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Research Article

REGENERATIVE ENDODONTICS IN IMMATURE PERMANENT TEETH: CLINICAL PROTOCOLS, OUTCOMES, AND FUTURE DIRECTIONS

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ABSTRACT

Regenerative endodontic procedures (REPs) have revolutionized the management of immature permanent teeth with necrotic pulps, representing a paradigm shift from traditional apexification techniques toward biologically driven regeneration. By integrating the triad of stem cells, scaffolds, and signalling molecules, REPs not only promote periapical healing but also allow continued root maturation and functional pulp-dentin regeneration. Despite encouraging clinical outcomes, variations in clinical protocols remain due to their empirical origins and evolving evidence. This review consolidates the biological foundation of REPs, case selection criteria, and current clinical approaches, while also comparing scaffold strategies and intracanal medicaments. Furthermore, it highlights reported success rates and explores future perspectives, including stem cell therapy, gene therapy, novel biomaterials, and 3D bioprinting, that may enhance predictability and long-term outcomes in regenerative endodontics.

Keywords: Regenerative Endodontics, Immature Teeth, Stem Cells, Clinical Protocols, Scaffolds, Tissue Engineering.

INTRODUCTION

Immature permanent teeth with necrotic pulps present a unique clinical challenge due to thin dentinal walls, open apices, and high susceptibility to fracture (Banchs F, & Trope M. 2004). Conventional strategies, such as long-term calcium hydroxide apexification or placement of mineral trioxide aggregate (MTA) apical barriers, achieve apical closure but do not restore pulp vitality or promote continued root development (Shaik I *et al.*2021). These limitations leave teeth vulnerable to long-term structural failure.

Regenerative endodontic procedures (REPs) emerged as an alternative aimed at true biological repair and regeneration (Saoud TMA *et al*., 2016; Saoud TM *et al.* 2015; Kim SG *et al*. 2018). The conceptual basis is derived from tissue engineering, combining three essential elements: stem cells, scaffolds, and signaling molecules (Brizuela C *et al*. 2020; Thalakiriyawa DS & Dissanayaka W L 2024). Stem cells from the apical papilla (SCAP) and dental pulp stem cells (hDPSCs) exhibit strong odontogenic potential and play a pivotal role in root maturation (Smeda, M *et al*., 2000). The role of bioactive molecules such as transforming growth factor-beta (TGF-β), bone morphogenetic proteins (BMPs), and vascular endothelial growth factor (VEGF) has been emphasized for their capacity to induce odontoblastic differentiation, angiogenesis, and migration of progenitor cells (Aksel H & Huang GT, 2017).

Clinical reports and systematic reviews consistently demonstrate high survival and healing rates following REPs, with evidence of continued root development in a significant proportion of treated cases (Li J *et al*., 2023). However, the lack of a universally standardized protocol continues to limit reproducibility and predictability (Kontakiotis EG *et al*., 2015). Variations exist in the choice of irrigants, intracanal medicaments, disinfection strategies, and scaffold induction methods. Therefore, this review

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provides a comprehensive synthesis of the biological basis, clinical protocols, scaffold and medicament strategies, reported outcomes, and future directions in regenerative endodontics.

BIOLOGICAL BASIS AND CASE SELECTION

The biological foundation of REPs is anchored in the tissue engineering triad. SCAP and hDPSCs are the principal stem cell sources responsible for odontoblastic differentiation and dentin-pulp regeneration (Huang GT, 2009). Growth factors embedded in dentin matrix, such as TGF-β, BMPs, and VEGF, are released during canal irrigation with agents like EDTA, which enhances signaling for stem cell homing and differentiation (Galler KM *et al*., 2016). Case selection is critical for achieving favourable outcomes. REPs are best indicated for immature permanent teeth with necrotic pulps, open apices, and minimal root resorption (Brizuela C *et al*., 2020; Thalakiriyawa DS & Dissanayaka W L 2024). Contraindications include fully developed roots, extensive periapical pathosis with poor healing potential, or systemic conditions impairing regenerative capacity (Figure 1).

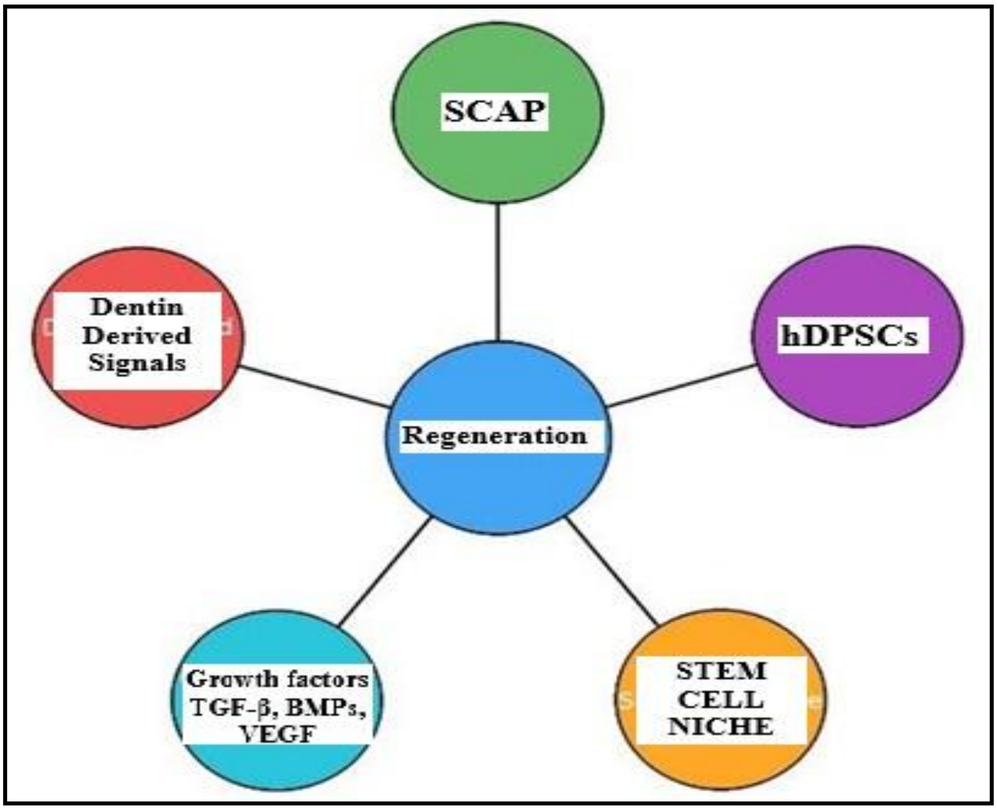

Figure 1. Conceptual mind map outlining the biological foundation of REPs, highlighting the roles of SCAP, HDPSCs, stem cell niche, dentin-released bioactive factors, and growth mediators.

CLINICAL PROTOCOLS

The clinical approach to REPs is typically completed in two or more visits (Wei X *et al*., 2022; Lin LM. & Kahler B. 2017; Brett A *et al*.,2025) (Figure 2): Diagnosis and Case Selection: Confirm pulp necrosis in immature teeth, often using vitality testing and radiographs. First Appointment (Disinfection): Access cavity preparation and canal irrigation with sodium hypochlorite (1.5–6%), followed by placement of intracanal medicaments such as triple antibiotic paste (TAP), modified TAP, double antibiotic paste (DAP), or calcium hydroxide. Second Appointment (Scaffold Induction): After irrigation with EDTA to release dentin-derived growth factors, a scaffold is introduced, typically through induced bleeding from the apical tissues or placement of platelet concentrates such as PRP, PRF, or CGF. Barrier Placement: A biocompatible material such as MTA or Biodentine is applied to seal the scaffold coronally. Final Restoration: Placement of coronal seal with bonded composite or equivalent to prevent reinfection. Follow-Up: Clinical and radiographic monitoring for signs of healing, root development, and sensibility responses.

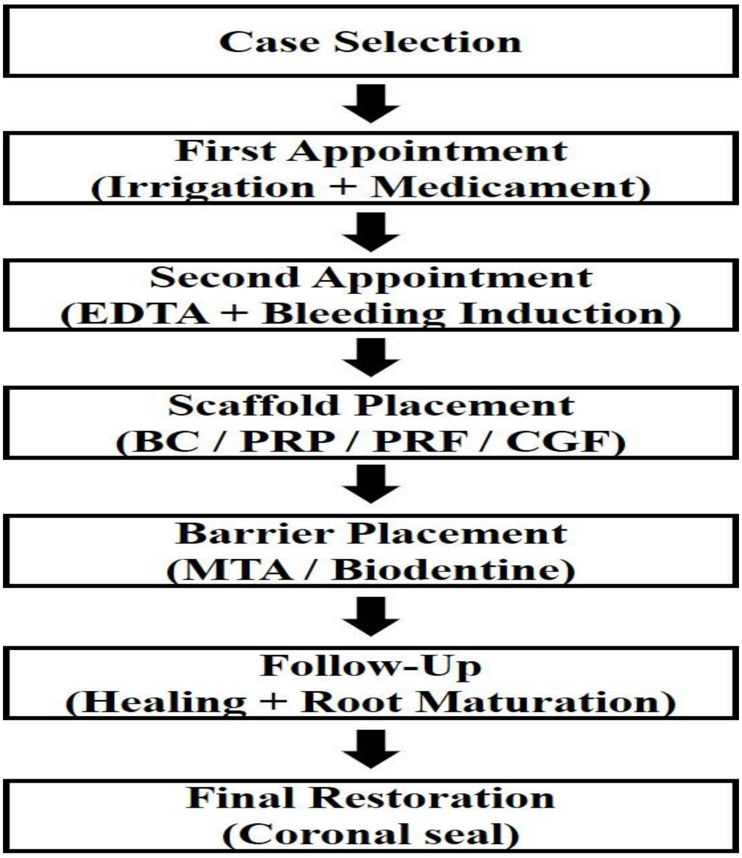

Figure 2. Stepwise schematic representation of the clinical protocol for regenerative endodontic procedures (REPs), covering patient selection, canal disinfection, scaffold induction, placement of a barrier material, definitive restoration, and post-treatment follow-up.

SCAFFOLD STRATEGIES

Scaffolds provide a 3D structure supporting stem cell proliferation and differentiation (Shahoon H *et al*. 2025; Araújo L *et al*.2022; Sequeira, DB *et al*.2023) (Table 1). Blood Clot (BC): Simple and cost-effective but may produce unpredictable volume. Platelet-Rich Plasma (PRP): Delivers high concentrations of growth factors, injectable and easy to manipulate, but requires centrifugation. Platelet-Rich Fibrin (PRF): Offers gradual release of factors, enhances angiogenesis, but has limited handling time. Concentrated Growth Factor (CGF): More advanced fibrin-based matrix, better stability and cell support. Synthetic Scaffolds: Experimental polymer- or hydrogel-based matrices with customizable properties.

Table 1. Scaffold Strategies for Regenerative Endodontic Procedures.

Scaffold	Composition/Source	Biological actions	Clinical Advantages	Limitations/Concerns
Induced Blood Clot (IBC)	Patient's own blood, provoked apical bleeding	Fibrin matrix: carries SCAP/DPSCs; releases dentin-derived growth factors (after EDTA conditioning)	Simple, low cost, no centrifuge required; widely reported clinical success	Unpredictable clot volume; risk of voids; less structural stability.
PRP (Platelet-Rich Plasma)	Autologous plasma rich in platelets (anticoagulant activator)	Burst release of PDGF, TGF-β, VEGF- promotes early angiogenesis	Injectable; can mix with cells or drugs; improved early bleeding	Requires centrifugation; short duration of growth factor release; protocol

				variability; higher cost
PRF (Platelet-Rich Fibrin)	Autologous fibrin clot without anticoagulant	Sustained growth factor release; fibrin supports migration/ proliferation	Easy preparation used as membrane/plasma; favourable handling	Short working time; variable compressibility; operator-dependent outcomes
CGF (Concentrated Growth Factor)	Fibrin-rich clot from variable-speed centrifugation	Denser fibrin matrix; prolonged GF release; supports neovascularization	Greater stability than PRF; easy to place under MTA/ Biodentine	Proprietary kits; higher cost; fewer long-term clinical trails
Synthetic Hydrogels/ECM-mimetic	Collagen hyaluronic acid, PEG, Chitosan, gelatin etc.	Tuneable porosity; can deliver antibiotics, growth factors or cells	Reproducible architecture; injectable; customizable degradation rates	Mostly preclinical use; regulatory hurdles; expensive
Electrospun nanofibres	PLGA, PCL, Collagen; sometimes bioactive glass composites	Biomimetic fiber network; promotes cell adhesion/differentiation	High surface area; controlled drug delivery possible	Still experimental; difficult to adapt to root canal anatomy

INTRACANAL MEDICAMENTS

The choice of intracanal medicament is central to disinfection while maintaining stem cell viability (Bhandi S *et al.*2022; Rahul M *et al.*2022; Diogenes A *et al.*2016; Ayoub S *et al.*2020) (Table 2). Triple Antibiotic Paste (TAP): Effective against diverse microbes but associated with tooth discoloration and stem cell toxicity. Modified TAP: Excludes minocycline to avoid staining. Calcium Hydroxide: Stimulates hard tissue deposition, but less effective against biofilm bacteria. Double Antibiotic Paste (DAP): Alternative with reduced discoloration risk.

Table 2. Intracanal Medicaments used in Regenerative Endodontic Procedures (REPs).

Intracanal Medicament	Composition	Mechanism of action	Advantages	Limitations/Concerns
Triple Antibiotic Paste (TAP)	Ciprofloxacin + Metronidazole + Minocycline	Broad spectrum antimicrobial activity against anaerobes and facultative bacteria	Effective disinfection; widely studied	Tooth discoloration (minocycline); possible cytotoxicity to stem cells; resistance risks
Modified TAP (m TAP)	Ciprofloxacin + Metronidazole + Amoxicillin / Clindamycin	Similar antibacterial spectrum without minocycline staining	Avoids tooth discoloration; effective antimicrobial	Cytotoxicity at high concentrations; less standardized
Double Antibiotic Paste (DAP)	Ciprofloxacin + Metronidazole	Target resistant anaerobic and facultative bacteria	Effective against most endodontic pathogens; avoids minocycline	May be cytotoxic to SCAP at high concentrations; reduced coverage compared to TAP
Calcium hydroxide [$Ca(OH)_2$]	Strong alkaline paste	High pH – antibacterial; induces release of bioactive dentin matrix proteins	Biocompatible; promotes hard tissue formation	Less effective than antibiotics against resistant bacteria; may weaken dentin long term

REPORTED OUTCOMES AND SUCCESS RATES

Clinical evidence demonstrates (Figure 3): Periapical healing: >90% success (Saoud *et al.*, 2016). Root lengthening: 70–80% (Arora *et al.*, 2020). Dentin wall thickening: 60–70% (Báez, V *et al.*, 2022). Pulp vitality recovery: ~40–50% in some cases (Shimizu E *et al.*, 2012).

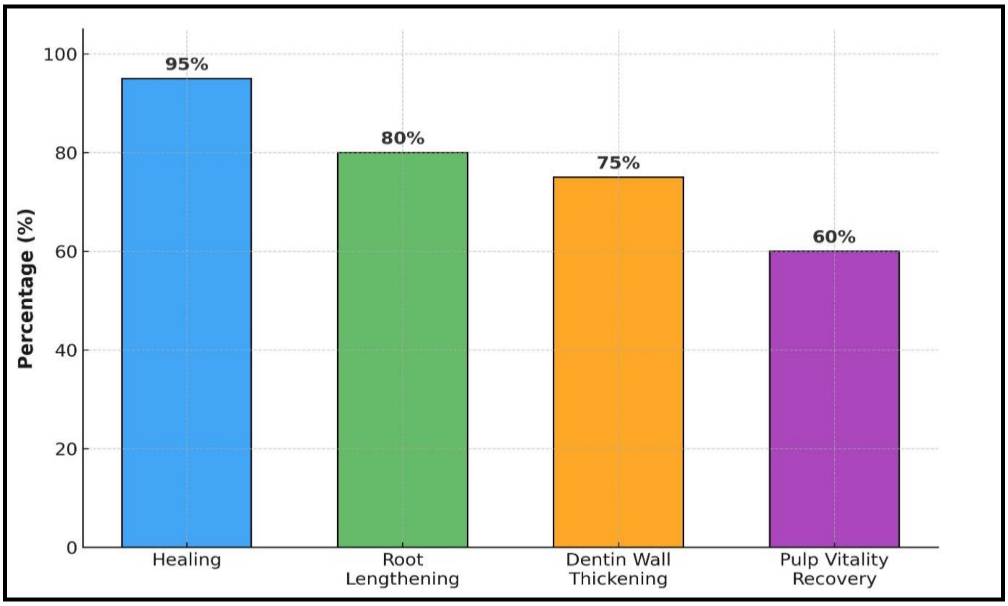

Figure 3. Bar chart representing success rates of REPs in clinical studies, highlighting healing, root lengthening, dentin wall thickening, and pulp vitality recovery.

FUTURE DIRECTIONS

Emerging strategies focus on enhancing predictability and biological fidelity: Stem Cell Therapy: Direct transplantation of DPSCs, SCAP, or induced pluripotent stem cells (iPSCs) (Duncan H F *et al*., 2016). Gene Therapy: Delivery of genes encoding growth factors such as BMP-2, VEGF, or TGF-β (Zeng, Q *et al*., 2016). Biomaterials: Nanofibrous scaffolds, injectable hydrogels, and bioactive ceramics (Galler KM *et al*., 2016). 3D Bioprinting: Enables fabrication of pulp-dentin complex structures with spatial control (Han, J., 2019). Personalized Regeneration: Leveraging patient-specific genetic and biological profiles for tailored therapies (Kim *et al*., 2018).

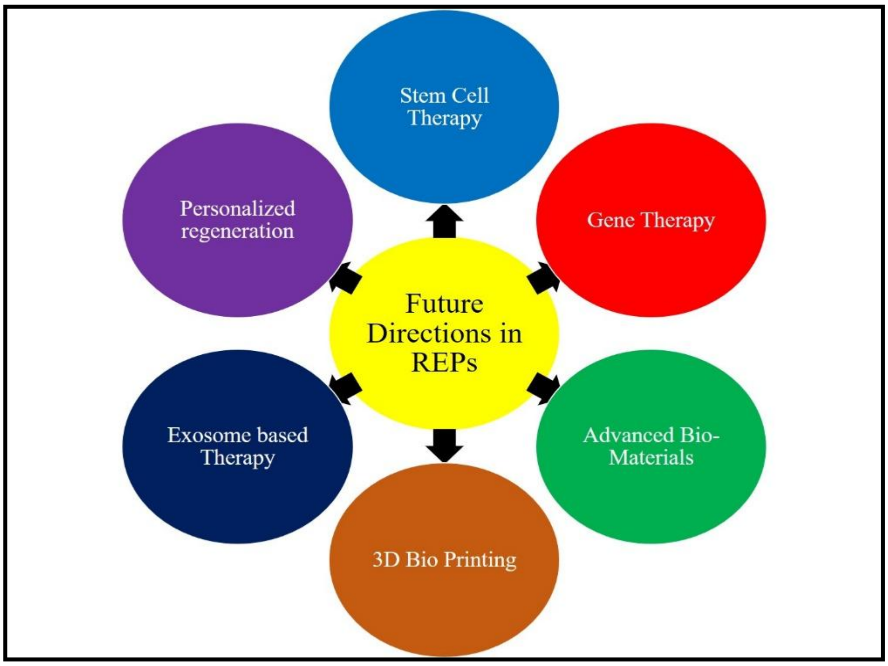

Figure 4. Mind map presenting emerging strategies in regenerative endodontics, including applications of stem cell-based therapy, gene modulation, advanced biomaterials, 3D bioprinting, and personalized regenerative approaches.

CONCLUSION

Regenerative endodontic procedures have matured into a clinically relevant strategy for managing immature necrotic teeth, producing high rates of periapical healing and frequent evidence of continued root maturation. Yet variability in technique and biological response limits universal predictability. Converging advances in stem cell science, biomaterials, and biofabrication are likely to narrow outcome variability and broaden applicability. Meanwhile, adopting pragmatic, evidence-informed protocols as outlined here will maximize the likelihood of clinical success.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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