather than repair is desired (Zhu *et al.*, 2023).

In pulp revascularization, MTA is commonly placed as a coronal barrier over the blood clot or scaffold after canal disinfection. Its sealing ability prevents bacterial microleakage, which is essential for maintaining a sterile environment that allows stem cell–mediated regeneration to occur. Clinical and systematic reviews have reported high success rates when MTA is used in regenerative endodontic

treatments, with continued root development and apical closure observed in immature teeth (Tawfeek *et al.*, 2023).

2.5.2 Bioceramic Materials

Bioceramic materials, particularly calcium silicate-based cements such as mineral trioxide aggregate (MTA) and newer bioceramic formulations, play a central role in regenerative endodontics. These materials are characterized by excellent biocompatibility, bioactivity, and sealing ability. Their high alkaline pH promotes antibacterial effects and stimulates the release of growth factors from dentin, which enhances the differentiation of dental pulp stem cells (DPSCs) into odontoblast-like cells (Alshawkani *et al.*, 2024). Furthermore, bioceramics support mineralization and dentin bridge formation, making them essential for creating a favorable microenvironment for pulp regeneration. Their ability to interact with periapical tissues has significantly improved the biological outcomes of regenerative procedures (Chen *et al.*, 2025).

2.5.3 Biodentine

Biodentine is a bioactive calcium silicate-based material developed as a dentin substitute and widely used in vital pulp therapy and regenerative procedures. Compared to traditional MTA, Biodentine offers improved handling properties, shorter setting time, and reduced risk of tooth discoloration. It promotes pulp cell proliferation and differentiation by releasing calcium ions and inducing mineralization. Biodentine has also been shown to stimulate tertiary dentin formation and enhance tissue repair, making it highly suitable for regenerative applications (Chen *et al.*, 2025). Its superior mechanical properties and sealing ability further contribute to its clinical success.

2.5.4 Collagen-Based Scaffolds

Collagen is the primary structural protein of the extracellular matrix (ECM) and is widely used as a natural scaffold in pulp regeneration. Collagen-based scaffolds provide an ideal three-dimensional environment that supports cell adhesion, proliferation, and differentiation. Due to its excellent biocompatibility, biodegradability, and low immunogenicity, collagen facilitates stem cell migration and angiogenesis, both of which are critical for pulp tissue regeneration (Chen *et al.*, 2025). However, its relatively low mechanical strength necessitates modification, such as cross-linking or combination with other biomaterials, to enhance its structural stability and regenerative efficiency.

2.5.5 Barrier Membranes

Barrier membranes are essential in regenerative procedures as they help create a protected environment for tissue regeneration. Their primary function is to prevent epithelial cell migration into the regenerative site while allowing selective repopulation by progenitor and stem cells. This process enhances tissue organization and vascularization. Most modern membranes are resorbable and commonly made from collagen or synthetic polymers. Recent advancements include the incorporation of bioactive components such as bioceramics into membrane structures to improve their regenerative potential (Chen *et al.*, 2025).

2.6 Disinfection of the Root Canal System

Disinfection of the root canal system represents a fundamental step in pulp revascularization and regenerative endodontic procedures (REPs), as successful regeneration depends on effective microbial control while maintaining a biologically favorable environment for stem cell survival. Unlike conventional root canal therapy, regenerative protocols emphasize minimal mechanical instrumentation in order to preserve dentinal integrity and protect stem cells residing in the apical papilla. Therefore, chemical disinfection plays the primary role in eliminating intracanal microorganisms while supporting tissue regeneration ((Diogenes *et al.*, 2016)). Irrigation protocols in regenerative endodontics are carefully designed to balance antimicrobial effectiveness with cytocompatibility. Sodium hypochlorite (NaOCl) remains the main irrigant because of its broad-spectrum antibacterial activity and tissue-dissolving capability. However, high concentrations of NaOCl have been shown to negatively affect stem cell viability and reduce dentin-derived growth factor activity. For this reason, current clinical guidelines recommend the use of lower concentrations ranging from 1.5% to 3% during regenerative procedures ((Lin *et al.*, 2021)). Following NaOCl irrigation, 17% ethylenediaminetetraacetic acid (EDTA) is applied to remove the smear layer and expose dentinal tubules. Importantly, EDTA promotes the release of bioactive molecules such as transforming growth factor-β (TGF-β), which enhances stem cell attachment and odontoblastic differentiation ((Noohi *et al.*, 2022)). The first step in the endodontic treatment of infected root canals involves disinfection through the use of chemical substances and mechanical instrumentation(Byström and Sundqvist, 1981). However, in immature teeth, the mechanical removal of microorganisms is not recommended due to the fragility of the thin root walls, requiring a decontamination restricted to the use of irrigant solutions and intracanal medication(Lovelace *et al.*, 2011).

2.6.1 Distinctions From Traditional Endodontic Therapy

The main goal of regenerative endodontic procedures (REPs) is the resolution of apical periodontitis, similar to conventional root canal therapy. However, REPs differ in that they are mainly applied to immature permanent teeth with thin canal walls and open apices. Unlike conventional endodontic treatment, mechanical instrumentation is minimized or avoided to preserve stem cells in the apical tissues and prevent further weakening of root canal walls(Iwaya, Ikawa and Kubota, 2001). Instead, chemical disinfection using irrigants and intracanal medicaments is preferred.

Disinfection in REPs must balance antimicrobial efficacy with stem cell survival. Sodium hypochlorite (NaOCl) is commonly used in concentrations ranging from 0.5% to 6%, but concentrations above 3% may be cytotoxic to stem cells of the apical papilla (SCAP) and reduce cell adhesion(Martin *et al.*, 2014). Therefore, lower concentrations are recommended according to recent guidelines from the American Association of Endodontists (AAE), along with reduced doses of calcium hydroxide or triple antibiotic paste (TAP)(Nikita B. Ruparel *et al.*, 2012).

Ethylenediaminetetraacetic acid (EDTA) is recommended as a final irrigant because it can release dentin-derived growth factors that promote differentiation of dental pulp stem cells (DPSCs) into odontoblast-like cells(Galler *et al.*, 2011).

Finally, REPs rely on tissue engineering principles, including stem cells, scaffolds, and growth factors. Induced intracanal bleeding helps deliver mesenchymal stem cells into the canal space. The formed blood clot acts as a natural scaffold and a reservoir of growth factors that support pulp-dentin regeneration(Nosrat, Homayounfar and Oloomi, 2012).

2.7 Intracanal medicaments

Pulp revascularization requires a bacteria-free environment to allow successful cell colonization(Turkistani and Hanno, 2011). Because root canal infections involve multiple bacterial species, a single antibiotic is insufficient(Windley III *et al.*, 2005). Triple Antibiotic Paste (TAP), composed of ciprofloxacin, metronidazole, and minocycline, has demonstrated effective bacterial elimination against endodontic pathogens. It typically consists of 400 mg Metronidazole, 250 mg Ciprofloxacin, and 50 mg Minocycline mixed in a propylene glycol vehicle. Nevertheless, concerns regarding cytotoxicity and tooth discoloration have led to the use of modified formulations or Double Antibiotic Paste (DAP), which excludes minocycline ((Diogenes *et al.*, 2016)). Calcium hydroxide has also been widely recommended as an alternative medicament due to its antimicrobial properties and superior compatibility with stem cells from the apical papilla. Studies indicate that calcium hydroxide promotes a more favorable environment for stem cell survival compared with high-concentration antibiotic pastes (Kontakiotis *et al.*, 2015). A key concept in regenerative endodontics is achieving "biologically based disinfection," where microbial reduction is accomplished without compromising regenerative potential. Excessive chemical irritation or aggressive antimicrobial strategies may impair stem cell function and negatively influence tissue regeneration. Consequently, modern regenerative protocols advocate gentle irrigation techniques, controlled medicament concentrations, and preservation of apical tissues to facilitate successful pulp–dentin complex regeneration (Murray, Garcia-Godoy and Hargreaves, 2007).

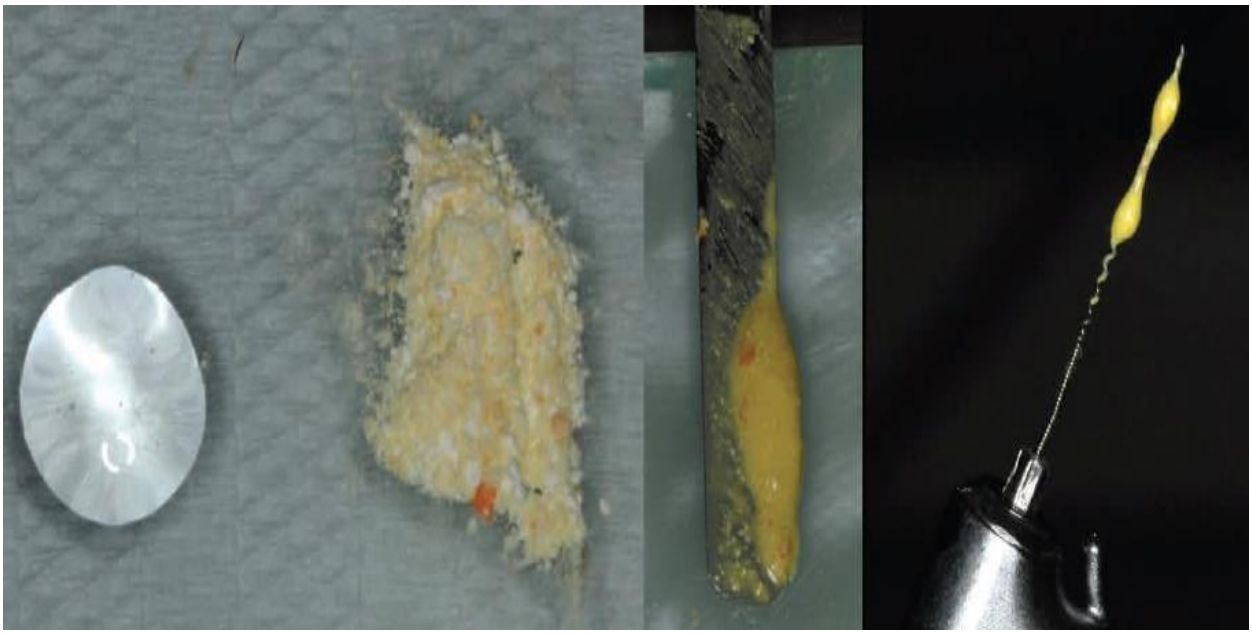

Figure 2 Images showing triple antibiotic paste consistence and insertion.

2.8 Protocol of Pulp Revascularization / Regenerative Endodontic Procedures:

First Appointment: Disinfection Phase

The initial step focuses on infection control while preserving stem cell viability. Local anesthesia without vasoconstrictor is often recommended to enhance bleeding induction during later stages. After rubber dam isolation, access cavity preparation is performed with minimal or no mechanical instrumentation to avoid weakening dentinal walls and damaging stem cells located in the apical papilla. Chemical irrigation represents the primary cleaning method. Sodium hypochlorite (NaOCl) at low concentrations (1.5–3%) is commonly used, followed by saline or EDTA irrigation. EDTA plays a crucial biological role by releasing growth factors embedded within dentin, which promotes stem cell attachment and differentiation

(Aga *et al.*, 2025). An intracanal medicament is then placed, typically calcium hydroxide or a low-concentration triple antibiotic paste, to eliminate residual bacteria. The canal is temporarily sealed for approximately 2–4 weeks.

irrigant Solutions

The most commonly used irrigant solutions worldwide are sodium hypochlorite (NaOCl) and chlorhexidine (CHX)37. NaOCl has strong antimicrobial activity against most endodontic pathogens38 and is used in concentrations ranging from 0.5% to 6%. In pulp revascularization, higher concentrations such as 2.5% and 6% are preferred to improve clinical outcomes(Nosrat, Seifi and Asgary, 2011b). Chlorhexidine has been used in concentrations of 2%15,39 and 0.12%40. However, despite their antimicrobial effectiveness, both NaOCl and CHX show limited biocompatibility, negatively affecting dental pulp stem cell survival and adhesion to dentinal walls(Ring *et al.*, 2008).

Chelating agents such as EDTA, citric acid, and MTAD are also used to remove the smear layer. MTAD, introduced by Torabinejad et al.(Torabinejad *et al.*, 2003), consists of 3% thiosulfate, 4.25% citric acid, and 0.5% polysorbate. EDTA is the most widely used chelating agent and can promote the release of growth factors from dentin, although its effect on stem cell proliferation during revascularization remains unclear(Hargreaves *et al.*, 2008).

Studies evaluating cytotoxicity have shown that NaOCl and CHX reduce stem cell adhesion to dentinal walls41. Other irrigants such as Aquatine Endodontic Cleanser (AquatineEC), Morinda Citrifolia™ (MCJ), sterile saline, EDTA, and MTAD have also been investigated. AquatineEC™, which contains hypochlorous acid (HOCl), demonstrates antimicrobial properties with better biocompatibility(Hargreaves *et al.*, 2008) and less cytotoxicity compared to NaOCl and CHX, allowing improved

stem cell adhesion(Ring *et al.*, 2008). Nevertheless, further research is needed to confirm its role in regenerative endodontics.

Second Appointment: Induction of Bleeding and Scaffold Formation

If the tooth is asymptomatic, the temporary restoration is removed and the canal is gently irrigated using EDTA solution. A sterile file is intentionally advanced beyond the apex to induce bleeding from the periapical tissues into the canal space. The resulting blood clot acts as a natural scaffold containing stem cells from the apical papilla (SCAP), growth factors, and signaling molecules essential for tissue regeneration. The blood clot is allowed to stabilize approximately 2–3 mm below the cemento-enamel junction.(N. B. Ruparel *et al.*, 2012)

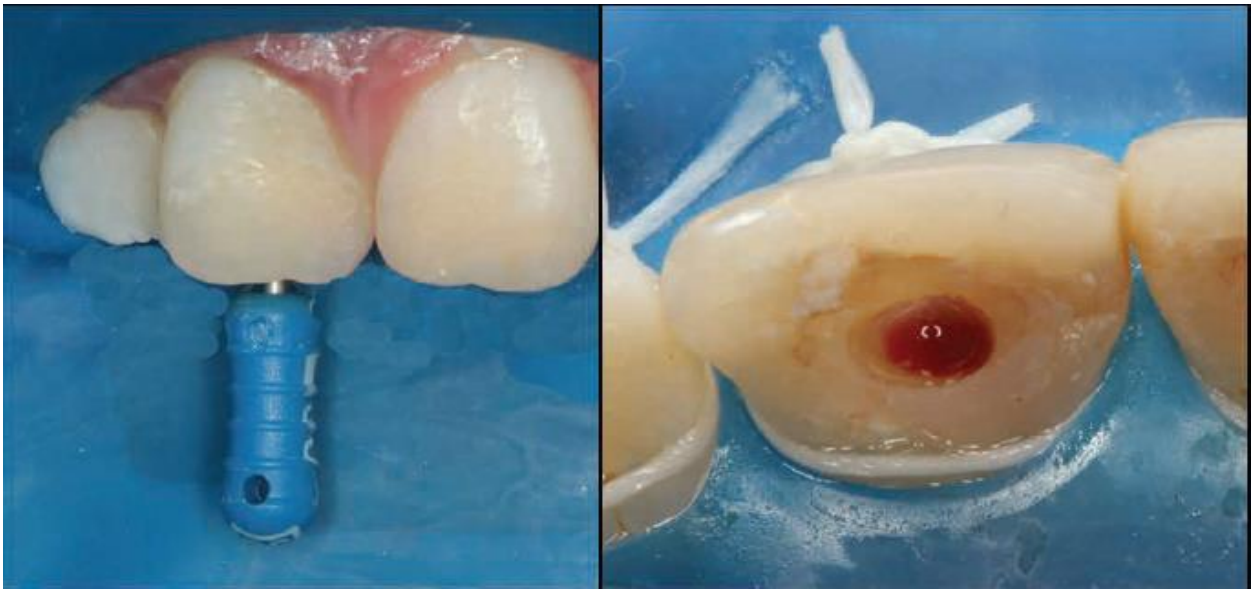

Figure 3 Blood clot stimulation with a manual endodontic file.

Coronal Barrier Placement

A biocompatible material such as mineral trioxide aggregate (MTA) or a bioceramic material is carefully placed over the clot to create a bacteria-tight seal. A permanent coronal restoration is subsequently placed to prevent reinfection.

Follow-Up and Outcome Evaluation

Patients are recalled periodically (3, 6, 12 months and annually thereafter). Treatment success is evaluated based on:

Resolution of clinical symptoms

Periapical healing

Continued root development and thickening of dentinal walls

Possible return of pulp sensibility

The primary goal remains elimination of infection, while continued root maturation is considered a secondary but highly desirable outcome (Lima *et al.*, 2025).

2.8.1 Clinical Procedure: Revascularization

Regenerative endodontic treatment (RET) is typically performed in two visits according to the American Academy of Endodontics (AAE)(Endodontists, 2016) and the European Society of Endodontics (ESE)(Galler *et al.*, 2016). The first visit focuses on infection control and inflammation reduction, while the second aims at pulp revascularization and regeneration. All procedures are performed under a dental microscope, except anesthesia and rubber dam placement.

Appointment No. 1

The aim is to control infection and reduce inflammation:

(1) **Isolation and anesthesia:** Local anesthesia is administered, and the tooth is isolated with a rubber dam.

(2) **Access preparation:** Complete removal of caries and unroofing of the pulp chamber.

(3) **Canal preparation:** Working length determination and initial apical file selection.

(4) **Irrigation:** Slow irrigation using side-vented needles into the apical third:

• 20 mL of 1.0% sodium hypochlorite (NaOCl) for 5 minutes.

• 20 mL of 17% EDTA for 5 minutes.

• Repeated EDTA irrigation followed by drying with sterile paper points.

Intra-canal medication:

The canal is filled with calcium hydroxide or low-concentration triple antibiotic paste to prevent reinfection. If triple antibiotic paste is used, it should be kept below the CEJ to avoid discoloration and sealed with a bonding agent. A 1:1:1 mixture of ciprofloxacin, metronidazole, and minocycline (0.1 mg/mL) can be used. The canal is sealed with a temporary material (IRM or glass ionomer), and the patient is recalled after 1–4 weeks(Banchs and Trope, 2004).

Appointment No. 2

Performed after 2–4 weeks if the patient is asymptomatic:

(1) **Disinfection and isolation:** Removal of temporary restoration and field preparation.

(2) **Irrigation:** Calcium hydroxide is removed using 20 mL of 17% EDTA for 5 minutes, followed by saline irrigation and drying.

(3) **Revascularization techniques:**

• **PRF method:** 10 mL of blood is centrifuged to obtain injectable platelet-rich

fibrin (i-PRF).

• **Induction of bleeding:** Over-instrumentation (2–3 mm beyond apical foramen) to allow blood clot formation up to 3 mm below CEJ (Banchs and Trope, 2004).

(4) **Canal sealing:**

i-PRF or blood clot is placed, followed by a 3-mm layer of MTA. Alternatives may be used in esthetic areas due to discoloration risk.

Follow-up evaluation:

- Absence of pain, swelling, or sinus tract.
- Resolution of apical radiolucency (6–12 months).
- Increased root wall thickness (12–24 months).
- Positive pulp vitality response.
- Continued root length development.

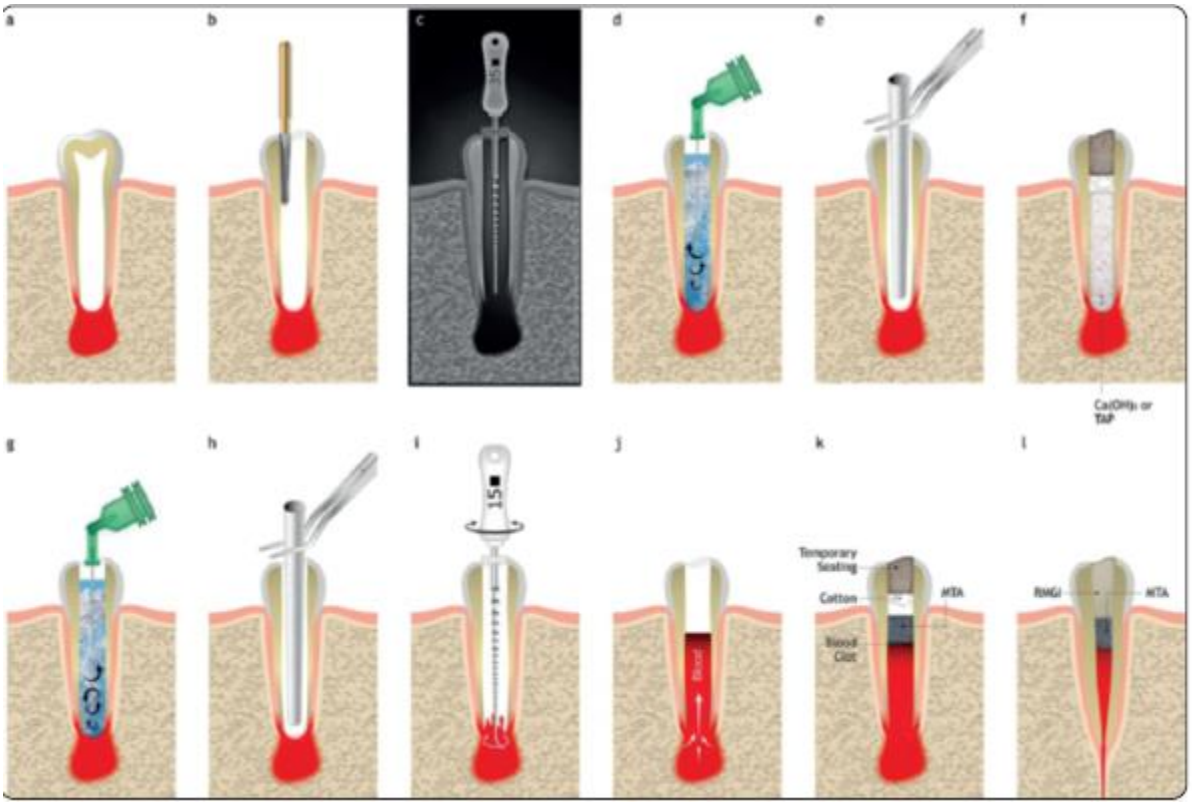

Fig. (4). Schematic illustration of revascularization procedure. Revascularization is considered for immature teeth with open apices (a). After accessing the opening (b), Gentle irrigation limited to coronal part of the chamber is performed. A radiograph with K-file insertion (c), Provides the approximate tooth length, which helps to determine working length. Low concentration of NaOCl (1.5 or less than 3%, 20 mL/canal, 5 min) is used for disinfection (d), Following which saline or 17% EDTA is used. After copious irrigation and canal drying with paper point (e) Intracanal medicaments, such as Ca(OH)2 or TAP were placed, and covered with temporary filling material (f) After confirming the absence of any signs of infection, the final step is initiated. Final irrigation is performed with sterile saline and 17% EDTA (g) After the canal has dried (h) Pre-curved K-file is introduced 2 mm past the apical foramen and rotated to induce bleeding (i) Blood fills the canal from the bottom and the blood clot can be identified after 15 min (j) After the blood clot is confirmed, capping materials such as MTA are placed over the blood clot (k) Regeneration of pulp-dentin leads to root development with thickening, lengthening, and apical closure, as well as maintenance of tooth vitality (l).

2.9 Clinical Issues and Limitations of Pulp Revascularization

Despite the promising outcomes of regenerative endodontic therapy, several biological and clinical challenges remain, limiting its predictability and universal application.

2.9.1. Unpredictable Tissue Regeneration

One of the main limitations is that true pulp regeneration does not always occur. Histological studies have demonstrated that the newly formed tissue may resemble cementum-like or bone-like tissue rather than authentic pulp tissue containing organized odontoblast layers. Therefore, many cases represent repair rather than complete regeneration (El Maroush, 2025).

2.9.2. Dependence on Stem Cell Availability

Successful regeneration depends heavily on the presence of viable stem cells from the apical papilla. In older patients or teeth with severe infection, stem cell populations may be reduced, resulting in compromised outcomes. Consequently, REPs show higher success rates in young patients with immature roots(Sonoyama *et al.*, 2008b).

2.9.3. Difficulty in Disinfection Balance

Achieving adequate disinfection while preserving stem cell viability is challenging. High concentrations of sodium hypochlorite or antibiotic pastes can be cytotoxic to stem cells and negatively affect regenerative potential. Clinicians must therefore balance antimicrobial efficacy with biological preservation.(N. B. Ruparel *et al.*, 2012).

2.9.4. Tooth Discoloration

Materials such as MTA or antibiotic pastes containing minocycline may cause crown discoloration, which represents an important esthetic concern, especially in anterior teeth.(Kim and Kim, 2026)

2.9.5. Lack of Standardization

Although general guidelines exist, variations still occur regarding irrigation protocols, intracanal medicaments, scaffold type, and coronal sealing materials. This heterogeneity contributes to inconsistent clinical outcomes among studies (Lima *et al.*, 2025).

Chapter Three

Discussion

Discussion

Pulp revascularization, also referred to as regenerative endodontic treatment (RET), represents a significant paradigm shift in modern endodontics, moving from traditional replacement-based therapy toward biologically driven tissue regeneration. Conventional apexification procedures using calcium hydroxide or mineral trioxide aggregate (MTA) primarily aim to induce apical barrier formation without promoting further root development. Consequently, treated teeth often remain structurally weak with thin dentinal walls and a higher susceptibility to fracture. In contrast, regenerative approaches aim to restore the pulp–dentin complex, enabling continued root maturation, apical closure, and functional recovery of immature permanent teeth with necrotic pulp (Rafter, 2005; Diogenes et al., 2016). The biological foundation of pulp revascularization is strongly based on tissue engineering principles involving stem cells, scaffolds, and signaling molecules. Stem cells from the apical papilla (SCAP), dental pulp stem cells (DPSCs), and other mesenchymal stem cell populations play a central role in regeneration due to their high proliferative capacity and ability to differentiate into odontoblast-like cells (Sonoyama et al., 2008; Gronthos et al., 2000). These cells are recruited into the disinfected root canal space through induced bleeding, where the resulting blood clot serves as a natural scaffold rich in fibrin matrix and growth factors that support cell attachment, proliferation, and differentiation (Banchs and Trope, 2004). One of the key determinants of success in regenerative endodontic procedures is effective disinfection of the root canal system while preserving stem cell viability. Sodium hypochlorite (NaOCl) remains the most widely used irrigant due to its strong antimicrobial activity; however, its cytotoxicity increases with concentration, negatively affecting stem cell survival and attachment (Martin et al., 2014). Therefore, lower concentrations (1.5%–3%) are recommended in regenerative protocols. Ethylenediaminetetraacetic acid (EDTA) plays a complementary role by removing the smear layer and releasing growth factors embedded in dentin, which enhances stem cell differentiation and tissue regeneration (Galler et al., 2011). This balance between antimicrobial efficacy and biological compatibility is critical, as excessive disinfection may impair regenerative potential. Intracanal medicaments further contribute to canal disinfection between treatment visits. Calcium hydroxide is commonly used due to its high pH and antimicrobial properties while maintaining relatively good

biocompatibility. Alternatively, triple antibiotic paste (TAP), consisting of ciprofloxacin, metronidazole, and minocycline, has demonstrated strong antimicrobial effectiveness against endodontic pathogens (Windley III et al., 2005). However, TAP is associated with disadvantages such as tooth discoloration and cytotoxic effects on stem cells, leading to the development of modified formulations such as double antibiotic paste (DAP) (N. B. Ruparel et al., 2012). These findings highlight the importance of selecting medicaments that maintain a balance between disinfection and preservation of regenerative capacity. Scaffold materials also play a fundamental role in supporting pulp regeneration. Natural scaffolds such as collagen, chitosan, gelatin, and platelet concentrates provide a biologically active environment that mimics the extracellular matrix and enhances cell adhesion and differentiation. Platelet-rich fibrin (PRF) and related concentrates are particularly valuable due to their sustained release of growth factors, which promotes angiogenesis and odontogenesis (Chai et al., 2019). Synthetic scaffolds such as polylactic-co-glycolic acid (PLGA), polyethylene glycol (PEG), and gelatin methacryloyl (GelMA) offer controlled degradation rates and mechanical stability, allowing precise regulation of tissue regeneration (Chen et al., 2023). More recently, bioceramic-based scaffolds have gained attention due to their excellent bioactivity, ability to induce mineralization, and antibacterial properties, making them highly suitable for pulp–dentin complex regeneration (Dong and Xu, 2023). Clinically, regenerative endodontic procedures have demonstrated promising outcomes. Reported benefits include resolution of apical periodontitis, continued root length development, thickening of dentinal walls, and, in some cases, recovery of pulp sensibility responses. These outcomes significantly improve the long-term prognosis of immature teeth by increasing structural resistance and reducing fracture risk (Kim et al., 2018). The use of MTA or bioceramic materials as coronal sealing agents further enhances success by providing an effective bacterial-tight seal and supporting hard tissue formation. MTA in particular has been widely regarded as a gold standard material due to its bioactivity and ability to stimulate hard tissue deposition (Zhu et al., 2023).

Despite these advantages, several limitations remain in regenerative endodontics. One major challenge is the unpredictability of the regenerated tissue, which may not always represent true pulp tissue histologically. Studies have shown that the tissue formed within the canal space may resemble cementum-like or bone-like

tissue rather than organized pulp-dentin complex with functional odontoblasts. This suggests that many cases represent repair rather than true regeneration (El Maroush, 2025). Another limitation is the dependence on stem cell availability, which is significantly higher in young patients with open apices, making outcomes less predictable in older individuals. Furthermore, achieving an optimal balance between disinfection and stem cell survival remains difficult. While aggressive antimicrobial protocols improve bacterial elimination, they may compromise the viability of SCAP and other regenerative cells. Additionally, esthetic complications such as crown discoloration, particularly associated with minocycline-containing antibiotic pastes and MTA, remain a clinical concern in anterior teeth (Kim and Kim, 2025). Finally, the absence of standardized clinical protocols regarding irrigant concentration, medicament type, scaffold selection, and sealing materials contributes to variability in clinical outcomes across studies (Lima et al., 2025).

Chapter Four

Conclusion

Conclusion

Pulp revascularization and regenerative endodontic procedures represent a major advancement in the treatment of immature permanent teeth with necrotic pulp. Unlike traditional apexification, which only promotes apical closure, regenerative approaches aim to restore the pulp–dentin complex and allow continued root development. This shift highlights the transition of endodontics toward a biologically based and tissue-engineering–driven discipline. The success of these procedures relies on the core principles of tissue engineering, involving stem cells, scaffolds, and signaling molecules. Dental stem cells such as DPSCs, SHED, and SCAP play a crucial role due to their ability to proliferate and differentiate into odontogenic tissues. When combined with appropriate natural or synthetic scaffolds, they contribute to the formation of vascularized pulp-like tissue within the root canal system. Effective yet conservative disinfection is essential, ensuring microbial control while preserving stem cell viability. The use of low-concentration irrigants, EDTA, and biocompatible medicaments reflects this balance. Additionally, bioactive materials like MTA and bioceramics support sealing and tissue regeneration. Clinically, regenerative procedures show promising results, including healing of periapical lesions, continued root maturation, and improved tooth strength. However, limitations such as inconsistent tissue regeneration, possible discoloration, and lack of standardized protocols remain challenges. Moreover, the regenerated tissue may not always fully replicate true pulp, indicating that complete regeneration is not consistently achieved.

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