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Clinical Protocols and Outcome Standardization in Regenerative Endodontic Procedures: A Contemporary Evidence-Based Review

By

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Abstract

Regenerative endodontic procedures (REPs) represent a paradigm shift in the management of immature and mature teeth with necrotic pulps, aiming to restore vitality and function by harnessing biological processes. However, the lack of uniformity in clinical protocols and outcome measures across studies has hindered the comparability and reproducibility of results. This review synthesizes current evidence on clinical protocols, disinfection strategies, scaffolds, biomaterials, and evaluation methods in REPs. It also emphasizes the need for standardization to achieve predictable clinical outcomes and facilitate meaningful inter-study comparisons.

Keywords*: Clinical protocol, Disinfection, Outcome standardization, Pulp regeneration Regenerative endodontics, Scaffold.*

Introduction

Regenerative Endodontic Procedures (REPs) have emerged as a biologically based alternative to conventional apexification for immature teeth with necrotic pulps. The primary goal of REPs is to restore pulp vitality, promote continued root development, and achieve functional recovery of the tooth [1]. Despite promising outcomes, there exists considerable variability in clinical protocols, including the choice of irrigants, medicaments, scaffold materials, and induction of bleeding. Such heterogeneity in methodology contributes to inconsistent outcomes and impedes systematic evaluation of treatment efficacy [2]. This review synthesizes current clinical protocols and focuses on outcome standardisation - a necessary step to move REP from promising therapy to consistently evidence-based care.

Search Strategy and Selection Criteria

This review draws upon major guideline documents (the American Association of Endodontists (AAE) Clinical Considerations for REPs), expert consensus papers, systematic reviews, umbrella reviews and clinical outcome studies published through to 2025. Search terms included "regenerative endodontic procedures", "regenerative endodontics", "clinical protocols", "outcome", "immature necrotic tooth", "scaffold" and "apical bleeding/PRF". Databases searched included PubMed, Web of Science and Scopus. Priority was given to systematic reviews with meta-analysis and well-documented clinical series. Several key reviews analysing protocol variability and outcome measures were identified. Because the literature remains heterogeneous, this review did not undertake a formal systematic review methodology but rather synthesised key evidence and distilled practical recommendations.

Indications and Case Selection

Appropriate case selection remains foundational to successful REPs. Ideal candidates generally include immature permanent teeth with necrotic pulp and open apices (wide apical foramen) where root development is incomplete and structural reinforcement is desirable. The presence of residual apical papilla stem/progenitor cells enhances the regenerative potential[3].

Contraindications or cautionary scenarios may include: non-restorable teeth, presence of severe root fracture, systemic conditions precluding regenerative approaches (e.g., bleeding disorders or immunocompromise), or cases where immediate mechanical reinforcement is critical and cannot await biological maturation [4][5].

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Effective case documentation should include baseline radiography (periapical and if justified CBCT), assessment of root development stage, pulp and periapical status, and discussion of alternative treatments (apexification, extraction). Clear informed consent that outlines potential outcomes, limitations and follow-up requirements is essential [6].

Core Clinical Protocol Components

Although considerable variation persists, consensus has emerged on major procedural components for REPs. Below we summarise key steps, and highlight areas of variation.

(1) Anesthesia, isolation and access

The clinician should use local anaesthesia without vasoconstrictor (e.g., 3% mepivacaine) when intended to induce apical bleeding, so as not to suppress vascular inflow. Rubber-dam isolation is mandatory, followed by conservative access that preserves tooth structure.

(2) Disinfection: Irrigation

Effective disinfection is required, yet must balance microbial elimination with preservation of stem/progenitor cell viability. Protocols widely use lower concentrations of sodium hypochlorite (NaOCl) than conventional root canal therapy — for example 1.5%–3% volumes of 10–20 mL per canal. The final irrigant often is EDTA (17%) to remove smear layer, release growth-factors, and mitigate NaOCl cytotoxicity.Many earlier studies used higher NaOCl concentrations (4–6 %), but data suggest lower concentrations are preferable in regenerative contexts.Activation of irrigation (ultrasonic or sonic) may improve disinfection, but standardised parameters (time, volume, activation method) remain lacking [7] [8].

(3) Intracanal medicament

Intracanal disinfection via medicaments is the next step. Historically the triple antibiotic paste (TAP: ciprofloxacin/metronidazole/minocycline) was commonly used; however, minocycline causes tooth staining and the paste is cytotoxic at higher concentrations. Thus, modified TAP (without minocycline) or calcium hydroxide (CH) have become prevalent alternatives. A systematic review noted non-antibiotic intracanal medicament REP protocols produced acceptable outcomes.Typical duration of medicament placement ranges from 1–4 weeks depending on canal status and absence of signs/symptoms [9] [10].

(4) Scaffold induction / placement

Placement of a scaffold that supports cell migration, growth-factor release and tissue formation is key. The most widely used scaffold is a blood clot induced by over-instrumentation or gentle irritating of the apical tissues beyond the foramen—this leverages the patient's endogenous stem cells and growth factors. Alternative scaffolds include platelet-rich plasma (PRP) or platelet-rich fibrin (PRF) and synthetic/biomimetic scaffolds. Comparative data are limited but suggest potential benefits with platelet concentrates in some cases.Documentation should specify scaffold type, method of induction (apical bleeding vs PRF/PRP), volume placed and time to barrier placement[11][12].

(5) Coronal barrier and restoration

Once the scaffold is placed, a bioceramic material (e.g., MTA, Biodentine, or newer bioceramics) is placed over the scaffold as a barrier (2–4 mm) followed by a permanent coronal restoration. Careful attention must be paid to avoid staining (minimize exposure of coronal dentin to antibiotic pastes; manage MTA handling). Rubber-dam isolation and high quality final restoration are essential for long-term success[13][14].

(6) Follow-up and monitoring

Follow-up intervals reported in the literature commonly are at 3, 6, 12 and 24 months, and annually thereafter. Clinical evaluation (pain/swelling/functional status), sensibility testing (cold/EPT) and radiographic imaging (standardised periapical or CBCT when indicated) should be documented. The clinician should track root length/width changes, apical closure and periapical healing[15].

Outcome Domains

For REPs to be meaningfully compared across studies, outcome domains must be defined and consistently reported. Below we outline recommended core domains and measurement considerations.

(1) Clinical success/signs & symptoms

The most basic domain: absence of pain, swelling, sinus tract, and retention of the tooth without further intervention. This should be assessed at each visit.

(2) Periapical healing

Radiographic evidence of resolution or reduction of periapical radiolucency is essential. Use of the Periapical Index (PAI) on periapical radiographs or volumetric CBCT measures is recommended. Report timing of healing and degree of reduction[16][17].

(3) Root maturation / structural change

Important for immature teeth: continued increase in root length, thickening of dentinal walls, and apical closure. Measures may include root length ratio (post-treatment vs baseline), incremental dentinal wall thickness (or root area on standardised radiographs), or volumetric CBCT assessment where available. Document method, calibration and examiner reliability[18][19].

(4) Apical closure

Definition of apical closure (complete, partial) should be specified. Ideal imaging standard is consistent baseline and follow-up orientation[20].

(5) Pulpal response / sensibility

Return of vitality or positive sensibility (cold test/EPT) may be recorded but must be interpreted cautiously: delayed response is common and false-negatives exist. Report method, time-point, and any caveats.

(6) Adverse events / complications

Report tooth discolouration, intracanal calcification (pulp canal obliteration), root fracture, need for retreatment or extraction, and scaffold/medicament-related complications[21][22][23].

*Corresponding Author: **Dr. Harsha Haridas** .

(7) Patient-centred outcomes

Where possible, record patient-reported outcomes such as pain scores, quality of life, satisfaction.

(8) Timing of assessment

Minimum follow-up time should be 12 months; 24 months or longer is preferred for root maturation outcomes. Report at consistent time-points (e.g., baseline, 3, 6, 12, 24 months). Longer term data (3-5 years) are desirable[24].

(9) Imaging standardisation

For periapical radiographs: use film/sensor holders, record angulation, magnification, and exposure settings. When CBCT used, report voxel size, field-of-view, segmentation method. Comparative data should use same imaging geometry for baseline and follow-up[25][26].

Discussion

Evidence Summary: Success Rates and Comparative Data

Multiple systematic reviews and meta-analyses have reported outcomes of REPs in both immature and mature teeth.

- A systematic review found that REP using a non-antibiotic medicament (calcium hydroxide) in immature teeth resulted in resolution of symptoms and periapical healing in all included cases, though root development outcomes were inconsistently reported[27].
- An umbrella review of 29 systematic reviews concluded that most reviews were of low to moderate quality and pointed to the need for higher-quality evidence[28].
- A systematic review and meta-analysis found that in mature permanent teeth with necrotic pulp and apical periodontitis, REPs exhibited no significant difference in success rate compared with conventional root canal treatment (RCT) (RR = 1.03; 95% CI: 0.92–1.15) [29].
- Another review reported an overall success rate of 96% (95% CI: 94%–98%) for REPs in mature permanent teeth, with no significant difference compared to non-surgical endodontic treatment (risk difference RD = 0.032; 95% CI: 0.023–0.087) [30].

These findings suggest that REPs are at least comparable to conventional treatment in selected scenarios, but the strength of evidence remains limited by small study numbers, heterogeneity in protocols and follow-up periods.

Table 1: Summary of Representative Clinical Studies / Series

Study (Year)	Sample (Teeth)	Protocol Highlights	Key Outcome Findings	Comments / Limitations
Bucchi et al. (2017) – systematic review of protocols	23 articles	NaOCl 1–6%; TAP in 9/11; EDTA	Protocol variability documented	Not primary outcome study; heterogeneity large
Giuliani (2016) – review of clinical protocols	51 studies, 357 teeth	Wide variation in irrigation, medicament, scaffold	No correlation between tooth type/etiology and success	Quality of included studies low
Alghamdi & Alsulaimani (2021) – systematic review of successful cases	18 studies, 250 successful cases	Healing of periapical lesion in 96%; root maturation in 45%	Long-term (≥2.5yrs) in 39% cases	Only successful cases included (reporting bias)
Dadpe et al. (2023) – systematic review of REPs in root resorption cases	14 studies (34 teeth)	Variable protocols for root-resorption management	Arrest of resorption in 33/34 teeth	Mostly case reports/series; low-moderate evidence
Meta-analysis (2021) of mature teeth REPs	3 RCTs	Compared REP vs conventional RCT	RR =1.03 for success (no difference)	RCTs small; short-term follow-up
Meta-analysis (2022) – overall mature teeth success	552 teeth	REPvs NSET(Non-surgical endodontic treatment)	Success rate ~96% for REP	Some studies single-arm; heterogeneity present

Common Complications and Their Management

While REPs generally demonstrate favourable outcomes, clinicians should be aware of and monitor for complications:

- Tooth discolouration: Frequently reported, often due to minocycline in TAP or grey-MTA. Prevention: use minocycline-free antibiotic paste, use white-MTA or newer bioceramics, adhesive seal of coronal dentine.
- Intracanal calcification / pulp-canal obliteration (PCO): Occurs in a subset of cases and may

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complicate future retreatment. Monitor canal anatomy on follow-up imaging [31].

- Failure (persistent infection or lack of maturation): In such cases, retreatment options include repeat REP, apexification, MTA plug or extraction depending on prognosis.
- Root fracture: Although data are limited, thin-walled roots remain at risk; the benefit of structural reinforcement post-maturation should be considered.
- Post-operative pain or swelling: Standard endodontic management applies; early assessment of disinfection and coronal seal is critical [32].

Practical Evidence-Based Recommendations

Based on current evidence and guideline consensus, the following practical recommendations are proposed:

1. Case selection: Choose immature necrotic teeth with open apices, good prognosis and patient ability to commit to follow-up.
2. Pre-operative documentation: Record baseline anatomy (radiographs/CBCT), pulp status, periapical status and root-development stage.
3. Irrigation protocol: Use lower concentration NaOCl (1.5–3%) with moderate volume (10–20 mL), side-vented needle, no extrusion. Follow with 17% EDTA rinse for 1–2 minutes. Activation (ultrasonic/sonic) may be used if available [33].
4. Intracanal medicament: Use calcium hydroxide or low-concentration antibiotic paste (without minocycline) for 1–4 weeks. Monitor symptom resolution before proceeding.
5. Scaffold induction: If using apical bleeding, gently penetrate apical tissues beyond the foramen to induce bleeding to about 2–3 mm below canal orifice. Alternatively, when platelets concentrate (PRF/PRP) is used, standardize preparation.
6. Coronal barrier: Place 2-4 mm bioceramic material (e.g., white-MTA, Biodentine or newer bioceramic) over the scaffold, then a permanent restoration with rubber-dam isolation to ensure coronal seal.
7. Follow-up protocol: Recommended follow-ups at 3, 6, 12 and 24 months minimum. At each visit: assess clinical signs/symptoms, sensibility (if appropriate), obtain standardised periapical radiograph (or CBCT if indicated) using reproducible geometry[34][35].
8. Outcome reporting: At each time-point document: (a) tooth retained and asymptomatic, (b) radiographic periapical healing (PAI or CBCT volumetric), (c) root length/width changes (report method), (d) apical closure (partial/complete), (e) sensibility test result, (f) adverse events (discolouration, PCO, fracture, retreatment).
9. Data transparency: Report full protocol details (irrigant type/concentration/volume/time, medicament type/duration, scaffold type/volume, barrier material, restoration details), imaging parameters, measurement methods, examiner calibration and follow-up losses.
10. Long-term monitoring: Plan for follow-up beyond 24 months (ideally 3–5 years or more) to assess long-term tooth survival, root integrity and late complications such as fracture or canal obliteration[36].

Research Gaps & Future Directions

Despite encouraging data, several gaps remain:

- Core outcome set development: There is no universally accepted core outcome set for REPs. Developing such a set with international consensus would facilitate comparative research.
- High-quality randomised controlled trials (RCTs): Few RCTs exist comparing different scaffolds (blood clot vs PRF/PRP vs synthetic), medicaments (CH vs TAP), or irrigation regimens, and many have short follow-up and small samples.
- Objective imaging metrics: Standardised, validated methods for quantifying root maturation (length/width/volume) are lacking. Automation or semi-automated segmentation may improve reliability.
- Long-term data: Follow-up longer than 3–5 years is required to report on survival, structural integrity (fracture risk), pulp canal obliteration and functional outcomes.
- Cost-effectiveness and clinical implementation: Studies comparing cost, chair-time, patient satisfaction and long-term outcomes versus conventional treatments are needed.
- Biologic adjuncts and scaffold innovation: More clinical data required for cell-based therapies, growth-factor enriched scaffolds, and biomimetic materials. While promising in vitro/animal studies exist, clinical translation remains limited [37].

Limitations of the Current Evidence

Several limitations must be acknowledged:

- Many studies are case reports/series, lacking controls and randomisation.
- Protocol heterogeneity is significant, making pooling of results difficult.
- Outcome reporting is inconsistent (different domains, time-points, imaging methods).
- Follow-up durations are often short (<12-24 months), limiting assessment of long-term maturation and survival.
- Publication bias: many reviews report primarily successful cases, under-reporting of failures is likely.
- Sensibility testing in immature teeth may be unreliable, and imaging assessments vary in standardisation.

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These limitations underscore the caution required when interpreting success rates and comparing across studies.

Conclusion

Regenerative endodontic procedures represent a major paradigm shift in the treatment of immature necrotic teeth, leveraging biologic principles to enable continued root development and preservation of tooth function. The core procedural elements are increasingly well defined, and preliminary success rates are highly encouraging. However, to convert potential into predictable routine practice, standardisation is essential: standardised clinical protocols, uniform outcome domains, reproducible imaging methods and long-term follow-up. Adoption of core outcome sets and rigorous trial design will improve evidence quality and guide clinical decision-making.

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