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# Regenerative Endodontic Treatment in Clinical Practice: A Systematic Review

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## Abstract

Recently, regenerative endodontic treatment (RET) has been recognized as a 'paradigm shift' regarding the management of immature teeth associated with pulp necrosis. Various reports have suggested the use of RET as a first-line therapeutic option when dealing with immature teeth, particularly when the roots are not yet fully developed. For that, a systematic electronic database search was conducted for relevant studies published from inception and till 24th June 2020 in seven databases. Several position statements and clinical recommendations regarding the use of RET in clinical practice have been released by the European Society of Endodontology (ESE) and the American Association of Endodontists (AAS). Such treatment modalities depend upon the tissue engineering concept components, including stem cells, scaffolds, and signaling molecules. In clinical practice, this treatment approach involves the process of disinfection and commonly the introduction of a blood clot into the root canal space. There are three main therapeutic goals of RET. The first goal is the resolution of associated clinical signs and symptoms. The second goal is to establish further root maturation. The final goal is the restoration of neurogenesis. Up till this point, RET has been unable to reach a proven result regarding the real regeneration of pulp tissue and dentine space. From a histological aspect, healing is achieved with repair from tissues that are originated mainly from the periodontal and osseous tissues, including bone-like tissues and cementum, rather than odontoblasts and pulp tissues. We have conducted the current literature review to provide an overview of the clinical and biological aspects of regenerative endodontic treatment.

***Keywords:*** *Regenerative; Endodontic; Treatment; Pulp: RET*

## Introduction

The main aim of pulp treatment is to maintain the structure of the tooth intact in order to preserve optimal functionality. Another purpose of pulp treatment is to maintain the vitality of the teeth that are damaged secondary to dental caries or trauma. Particularly, in

the case of an immature permanent tooth, maintain pulp vitality is of critical value for continuous root development and apical closure. In cases where the pulp of an immature permanent tooth is an infection, apexification with the removal of the infected pulp along with the application of calcium hydroxide is a traditional approach in clinical practice [1]. Calcium hydroxide is commonly used as the material of choice in closing the apical foramen. Despite the fact that apexification can result in apical closure; however, it cannot maintain pulp vitality [2]. In recent years, regenerative endodontic treatment (RET) has been suggested in an attempt to replace damaged pulp tissues with viable ones. That is of particular importance when the tooth is damaged secondary to trauma. In this case, pulp RET is approached because the pulp tissue is not infected. On the other hand, in circumstances when apical periodontitis is correlated with a tooth infection, RET is not considered. However, recently, a case report highlighted optimal outcomes in terms of continuous root development and apical closure with the application of antibiotics [3]. The procedures that are used in this treatment approach are based on the concept that stem cells have a potent regenerative capacity, being multipotent at the same time. Those stem cells are used for the induction of pulp regeneration in order to treat immature permanent teeth in a conservative approach [4].

During the apexification process, calcium hydroxide is commonly used as an intracanal medication. This requires its replacement every 3 months, combined with a long-term follow-up. In this context, the long-term application of intracanal medications increases the liability of root fracture and subsequent tooth defects in the root walls because of its porous characteristics [1,2]. Meanwhile, RET has been shown to result in complete root development, especially after a short-term procedure. Therefore, RET results in increased root length and thickness [3,5].

RET is a procedure that includes root canal disinfection through the use of several antibiotics. Multiple aerobic and anaerobic bacteria can result in root canal infection, and thus, it would be challenging to effectively disinfect these root canals with just one type/category of antibiotics. Therefore, it has been recommended that a combination of three antibiotics should be used, including ciprofloxacin, metronidazole, and minocycline [6,7]. That being said, there is not much known about the clinical and biological aspects of RET. Therefore, we conducted the current investigation to systematically review the available literature in order to discuss the clinical features, biological aspects, outcomes, and limitations of regenerative endodontic treatment.

## Methods

### Search strategy and study selection

The study process was conducted following the accepted methodology recommendations of the PRISMA checklist for systematic review [8]. A systematic electronic database search was conducted for relevant studies published from inception and till 24$^{th}$ June 2020 in seven databases including Google Scholar, Scopus, Web of Science (ISI), PubMed, Cochrane Central Register of Controlled Trials (CENTRAL), Embase and CINAHL using keywords, medical subject (MeSH) terms. In databases not supporting MeSH terms, combinations of all possible terms were used. Moreover, We conducted a manual search of references from the included articles by searching the primary studies that had cited our included papers and scanning references of the relevant papers in PubMed and Google Scholar to avoid missing any relevant publications [9].

We included the most appropriate published studies that reported regenerative endodontic treatment in clinical practice. Papers were excluded if there were one of the following exclusion criteria: non-human (*in vitro* or animal) studies, duplicate records, data could not be reliably extracted or incomplete reports, abstract only articles, thesis, books, conference papers, and poor quality papers. The title and abstract screening were done independently by four reviewers. Then, three independent reviewers performed a full-text screening to ensure the inclusion of relevant papers in our systematic review. Any disagreement was resolved by discussion and referring to the senior author when necessary.

### Data extraction

Two authors developed the data extraction sheet using the Microsoft Excel software. Data extraction was performed by three independent reviewers using the excel sheet. The fourth independent reviewer performed data checking to ensure the extracted data accuracy. All the disagreements and discrepancies were resolved by discussion and consultation with the senior author when necessary.

### Quality assessment

Three independent reviewers evaluated the risk of bias in the included studies. The National Institutes of Health (NIH) quality assessment tools were used to determine the quality of included studies, according to their study design [10]. Any discrepancy between the reviewers was solved through discussion. This step was done only to assess the quality of the evidence to include only studies with fair to good quality (and exclude studies with poor quality), that is why no reporting of individual studies was provided.

## Results and Discussion

### Search results

We identified 2,310 records after excluding of 420 duplicates using the Endnote X9 software. Title and abstract screening resulted in 97 records, which were reduced to 41 papers following full-text screening. Moreover, four papers were added after performing manual search trials. Finally, we included 45 of the different study designs for this systematic review (Figure 1).

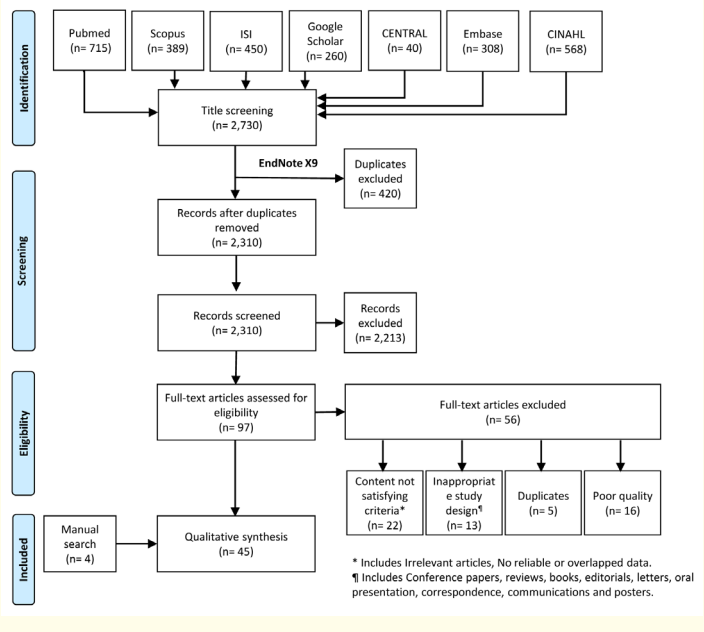


***Figure 1:*** *PRISMA flowchart of the search and screening process.*

### Current approaches for pulp-dentine regeneration

The pulp tissue engineering strategy requires the interaction of three essential factors: stem cells from the apical papilla (SCAP), scaffolds, and signaling molecules [11]. Whilst stem cells from the pulp are through to have the greatest potential to form odontoblast-like

cells [12] these may also be derived from the periodontal ligament and bone marrow cells. Also, it is believed that the pulp micro-environment is of utmost importance and that the fate of the stem cells could be dependent on the presence of infection, biofilms, and other factors [11,13]. Meanwhile, other scaffolds can be manufactured, consisting of various biodegradable polymers [14]. In the majority of clinical techniques, the scaffold is provided either by a blood clot, or the addition of platelet-rich plasma (PRP), or platelet-rich fibrin (PRF). A recent report has shown that PRP, PRF, and platelet-pellet (PP) can also yield similar clinical and radiological outcomes to a created blood clot, even without the need for previous apical bleeding. This is also associated with significantly less tendency for root canal closure [15]. In the same context, signaling molecules can be released from the demineralized dentine matrix and are believed to be chemotactic and to promote cellular adhesion. Furthermore, blood-derived proteins can also act as signaling molecules, and they are found in the blood clot that is formed or created in the canal [16].

## The current recommendations of AAE regarding RET procedures

Since there are many suggested procedures of RET, it is of particular importance to acknowledge the procedure that is commonly and generally used in practice. The American Association of Endodontics (AAE) recommends the use of RET in a compliant patient with an immature tooth, necrotic pulp, immature apex, and pulp space not required for post and core (Figure 2). Prior to initiating RET procedures, it is essential to discuss all of the benefits as well as all of the potential risks of RET with the patient following the collection of relevant clinical data and establishing a pulpal and peri-radicular diagnosis. The tooth is then anesthetized and isolated with rubber dam in order to create an access point. Copious, gentle irrigation with 20 mL sodium hypochlorite (NaOCl) is recommended through the use of an irrigation system that limits the possibility of extrusion of irrigants into the periapical space. Moreover, the use of lower concentrations of NaOcl is also advised to minimize cytotoxicity to stem cells in the apical space. After that, the root canal is dried with sterile paper points, while the antimicrobial medicament is being applied into the canal space. Hereafter, a paste containing three antibiotics is applied to disinfect the canal space [17]. This triple antibiotic paste has the advantage of being very effective against intracanal bacteria [6,7]. Meanwhile, even though calcium hydroxide has the advantage of being widely used in other areas, its use in RET may be cytotoxic to stem cells [18]. Following the application of antimicrobial medicament, the tooth is sealed off with a temporary filling, and the patient is advised to visit for a check-up after 3 to 4 weeks.

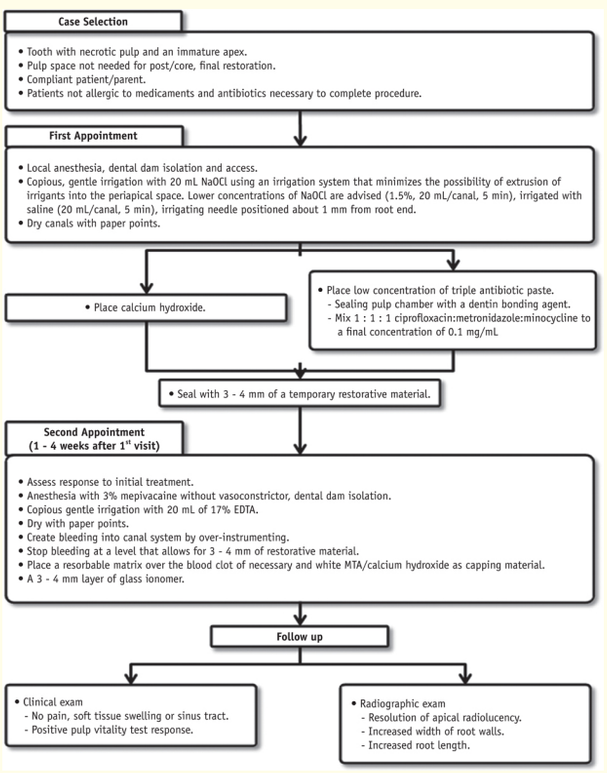


***Figure 2:*** *Current consideration for RET (Courtesy of the AAE). NaOCl: Sodium hypochlorite; EDTA: Ethylenediaminetetraacetic Acid; MTA: Mineral Trioxide Aggregate [19].*

During the follow-up appointment, the patient is examined for the resolution of inflammation (the disappearance of signs and symptoms of acute infection). When the resolution is achieved, it is proper to proceed with the following step of RET. At this step, local anesthetic without vaso-constrictors is recommended in order to avoid the disturbance of intracanal bleeding. Following rubber dam isolation and the formation of a coronal access point, the tooth is then irrigated with 20 mL ethylenediaminetetraacetic acid, followed by normal saline, while antimicrobial medicament is carefully withdrawn. After canal drying through a paper point, a file is placed within a few millimeters beyond the apical foramen, while bleeding is being induced up to 3 mm from the cementoenamel junction (CEJ). In order to place the mineral trioxide aggregate (MTA), a colla-plug, which acts as a resorbable matrix, is then placed into the canal. After that, 3 mm of MTA is placed, followed by positioning the final restoration [17]. Finally, a 12- to 18-month recall time is proposed as the minimal time to radiographically judge the evidence of root development along with the need for further clinical examination [18].

### Case selection

RET can be considered as a viable treatment option for immature teeth with necrotic pulp. It has been suggested that RET is the treatment of choice for teeth with Class 1 - 3 root conditions, as classified in 1992 [20]. For teeth with more mature root development, the MTA barrier technique may be more appropriate [21]. However, mature teeth have also been treated successfully with RET [22] but this would not be appropriate for teeth requiring post-placement in a prosthodontic rehabilitation situation. Patient compliance is also a consideration since multiple visits are needed.

### Informed consent/alternative treatment options

The informed consent includes the discussion with the patients, but if the patient is under the age of consent, a discussion with a parent or a guardian should be considered. The treatment generally involves two visits and further follow-up appointments. Certain antibiotics may cause allergic reactions to certain and liable patients. Adverse events can include pain, swelling, tooth discoloration, as well as non-healing. The informed consent should also include alternative treatment modalities such as apexification, apical barrier techniques with bioactive endodontic cements, extraction, and the risks involved in case the treatment is not proceeded [23].

### Root canal disinfection

The preservation of stem cells is critical in RET; however, if the infection is not controlled or properly managed, not only the regeneration but also the repair will not occur [24]. Therefore, in RET, intra-radicular infections should be under control to enable the realization of pulp tissue regeneration [25].

### Antiseptic irrigation

- Sodium hypochlorite, NaOCl, is the most commonly used antiseptic irrigating solution in root canal treatment [26]. Concentrations of 1 to 6% of NaOCl have been used in RET [27]. The AAE “Clinical Considerations for a Regenerative Procedure” recommends the use of 1.5% NaOCl followed by 17% EDTA [23].

- EDTA is a chelating agent that is used to remove the smear layer in conventional root canal treatment, as well as to promote the release of growth factors from the dentine matrix in RET [28]. EDTA in RET has also been tested against SCAP *in vitro*, rather than in clinical scenario with intracanal bacteria and biofilms *in vivo* [28]. The use of 17% EDTA resulted in an increased expression of SCAP survival, as well as partially reversed the deleterious effects of NaOCl [29]. EDTA demineralizes dentine and exposes the dentine matrix to the released growth factors [28,30]. In cases where the minimal filling was undertaken, the use of EDTA and the removal of the smear layer may expose the binding sites for attachment of newly formed tissue to the canal walls and promotes stem cell differentiation, migration and adhesion to dentine [30]. EDTA conditioning promoted the adhesion, mi-

gration and differentiation of dental pulp stem cells towards or onto the dentine [31]. Therefore, a final rinse with EDTA before the creation of a blood clot is recommended.

- Chlorhexidine is cytotoxic to stem cells [32] and thus, it is not recommended in RET.

### Medicaments

- Calcium hydroxide is recommended as an intracanal medication in RET because of its good antimicrobial properties [33]. It has been tested on the survival of SCAP *in vitro*, rather than on the elimination of intracanal bacteria *in vivo* [29,32]. Furthermore, the possible effects of calcium hydroxide on the biological properties of the dentine matrix-derived growth factors need to be investigated in RET. Bose., *et al.* [18] reported that when calcium hydroxide was radiographically restricted to the coronal half of the root canal system, the median percentage increase in the dentinal wall thickness was 53.8% compared with an increase of 3.3%, observed when it was placed in the apical half of the root canal. However, when calcium hydroxide was placed in the apical canal, the percentage increase in root length was not affected [18]. A recent *in vitro* study had shown that the attachment of human apical cells to the root dentine was higher when it was treated with calcium hydroxide compared with TAP [34]. It has been speculated that a long-term intracanal dressing with calcium hydroxide might increase the risk of root fracture [35]. However, a recent study used lamb mandibular anterior teeth dressed with three commercial products of calcium hydroxide during a period up to 9 months, and then loaded the teeth until fracture in a universal testing machine and observed no statistical difference between experimental and control groups [36]. The authors concluded that root fracture occurred after using calcium hydroxide as dressing material might be more related to the stage of root development than to the long-term use of calcium hydroxide [36].

- TPA has been used by Hoshino., *et al*. [6] and Sato., *et al*. [7] to sterilize infected root canals *in vitro*. One major concern with the use of antibiotics is the possibility of a systemic allergic reaction to occur, so the medical history should be carefully taken into account. When performing RET in immature permanent teeth with infected necrotic pulp tissue, TAP (minocycline, ciprofloxacin, and metronidazole) has been recommended as an intracanal medication [23] based on *in vitro* studies that observed excellent antimicrobial activity of TAP against bacterial species existing in infected root canals [6,7] TAP has also been tested as to its effects on the survival of SCAP *in vitro* [37] and is recommended to be used at a concentration no greater than 1 mg/ml (from 0.1 to 1 mg/m) in RET to avoid damaging SCAP [23].

### Blood clots

### Creation of a blood clot or protein scaffold in the canal

After the disinfection of the canal and resolution of symptoms, RET usually involves lacerating the periapical tissues to initiate bleeding or the use of PRP or PRF. RET recommends that after irrigation of the intracanal medicament with 17% EDTA (30 ml for 10 minutes), bleeding should be induced by rotating a pre-curved size 25 K-file at 2 mm past the apical foramen with the objective of filling the canal with blood until the CEJ level [23]. In RET, it is advisable to use a local anesthetic without a vasoconstrictor to encourage bleeding [38]. It may take 15 minutes for a blood clot to form. If bleeding does not occur, a further appointment can be scheduled, or a more traditional approach such as an MTA apical barrier or calcium hydroxide apexification can be used to treat the immature tooth [38]. An important study demonstrated that the evoked-bleeding step in RET triggers a significant accumulation of undifferentiated stem cells into the canal space [39]. Contemporary regenerative endodontics is based on bioengineering principles; the interaction between stem cells, scaffolds, and growth factors is key for the formation of engineered tissues [16].

### Intracanal barrier

### Effective coronal seal

Once a blood clot or a scaffold is in place within the canal, a barrier is placed to prevent the coronal leakage of microorganisms. In 2015, 85% of studies used MTA for this purpose [27]. Current protocols recommend to carefully place a premeasured piece of CollaPlug on top of a blood clot that has just been formed, to serve as an internal matrix for the placement of approximately 3 mm of white MTA followed by a 3 to 4 mm glass ionomer layer over the MTA. A bonded reinforced composite resin restoration is then placed over the glass ionomer [23]. MTA is a biocompatible material with bioactive properties resistant to bacterial contamination.

### Follow-up

Most studies have undertaken 6-month radiographic reviews to assess further root maturation. Bose., *et al*. [18] showed that radiographic changes could be detected as early as 6 months, although greater percentage changes occurred between 12 to 30 months. A study utilizing quantitative analysis showed progressive maturation of two teeth at 1- and 36-month reviews [40].

### Types of root maturation

Chen., *et al*. [41] described five types of calcific response radiographically following RET:

- Type 1: Canal walls (increase in thickening); root maturation (continued).
- Type 2: Root maturation (not significantly continued); root apex (blunt and obliterated).
- Type 3: Root maturation (continued); apical foramen (still open).
- Type 4: Canal space (severe calcification/obliteration).
- Type 5: The development of a hard tissue barrier inside the canal just between the coronal MTA plug and the root apex.

The variations in these responses may be explained by the continued viability of the Hertwig's epithelial sheath (HERS) and its ability to withstand trauma; HERS can be damaged by apical periodontitis or abscess, as well as physically damaged by the laceration of the periapical tissue during the creation of blood clot.

### Intracanal calcification

Song., *et al*. [42] reported that intracanal calcification occurred in 72.2% of 29 teeth that were treated with RET. The revascularization-associated intracanal calcification (RAIC) occurred more frequently in teeth dressed with calcium hydroxide (76.9%) compared with teeth treated with antibiotic pastes (46.2%).

### Regeneration or repair

Regeneration is defined as the restoration of the tissue architecture and biological function of damaged tissues with tissue similar to the original [43]. Repair is the replacement of damaged tissue by tissue different from the original tissue and loss of biological function [43]. In animal and human studies, after RET, the damaged pulp tissue in the canal space of immature teeth is replaced by bone-, cementum-, and periodontal ligament-like tissue. Therefore, RET is considered a reparative and not a regenerative process histologically [44]. Repair is not the ideal wound healing process because the damaged tissue loses its physiological function.

### Histological characterization of tissues formed in the canal space

Many clinical case reports and case series have shown that, after RET, immature permanent teeth with necrotic pulp may result in the radiographic thickening of the canal walls and/or continued root maturation or apical closure. In addition, the tissue engineering triad component (blood clot, mesenchymal stem cells, and bioactive growth factors) have been brought into the root canal space during the induction of periapical bleeding in RET. However, the nature of tissues formed in the canal space can only be determined by histological examination. From the many histological studies in animals and humans, RET of immature teeth with necrotic pulp and apical periodontitis revealed that the tissues formed in the canal space were mineralized tissue, similar to cementum and bone, and fibrous connective tissue resembling the periodontal ligament [45-47]. The thickening of the canal walls and/or apical closure were due to the apposition of cementum- or bone-like tissue and not dentine. In some cases, the apical root canal space was almost completely filled with mineralized tissue. In other studies, there was minimal thickening of the canal walls, and the canal space contained fibrous connective tissue [48]. The ingrowth of apical alveolar bone into the apical canal space was discernible in some cases.

Histologically, RET of human immature permanent teeth with necrotic pulp is considered reparative and not regenerative. The radiographic thickening of the canal walls and the continued root maturation of immature permanent teeth with necrotic pulp after RET should not be regarded as a regeneration process of the dentine pulp complex without histological confirmation. If the primary goal of RET is the elimination of clinical symptoms/signs and the resolution of apical periodontitis [23] then repair, although not being the ideal wound healing process, should not be considered as treatment failure.

### Adverse outcomes and limitations of RET

### Discoloration

Many studies have shown that discoloration is a significant problem following RET. This is of particular concern for traumatized anterior teeth since pleasing aesthetics is a patient-centered outcome. The discoloration is more often associated with TAP that includes minocycline, although discoloration has also been reported with calcium hydroxide. In a prospective study, Kahler., *et al*. [40] noted discoloration in 10 out of 16 cases (62.5%), of which 13 were incisors when minocycline was substituted with amoxicillin in the TAP.

Bleaching is generally effective in improving the aesthetic outcomes. As stated in the AEE guidelines, patients should be advised that discoloration of teeth is often associated with RET [23].

### Other adverse events

Studies have shown that most failures associated with RET are due to inadequate disinfection and persistence of infection [29,49]. Failed cases are primarily attributed to inadequate removal of biofilms, possibly due to minimal instrumentation, or inadequate disinfection. Failure has also been associated with reinfection of the root canal system, which may be attributed to failed restoration that allows coronal leakage.

Three recent studies have shown further root maturation despite persistent apical periodontitis [50-52]. While this phenomenon is likely related to HERS, the primary goal of RET is to eliminate the signs and symptoms of apical periodontitis. The treatment of immature permanent teeth after failed RET includes nonsurgical root canal treatment, RET, or apexification.

Root fractures in teeth treated with RET have been reported [29,53]. A limitation of this technique is that root thickening cannot occur in the cervical third of the root where the intracanal barrier is placed and where teeth are susceptible to further root fracture at this level.

### Outcome studies

A number of studies have shown that apexification, MTA apical barrier techniques, and RET are viable treatment options for treating immature teeth with apical periodontitis [54-56]. However, only RET has the potential to promote further root maturation. Therefore, immature teeth with less than 2/3 of root development should be treated with RET in the 1st instance, although the increase of further root maturation is variable [40]. There remains a lack of high-quality studies that directly compare outcomes of RET with apical barrier techniques, and thus, further research is still warranted.

## Conclusion

RET can be considered as the first treatment option for immature teeth with pulp necrosis, particularly when the root has developed less than three-fourths, as described in the literature. There are reports of RET being utilized for mature teeth where the tissue engineering concept components, stem cells, scaffolds, and signaling molecules, provide biologically-based treatment. Currently, there is much research being under-conduction to improve the clinical outcomes and to achieve true regeneration. There are still many unanswered questions about the cellular and molecular processes that allow for the deposition of new calcific material and neurogenesis with RET. Current techniques may offer patients improved outcomes compared with apexification and apical barrier techniques.

## Funding

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## Conflicts of Interest

No conflicts related to this work.

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