Regenerative endodontic treatment: a clinical focus and review

KEY WORDS
immature teeth, pulp necrosis, regenerative endodontic treatment, stem cells, tissue engineering

ABSTRACT
Regenerative endodontic treatment (RET) has been described as a ‘paradigm shift’ in the treatment of immature teeth with pulp necrosis. It has been suggested that RET should be the first option for treating immature teeth when the roots are not yet fully developed. The European Society of Endodontology and the American Association of Endodontists have released position statements and clinical considerations for RET. Treatment modalities rely on the tissue engineering concept components stem cells, scaffolds and signalling molecules. Clinically, the treatment involves disinfection of, and often the introduction of a blood clot into, the root canal space. RET has three goals: 1) resolution of clinical signs and symptoms; 2) further root maturation; and 3) restoration of neurogenesis. To date, RET has been unable to establish a real regeneration of the pulp tissue and dentine space. Histologically, healing is achieved with repair from tissues derived primarily for the periodontal and osseous tissues such as bone-like tissue and cementum, rather than pulp tissue and odontoblasts. This review aims to present a clinical focus on RET and to provide a current view on this emerging clinical technique.

Introduction
Immature permanent teeth with necrotic pulp or apical periodontitis have traditionally been treated with an apexification procedure using calcium hydroxide to induce the apical hard tissue barrier formation, or with an apical mineral trioxide aggregate (MTA) plug, before root canal filling1-3. The disadvantages of the calcium hydroxide apexification include the need for multiple treatment visits, over an extended period of time3. In addition, there is an increased risk of root fracture in immature permanent teeth that are exposed to prolonged calcium hydroxide dressing4. Barrier techniques such as an apical MTA plug can shorten the treatment time3. The treatment outcomes of calcium hydroxide apexification and the apical MTA plug appear to be comparable3,5,6. However, an apexification procedure has no ability to restore the vitality of the damaged tissue and to promote root maturation (thickening of the root canal walls and/or apical closure) of immature permanent teeth with necrotic pulp. In 2001, a new treatment option termed ‘revascularisation’ was introduced in endodontics to manage immature permanent teeth with apical periodontitis and sinus tract7.

History and terminology
The term ‘revascularisation’ was first used by Iwaya et al7. Later, ‘revitalisation’ was proposed instead of ‘revascularisation’, as a more applicable term since the tissues regenerated in the root canal space were not only blood vessels but also hard and soft tissues8. The term ‘regenerative endodontics’ was adopted by the American Association
Regenerative endodontic treatment (RET) was defined as “biologically based procedures designed to replace damaged tooth structures, including dentine and root structures, as well as cells of the pulp-dentine complex”\(^9\). Based on this definition, regenerative endodontic treatment (RET) is aimed at regenerating the pulp-dentine complex damaged by infection, trauma or developmental anomaly of immature permanent teeth with necrotic pulp.

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 signalling molecules\(^17\). Whilst stem cells from the pulp are thought to have the greatest potential to form odontoblast-like cells\(^18,19\), these may also be derived from the periodontal ligament and bone marrow cells. Also, it is believed that the pulp microenvironment is of utmost importance, and that the fate of the stem cells could be dependent on the presence of infection, biofilms and other factors\(^17,20\). Other scaffolds can be manufactured and consist of a number of biodegradable polymers\(^21\). In most 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 study has shown that PRP, PRF, and a platelet-pellet (PP) can also yield similar clinical and radiographic outcomes to a created blood clot, without the need for prior apical bleeding and with significantly less tendency for root canal obliteration\(^22\). Signalling molecules can be released from the demineralised dentine matrix and are believed to be chemotactic and to promote cell adhesion. Blood-derived proteins also act as signalling molecules and are found in the blood clot created in the canal\(^23\).

Materials and methods

Clinical procedures

The clinical procedures are outlined below and have been reproduced from Kahler and Lin\(^24\).
These recommendations reflect the clinical procedures developed by the AAE, “Clinical Considerations for a Regenerative Endodontic Procedure”\textsuperscript{15}, revised in 2018. These recommendations should be considered as a possible source of information and, given the rapid evolving nature of this field, clinicians should also actively review new findings elsewhere as they become available.

**Case selection**

- Tooth with necrotic pulp and an immature apex.
- Pulp space not needed for post and core placement in the final restoration.
- Patient or parental compliance.
- Patients who are not allergic to medicaments and antibiotics required to complete the procedure (according to the American Society of Anesthesiologists [ASA] classification; patients classified as ASA 1 or 2).

**Informed consent**

- Two (or more) appointments.
- Use of antimicrobial(s).
- Possible adverse effects: staining of crown/root, lack of response to treatment, and pain/infection.
- Alternative treatments: MTA apexification, no treatment, extraction (when deemed non-salvageable).

**Clinical procedure – first appointment**

- Local anaesthesia, dental dam isolation and access.
- Copious, gentle irrigation with 20 ml of NaOCl using an irrigation system that minimises the possibility of extrusion of irrigants into the periapical space (e.g. needle with closed end and side-vents, or EndoVac). Lower concentrations of NaOCl are advised (20 ml of 1.5% NaOCl/canal, for 5 minutes) and then irrigation with saline or ethylenediamine tetraacetic acid (EDTA) (20 ml/canal, for 5 minutes), with the needle positioned about 1 mm from the root end, to minimise cytotoxicity to the stem cells in the apical tissues.

**Clinical procedure – second appointment**  
*(1 to 4 weeks after the first visit)*

- Dry the canals with paper points.
- Place calcium hydroxide, Ca(OH)\textsubscript{2}, or a low concentration TAP. If TAP is used: 1) consider sealing the pulp chamber with a dentine bonding agent (to minimise the risk of staining); and 2) mix ciprofloxacin, metronidazole, and minocycline equally (1:1:1) to a final concentration of 1 to 5 mg/ml.
- Deliver this solution into the canal system via a syringe. The TAP has been associated with tooth discoloration. A double antibiotic paste (DAP) without minocycline paste or the substitution of minocycline with another antibiotic (e.g. clindamycin, amoxicillin or cefaclor) is another possible alternative as a root canal disinfectant. Clinicians should be aware that some studies have already assessed higher concentrations of TAP/DAP, but recommendations to use higher concentrations of these pastes are not currently available due to the limited number of studies.
- Deliver the solution into the canal system via a syringe.
- If a triple antibiotic is used, the clinician should ensure that it remains below the cementoenamel junction (CEJ) to minimise crown staining.
- Seal with 3 to 4 mm of provisional restorative material such as Cavit, intermediate restorative material (IRM), glass ionomer or other provisional material.
- Dismiss the patient for 1 to 4 weeks.
at 2 mm past the apical foramen with the goal of having the entire canal filled with blood until the CEJ level. An alternative to creating a blood clot is to use a PRP, PRF or an autologous fibrin matrix (AFM).

- Stop bleeding at a level that allows the placement of 3 to 4 mm of restorative material.
- Place a resorbable matrix such as CollaPlug (Zimmer Dental, Warsaw, IN, USA), CollaCote (Zimmer Dental), CollaTape (Zimmer Dental) or another material over the blood clot, if necessary, and place MTA as a capping material.
- A 3 to 4 mm layer of a glass ionomer (e.g. Fuji IX, GC America, Alsip, IL, USA) should be flowed gently over the capping material and light-cured for 40 seconds. MTA has been associated with discoloration. Alternatives to MTA, such as resin-modified glass ionomers (RMGI) or bioceramics (e.g. Biodentine [Septodont, Saint-Maur-des-Fossés, France] or EndoSequence BC RRM-Fast Set Putty [Brasseler USA, Savannah, GA, USA]) should be considered when there is an aesthetic concern.
- Anterior and premolar teeth: consider the use of CollaTape or CollaPlug and restoring with 3 mm of a nonstaining restorative material, followed by bonding a filled composite to the bevelled enamel margin.
- Molar teeth or teeth with a porcelain-fused-to-metal (PFM) crown: consider the use of CollaTape or CollaPlug and restoring with 3 mm of MTA, followed by a RMGI, composite or alloy.

Follow-up (6, 12 and 24 months)

- A clinical and radiographic exam.
- No pain, soft tissue swelling or sinus tract (often observed between the first and second appointments).
- The resolution of apical radiolucency (often observed 6 to 12 months after treatment).
- Increased width of the root walls (this is generally observed before an apparent increase in the root length and often occurs 12 to 24 months after treatment).
- Increased root length.
- A positive pulp vitality test response.
- Follow-up recommended yearly after the first 2 years.
- A cone beam computed tomography (CBCT) is highly recommended for initial valuation and follow-up visits.

Follow-up goals

RET degree of success is largely measured by the extent to which it is possible to attain primary, secondary and tertiary goals.

- Primary goals: the elimination of symptoms and evidence of bone healing.
- Secondary goals: an increased root wall thickness and/or increased root length (desirable, but perhaps not essential).
- Tertiary goals: a positive response to vitality testing (which if achieved, may indicate a more organised vital pulp tissue).

Outcome criteria

Outcome criteria are defined by primary, secondary and tertiary goals15. The primary goal is the elimination of symptoms and evidence of bone healing. The secondary goal is further root maturation (increased length, increased canal wall thickness and apical closure), which is considered desirable but not essential. The tertiary goal is eliciting a positive response to pulp sensibility testing15.

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 to 3 root conditions, as classified by Cvek in 199225. For teeth with a more mature root development, the MTA barrier technique may be more appropriate26. However, mature teeth have also been treated successfully with RET27,28 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 required.
Informed consent/alternative treatment options

The informed consent includes a discussion with the patient, but if the patient is under the age of consent, a discussion with the parents or guardians should be conducted; the treatment generally involves two visits and further follow-up appointments. Certain antibiotics may cause allergic reactions to patients. Adverse reactions can include pain, swelling and tooth discolouration, as well as nonhealing. The informed consent should also include alternative treatment options such as apexification, apical barrier techniques with bioactive endodontic cements, extraction and the risks involved in case the treatment is not proceeded.

Root canal disinfection

The preservation of stem cells is important in RET; however, if the infection is not under control, not only the regeneration but also the repair will not occur. Therefore, in RET, intraradicular infections should be under control to enable the realisation of pulp tissue regeneration.

Antiseptic irrigants

- Sodium hypochlorite, NaOCl, is the most commonly used antiseptic irrigating solution in root canal treatments. Concentrations of 1 to 6% NaOCl have been used in RET. The AAE ‘Clinical Considerations for a Regenerative Procedure’ recommends the use of 1.5% NaOCl followed by 17% EDTA.
- EDTA is a chelating agent used to remove the smear layer in conventional root canal treatments and to promote the release of growth factors from the dentine matrix in RET. EDTA in RET has also been tested against SCAP in vitro, rather than on the elimination of intracanal bacteria in vivo. Furthermore, the possible effects of calcium hydroxide on the biological properties of the dentine matrix-derived growth factors needs to be investigated in RET. Bose et al. 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. A recent in vitro study has shown that the attachment of human apical cells to the root dentine was higher when it was treated with calcium hydroxide compared with TAP. It has been speculated that a long-term intracanal dressing with calcium hydroxide might increase the risk of root fracture. 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. The authors concluded that root fracture occurring 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.

- TAP has been used by Hoshino et al. and Sato et al. to sterilise 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, metronidazole) has been recommended as an intracanal medication based on in vitro studies that observed excellent antimicrobial activity of TAP against bacterial species existing in infected root canals. TAP has also been tested as to its effects on the survival of SCAP in vitro, and is recommended to be used at a concentration no greater than 1 mg/ml (from 0.1 to 1 mg/ml) in RET to avoid damaging SCAP. However, some studies have suggested that Augmentin may be as effective as TAP in RET. Nevertheless, the ESE position statement on the use of antibiotics in endodontics suggests that in the absence of strong evidence supporting the use of antibiotics in RET, they should be avoided. Many studies have shown that root canal irrigating solutions and intracanal medicaments are not able to completely eliminate bacterial biofilms in infected root canals. Therefore, mechanical debridement has been recommended as part of root canal disinfection procedures against biofilms in infected root canals. In RET, mechanical debridement is not recommended in immature permanent teeth with necrotic pulp because removing the root canal dentine may weaken the already thin and fragile root and render it more prone to fracture. However, the effectiveness of disinfection protocols in RET has been questioned. In both nonsurgical and surgical endodontic treatments, the control of root canal infections is the key to a successful RET.

**Blood clot**

**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. In RET, it is advisable to use a local anaesthetic without a vasoconstrictor to encourage bleeding. 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 a MTA apical barrier or calcium hydroxide apexification can be used to treat the tooth. An important study demonstrated that the evoked-bleeding step in RET triggers a significant accumulation of undifferentiated stem cells into the canal space. 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.

**Intracanal barrier**

**Effective coronal seal**

Once a blood clot or 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. 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 (Dentsply, Tulsa, OK, USA) 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. 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\(^4\) showed that radiographic changes could be detected as early as 6 months although greater percentage changes occurred between 12 and 30 months. A study utilising quantitative analysis showed progressive maturation of two teeth at 1- and 36-month reviews\(^6\).

Types of root maturation

Chen et al\(^6\) described five types of calcific responses radiographically following RET:
- Type 1, increased thickening of the canal walls and continued root maturation.
- Type 2, no significant continuation of root development with the root apex becoming blunt and closed.
- Type 3, continued root development with the apical foramen remaining open.
- Type 4, severe calcification (obliteration) of the canal space.
- Type 5, a hard tissue barrier, formed in the canal between the coronal MTA plug and the root apex.

The variations in these responses may be explained by the continuous viability of the Hertwig's epithelial root sheath (HERS) and its ability to withstand trauma; HERS can be damaged by apical periodontitis or abscesses, as well as physically damaged by the laceration of the periapical tissue during the creation of the blood clot\(^6\).

Intracanal calcification

Song et al\(^7\) reported that intracanal calcification occurred in 72.2% of 29 teeth that were treated with RET. The revascularisation-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\(^7\). Repair is the replacement of damaged tissue by tissue different from the original tissue and loss of biological function\(^7\). 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\(^7\). Repair is not the ideal wound healing process because the damaged tissue loses its physiological function.

Histological characterisation 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 components (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 mineralised tissue, similar to cementum and bone, and fibrous connective tissue resembling the periodontal ligament\(^7\). The thickening of the canal walls and/or apical closure were due to apposition of cementum- or bone-like tissue and not dentine. In some cases, the apical root canal space was almost completely filled with mineralised tissue\(^8\). In other studies, there was minimal thickening of the canal walls and the canal space contained fibrous connective tissue\(^8\). The ingrowth of apical alveolar bone into the apical canal space was discernible in some cases\(^8\).
Histologically, RET of human immature permanent teeth with necrotic pulp are considered reparative and not regenerative\textsuperscript{73,74}. 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 a histological confirmation. If the primary goal of RET is the elimination of clinical symptoms/signs and the resolution of apical periodontitis\textsuperscript{15}, then repair, although not being the ideal wound healing process, should not be considered as treatment failure\textsuperscript{73,74}.

\textbf{Is root maturation predictable?}

A recent systematic review reported that 97 of 101 teeth (96\%) treated with RET between 2001 and 2014 were successfully treated as measured by some degree of apexogenesis, including increased root length, increased root thickness and apical closure. Only 4 teeth (4\%) failed to show further root maturation. Apical closure was detected in fewer cases (55.4\%) compared with teeth with increased root length (76.2\%) and increased root width (79.2\%), which were reported more frequently\textsuperscript{84}.

\textbf{Discolouration}

Many studies have shown that discolouration is a significant problem following RET\textsuperscript{85}. This is of a particular concern for traumatised anterior teeth since pleasing aesthetics is a patient-centred outcome. Discolouration is more often associated with TAP that includes minocycline although discolouration has also been reported with calcium hydroxide. In a prospective study, Kahler et al\textsuperscript{68} noted discolouration in 10 out of 16 cases (62.5\%), of which 13 were incisors, when minocycline was substituted with amoxicillin in the TAP.

Dabbagh et al\textsuperscript{86} replaced minocycline with cefaclor, which resolved the issue of discolouration. It also appears that this problem is less likely when calcium hydroxide is used as an intracanal medicament; Nagata et al\textsuperscript{60} reported only 3 discoloured teeth out of 11 during treatment with calcium hydroxide and chlorhexidine, as opposed to 10 out of 12 with TAP (which included minocycline). In a spectrophotometric analysis of discolouration induced by various antibiotic pastes used in revascularisation, TAP had a significant association with discolouration; however, calcium hydroxide showed no colour changes exceeding the perceptibility threshold\textsuperscript{87}. MTA, which is the most commonly used material as intracanal barrier, has also been shown to discolour teeth\textsuperscript{88}. To minimise the risk of discolouration, Biodentine (Septodont) can be used instead of MTA\textsuperscript{89-91}.

Bleaching is generally effective to improve the aesthetic outcome\textsuperscript{92}. As stated in the AAE guidelines, patients should be advised that discolouration of teeth is often associated with RET\textsuperscript{15}.

\textbf{Other adverse outcomes and limitations}

Studies have shown that most failures associated with RET are due to inadequate disinfection and persistence of infection\textsuperscript{59,78,93}. Failed cases are primarily attributed to inadequate removal of biofilms possibly due to minimal instrumentation\textsuperscript{59}, or inadequate disinfection\textsuperscript{94,95}. Failure has also been associated with reinfection of the root canal system\textsuperscript{96}, which may be attributed to failed restorations that allow coronal leakage.

Three recent studies have shown further root maturation despite persistent apical periodontitis\textsuperscript{95,97,98}. While this phenomenon is likely related to HERS, the primary goal of RET is to eliminate the signs and symptoms of apical periodontitis\textsuperscript{15,26}. The treatment of immature permanent teeth after failed RET includes nonsurgical root canal treatment\textsuperscript{95}, regenerative endodontic retreatment\textsuperscript{99}, or apexification\textsuperscript{63}.

Root fractures in teeth treated with RET have been reported\textsuperscript{78,79,82}. 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\textsuperscript{6,100,101}. However, only RET has the potential to promote further root maturation. Therefore, immature teeth with less than two-thirds of root development should be treated with RET in the first instance, although the increase of further root maturation is variable\textsuperscript{68,101,102}. There remains a lack of high-quality studies that directly compare outcomes of RET with apical barrier techniques and, hence, further research is warranted\textsuperscript{103}.

Case reports

Figures 1 to 8 illustrate cases treated with RET under similar protocols for immature teeth with pulp necrosis and apical periodontitis and/or abscesses with either an aetiology of fractured dens evaginatus tubercle, trauma or dens evaginatus\textsuperscript{104,105}. Lin et al\textsuperscript{100} reported that dens evaginatus cases had a better prognosis than trauma cases, after RET. However, other studies consider RET to have comparable outcomes to traditional apexification or apical barrier techniques\textsuperscript{6,100,106}. Other authors recommend that RET should be considered as a first treatment option for immature teeth with pulp necrosis\textsuperscript{107}, especially when the root is in the earlier stages of development\textsuperscript{26}.

Figure 2 is a case that demonstrates the importance of HERS survival in promoting continued arrested root development in immature permanent teeth with necrotic pulp/apical periodontitis after a regenerative endodontic protocol. When the pulp is infected and inflamed, the biological function of HERS cells and stem cells in the inflamed periapical tissues is inhibited by proinflammatory cytokines\textsuperscript{108,109}. After RET, the infection and inflammation of the pulp and periapical tissues gradually subsides, and the biological function of HERS and stem cells in the periapical area is reactivated. HERS cells regulate the development of
not only the root but also the root canal system. Therefore, in this case, continued root development in length and lateral canal formation were observed radiographically due to HERS survival. HERS is capable of signalling cells in the apical papilla to differentiate into apical odontoblasts, and cells in the dental follicle to differentiate into cementoblasts for physiological root maturation. Although the tissue formed in this continued root development is not confirmed without an histological examination, the patient- and clinician-centred outcomes were successfully achieved in this case.

**Future directions**

Currently, there are two approaches to pulp tissue regeneration in regenerative endodontics, namely cell-free and cell-based. Both approaches are based on the tissue engineering triad, which is to apply stem cells, bioactive growth/differentiation factors and biomimetic scaffolds for the formation of engineered tissue. The cell-free approach involves homing of endogenous stem cells, transplantation of a scaffold and growth factors or chemo-attractants in the canal space. A clinical RET can be considered a cell-free approach.
**Fig 4a to d**  
(a) Traumatic injuries to both maxillary central incisors resulting in a chronic periapical abscess in the right central incisor and apical periodontitis in the left central incisor; both were treated with regenerative endodontic treatment (RET).  
(b) An 18-month review radiograph showing intracanal calcification of the maxillary left central incisor and resolution of signs of infection.  
(c) A 3-year review radiograph showing further calcification of the maxillary left central incisor.  
(d) A 5-year review radiograph showing near-complete calcification of the maxillary left central incisor. The maxillary right central incisor has not shown further root development. There was a deposition of calcific material adjacent to the mineral trioxide aggregate (MTA) intracanal barrier. Images reproduced and adapted with permission.  

**Fig 5a to d**  
(a) A preoperative radiograph of a maxillary right central incisor that had sustained an uncomplicated crown fracture and developed pulp necrosis and asymptomatic apical periodontitis. This was noted during an orthodontic assessment and the tooth was subsequently treated with regenerative endodontic treatment (RET).  
(b) Postoperative radiograph.  
(c) An 18-month review radiograph showing no apical closure and some increase in the periodontal ligament space.  
(d) A 6-year review radiograph showing further root development and apical closure. Some calcific material was evident in the apical third of the root. Reproduced with permission.  

**Fig 6a to d**  
(a) Preoperative radiograph of a maxillary left central incisor that had sustained an uncomplicated crown fracture and then developed pulp necrosis and symptomatic apical periodontitis. The tooth was treated with regenerative endodontic treatment (RET).  
(b) At the 3-year follow-up, there appeared to be no significant changes in the root length or width compared with the preoperative radiograph. A calcific opacity was noted at the apex, which most likely was the proliferation of the Hertwig’s epithelial root sheath (HERS), resulting in some root development.  
(c) A 5-year review radiograph.  
(d) A 9-year review radiograph. A calcific barrier was also present in the root canal space (white arrow). Reproduced and adapted with permission.
The cell-based approach employs transplantation of exogenous stem cells by ex vivo expansion and a scaffold in the canal space. Technically, a cell-free is simpler than a cell-based approach because the former does not have to be concerned about stem cell source and isolation. However, in the cell-free approach the endogenous stem cells are not pulp tissue-specific. The cell-based approach employs pulp tissue-specific stem cells, such as dental pulp stem cells, stem cells from exfoliated primary teeth and SCAP. These stem cells have been shown to be capable of differentiating into odontoblasts and produce dentine. Hurdles to the cell-based approach include the limited availability of stem cell sources, cell harvest and expansion, logistics, higher cost and regulatory requirements. However, for the future pulp tissue regeneration therapy to be an attainable goal, the focus should be on the concept of stem cell-based pulp tissue engineering.

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 by Cvek. There are reports of RET being utilized for mature teeth where the tissue engineering components, stem cells, scaffolds and signalling molecules, provide biologically-based treatments. Currently, there is much research being undertaken to improve the clinical outcomes and to achieve a ‘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.
Regenerative endodontic treatment


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