RESEARCH PAPER

Local Drug Delivery Approaches In Regenerative Endodontics: Intracanal Medicaments, Biomimetic Scaffolds, And Emerging Clinical Strategies

Ziyad Mohammed Alabdulsalam[1], Rayyan A. Alwazzan[2], Yaser M Almazrou[3], Ayman S. AlBihlal[4]
[1]Consultant endodontist, General Department of Medical Services in Presidency of State Security, Riyadh, Saudi Arabia. BDS, SSC - endo
[2]AEGD, McDPH, Ministry of Interior, Riyadh, Saudi Arabia
[3]Consultant Endodontics, Prince Naif Security Campus, Riyadh, Saudi Arabia. BDS, SBE
[4]Consultant in Endodontics, Presidency of State Security, Riyadh, Saudi Arabia. BDS, SBE

Abstract: Regenerative endodontic therapy (RET) is a biologically based treatment modality for immature permanent teeth with necrotic pulp and incomplete root formation, developed to overcome the limitations of apexification, which does not restore pulp vitality or predictably support further root maturation. RET is founded on tissue-engineering principles involving stem cells, scaffolds, growth factors, and a disinfected canal environment. Among these, local drug delivery is a central component, as intracanal irrigants, medicaments, and scaffold-associated bioactive systems directly influence disinfection, stem cell survival, and regenerative outcomes.

This review summarizes current concepts in regenerative endodontics with emphasis on intracanal drug delivery and biomimetic scaffold design. It outlines the biologic rationale of RET, recommended clinical protocols, case selection criteria, and treatment outcomes, while highlighting the role of low-concentration sodium hypochlorite, EDTA, calcium hydroxide, and antibiotic pastes in achieving canal disinfection with minimal toxicity to apical stem cells. The review also discusses scaffold-based strategies, including blood clot, platelet-rich derivatives, collagen, hydrogel, and other bioengineered matrices, as well as emerging delivery platforms such as antibiotic-loaded nanofibers and patient-specific three-dimensional constructs.

Available evidence indicates that RET achieves favorable rates of symptom resolution and periapical healing, although continued root development, canal wall thickening, and true pulp–dentin complex regeneration remain variable and less predictable. Current limitations include discoloration, intracanal calcification, reinfection, and inconsistent histologic outcomes. Nevertheless, advances in scaffold technology, local therapeutic delivery, stem cell transplantation, and cell-homing approaches continue to expand the regenerative potential of endodontic therapy. RET therefore represents a promising intersection of endodontics, biomaterials, and localized drug-delivery science with growing translational relevance.

Keywords: Regenerative endodontics; Regenerative endodontic therapy; Intracanal drug delivery; Scaffolds; Stem cells; Growth factors; Immature necrotic teeth; Biomimetic materials; Tissue engineering; Root maturation

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Introduction

Permanent teeth with necrotic pulp/apical periodontitis without complete root formation/apical closure cannot be treated by conventional endodontic treatment and have been previously treated with apexification procedures.[1-3] Traditionally, apexification procedure involved use of calcium hydroxide to induce apical hard tissue barrier formation and after the advent of Mineral Trioxide aggregate (MTA) apical MTA plug is formed followed by root canal filling using gutta percha.[3] But in teeth with incomplete root formation these procedures are associated with certain disadvantages. Use of calcium hydroxide for apexification usually involves multiple treatment visits over an extended period of time and exposing already weak roots to prolonged calcium hydroxide dressings is anticipated to increase the risk of root fracture.[4] While MTA apexification is performed in one or two visits only thereby reducing the treatment time however outcome of both the techniques appears to be similar with none of them strengthening the already weak roots by promoting the maturation of roots or restoring the vitality of damaged pulp tissue.[4,5] To overcome these issues a new treatment option of revascularization was introduced by Iwaya et al in year 2001 to manage an immature permanent tooth with apical periodontitis and sinus tract.[6] Over a period of time the term revascularization was proposed to be renamed as revitalization due to observation of both hard and soft tissues in canal space other than blood vessels.[7]

American association of endodontists adopted the term Regenerative Endodontics in 2007 based on the concept of tissue engineering. Regenerative endodontics is 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[8]. Based on this definition, regenerative endodontic therapy (RET) is aimed to regenerate the pulp–dentine complex damaged by infection, trauma or developmental anomaly of immature permanent teeth with necrotic pulp.

Pioneering experimental studies in regenerative endodontics were done by Nygaard-Ostby[9] and Nygarrd-Ostby & Hjortdal.[10] Nygarrd-Ostby & Hjortdal observed fibrous connective tissue and cellular cementum were formed in the apical canal space of teeth originally containing vital pulp on histological examination 9 days to 3 years after induced bleeding from the periapical tissues into the chemo-mechanically debrided canal space of teeth, which was partly filled with root filling.[10] However, in teeth with necrotic pulp no repair tissue was formed in the apical canal space. Clinically, Iwaya et al. were the first group to apply the concept of revascularization to treat immature permanent teeth with apical periodontitis and sinus tract.[6]

Despite its potential, the clinical efficacy and, more importantly, the consistency of RET in achieving predictable root development remain debatable.[11-13] To improve the outcomes of RET, recent advancements have mainly concentrated on incorporating principles of tissue engineering to refine this technique.

Successful biological outcomes in tissue engineering rely on the meticulous integration of three key components: Stem cells, scaffolds and growth factors, all within a sterile environment. The lack or insufficiency of any of these elements can jeopardise the success of treatment.[14]

Stem cells

Different populations of adult stem cells that can be induced to differentiate into odontoblast-like cells in specific conditions have been identified. These include dental pulp stem cells (DPSCs), stem cells of the apical papilla (SCAPs), periodontal ligament stem cells (PDLSCs), inflammatory periapical progenitor cells (iPAPCs) and bone marrow stem cells (BMSCs).[15] Some residual vital pulp tissues may exist in the apical region even when teeth develop pulp necrosis, apical periodontitis or periapical abscess and can be used in RET to promote tissue regeneration.[16] SCAPs from the apical tissue have capacity of proliferation and odontogenic differentiation that is beneficial for root development [17–20] with added benefit of their location proximal to teeth apices making them the most promising stem cell source for RET. PDLSCs and BMSCs are also the potential stem cell sources for RET as evoked bleeding from the apical tissue may induce the release of these cells.[15,21] The iPAPCs appear to be largely localized in the vasculature within apical granulomatous tissues, and represent another important potential source of stem cells for RET.[22]

Growth factors

Growth factors are polypeptides produced by immuno-inflammatory and tissue cells and bound to extracellular matrix. They regulate many aspect of cellular function, including survival, proliferation, migration and differentiation.[23] Growth factors typically have a short half-life and are rapidly eliminated. They usually have specific temporal and spatial expression during tissue regeneration and repair.[23] Growth factors determine the fate of stem/progenitor cells and are often immobilized in scaffold to help promote tissue regeneration in tissue engineering.

Dentin matrix acts as a reservoir of growth factors,[24] which may be released through demineralization of dentin matrix by bacterial acid, irrigation with sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA), stimulation by calcium hydroxide and silica-calcium biomaterials
such as MTA and Biodentine [25–27] These dentine matrix molecules include growth factors, noncollagenous proteins and glycosaminoglycans.[28] Besides, the blood clot formed during RET also contains certain growth factors.[29] The dentin-derived growth factors are believed to play a key role in progenitor cell recruitment, proliferation, differentiation, and promoting tissue regeneration.[24,30] For example, transforming growth factor-β1 (TGF-ß1) and fibroblast growth factor 2 (FGF2) have been implicated in promoting cell migration and proliferation.[21] Vascular endothelial growth factor (VEGF) plays an important role in cell proliferation and regulation of angiogenesis while bone morphogenetic protein (BMP) and FGF2 mediate the signaling in dentin formation.[21] Non-collagenous proteins (NCPs) including dentin matrix protein and dentin phosphoprotein may be involved in odontogenesis.[28]

Exogenous growth factors have also been used to produce synergistic effect with autologous growth factors in RET.[31,32] Collagen scaffolds loaded with human recombinant platelet-derived growth factor (rPDGF) have successfully promoted root maturation in an immature tooth with pulp necrosis.[31] And a clinical trial demonstrated that injectable hydrogel scaffolds impregnated with basic fibroblast growth factor (bFGF) achieved apical healing and continued root development in teeth with pulp necrosis.[33]

Scaffolds

A scaffold is a key element for tissue engineering to guide stem cells location and regulate cell proliferation, differentiation or metabolism. It could also help to promote nutrient and gaseous exchanges.[34] Blood clot, autologous platelet concentrates and synthetic biomaterials could serve as the scaffolds. Among them, blood clot and autologous platelet concentrates are the most commonly used scaffolds during RET. Blood clot has been induced as a scaffold in most of the RET cases,[31,35–37] which is a relatively straightforward and simple approach. It allows integrators on cell surfaces to adhere to fibrous components and selectively adsorb cells, supplying growth factors to promote tissue regeneration. However, blood clot is not easy to obtain, and lacks some properties as an ideal scaffold including easy delivery, good mechanical properties, controllable biodegradation, and incorporation of growthfactors.[38] Moreover, the blood clot contains numerous hematopoietic cells, which could release toxic intracellular enzymes during cell death into the microenvironment, compromising stem cell survival.[39]

Another approach for creating a scaffold is the use of autologous platelet concentrates, including platelet-rich plasma (PRP), platelet- rich brin (PRF) and concentrated growth factor (CGF). These autologous scaffolds require minimal manipulation in vitro and are easy to prepare. They consist of three-dimensional fibrin matrix and abundant bioactive molecules, and can degrade over time. Several RET cases have achieved success with these autologous scaffolds.[40-42] However, drawbacks are also presented in the clinical use, such as requiring collection of intravenous blood with special equipments and difficult to control the types and concentration of growth factors during preparation. Also, their lack of temporal degradation control and inadequate mechanical strength to support the coronal restoration further compromise their application.[39]

Several exogenous scaffolds including collagen type 1,[31] hydrogel,[33] and collagen- hydroxyapatite[43] have been clinically used in RET. These scaffolds are usually loaded with growth factors before placing into immature root canals. The resolution of clinical symptom and apical radiographic radiolucency, as well as the continued root development, has indicated its success in clinical application. In addition, decellularized dental pulp has gained some interest in studies as a potential scaffold for pulp regeneration. An in vivo study showed that decellularized dental pulp of swine implanted into pulpectomized teeth in dogs could induce the formation of a vascularized pulp-like tissue with expression of odontoblastic markers.[44]

Clinical protocol of RET

A data analysis of clinical protocol of RET has revealed that RET protocols varied considerably amongst all studies.[32] Despite the variations in clinical protocols, the key steps of conducting RET are (a) Minimal instrumentation of canal wall; (b) Disinfection using irrigants (c) Dressing with an intracanal medicament; (d) Introducing bleeding into the canal space and creating a blood clot; (e) Capping with bioceramic materials; (f) An effective coronal seal as summarised in Table 1.[45] These issues should be considered when implementing the treatment procedures.

Table 1. Treatment Procedures of REPs

Appointments	Procedural Steps	Recommendations
	Adequate local anaesthesia	No emphasis on the use of vasoconstrictors
	Rubber dam isolation Superficial disinfection by 2% chlorhexidine or 2% povidone iodine Removal of coronal infected tissue under dental operating microscope Access cavity preparation	
	Working length determination	By radiograph with a file positioned at 1 mm from apex
	Minimal instrumentation	Using larger size of les or reamers such as Hedström files Circumferentially "brushing" the canal walls without major dentin removal
Procedures of first appointment	Irrigation with 1.5~3% NaOCl (20 mL per canal, 5 min)	Bleeding or exudate may require extended irrigation until they can be controlled with paper points Irrigating needle is suggested to be positioned 1~2 mm from the root end
	Irrigation with sterile saline (5 mL per canal)	Irrigating needle is suggested to be positioned 1~2 mm from the root end
	Dry with paper points Irrigation with 17% EDTA (20 mL per canal, 5 min)	
	Dry with paper points	AAE recommends the use of Ca(OH)2 or 1~5 mg·mL^{-1} antibiotic dressing ESE recommends the used of Ca(OH)2

	Intra-canal medicament with Ca(OH)2 or a low concentration of antibiotic dressing	The medicament should be placed below the cementoenamel junction (CEJ)
	Access restoration with temporary restorative material	Cavit or glass-ionomer can be used Restoration should be at least 3~4 mm
Procedures of second appointment	Clinical assessment of response to the first treatment	AAE recommends the recall of 1~4 weeks after the first visit ESE recommends the recall of 2~4 weeks after the first visit Repeat the treatment procedures of the first appointment if there are signs or symptoms of persistent infection
	Local anesthesia	3% mepivacaine without vasoconstrictor (epinephrine)
	Rubber dam isolation	
	Superficial disinfection	2% chlorhexidine or 2% povidone iodine is used
	Removal of temporary seal	
	Gentle irrigation with 17% EDTA under microscope to remove the intracanal medicament	AAE has no requirement for the volume and time of irrigation with EDTA ESE suggests the irrigation of 20 mL EDTA in 5 mins followed by 5 mL sterile saline Use ultrasonic activation if necessary
	Dry with paper points	
	Induction of bleeding	By rotating a pre-curved K- le with larger size (for example size #25) at 2 mm past the apical foramen until the whole canal filled with blood below the CEJ level Wait for 15 min for a blood clot formation
	Placement of resorbable matrix over the blood clot	CollaPlug , Collacote or CollaTape can be used. The matrix is trimmed into a diameter slightly larger than the coronal part of the root canal
	Placement of tricalcium silicate biomaterial	MTA, Biodentine®, EndoSequence® BC RRM-Fast Set Putty, etc. can be used. Capping material should be approximately 2 mm underneath the CEJ
	Application of light-cured glass ionomer	The layer should be at least 3~4 mm over the capping material
	Refreshment of the cavity walls with a diamond bur	Bonded reinforced composite resin is suggested

	Permanent restoration	
Follow-up requirement	The patients should be reviewed at 3 months, 6 months, 12 months, and yearly for a total of 5 years for regular clinical and radiographic examinations. CBCT is highly recommended for initial evaluation and follow - up visits.	

Minimal instrumentation

Immature permanent teeth apical diameters usually exceed the diameter of largest files, making mechanical instrumentation a challenging task with chances of further weakening the fragile and thin dentin wall of roots. Although there is no mechanical instrumentation recommended in the statement of ESE and the most recent guideline of AAE but without mechanical instrumentation, bacterial biofilm is likely to remain within dentinal tubules which may lead to a failure in RET.[46] Thus minimal instrumentation should be considered in RET in which canal walls are lightly brushed circumferentially with endodontic instruments such as K filles with larger size and Hedström filles to disrupt the bacterial biofilms without any aggressive removal of dentin.[45]

Chemical disinfection

Preservation of stem cells is important in RET. However, if infection is not under control, not only regeneration but also repair will not occur.[47] The presence of prior infection could negatively affect the process of pulp tissue regeneration by damaging tissue forming cells as well as stem cells in the periapical tissues.[48] Therefore, intra-radicular infection should be controlled for possibly pulp tissue regeneration to occur in RET.[49]

Sodium hypochlorite (NaOCl) with concentrations of 1–6% have been used in RET.[32] The NaOCl is the favorable irrigant in most RET studies due to its broad antibacterial spectrum and tissue dissolution properties. Although 6% NaOCl has been used in RET cases and achieved success,[50] several in vitro studies have shown that NaOCl had a concentration-dependent effect on the survival of SCAPs, with 6% NaOCl significantly reducing the stem cell survival.[51,52] Thus, a low concentration of NaOCl, is recommended in RET. On the other hand, 17% EDTA has been proved to rescue the detrimental side effects of NaOCl on stem cells attachment, survival and differentiation,[53] and promote the release of endogenous growth factors from the dentin.[54] Based on these evidence, the guidelines of AAE[55] and ESE[56] recommend the use of 1.5%~3% NaOCl followed by 17% EDTA in the first appointment and 17% EDTA in the second appointment of RET. This recommendation is mainly based on the studies that show the cytotoxic effect of sodium hypochlorite on survival of stem cells from the apical papilla in vitro rather than on killing of the intracanal bacteria in vivo.[51,52] Noteworthily, chlorhexidine is not recommended for RET because it does not have tissue dissolution capability and has been shown to be cytotoxic to stem cells.[57]

To enhance the efficacy of disinfection in root canals, irrigation methods such as negative pressure irrigation, passive ultrasonic irrigation (PUI), photon-induced photoacoustic streaming (PIPS) or laser, can be used.[49] Negative pressure irrigation demonstrates beneficial effect in minimizing the risk of irrigant extrusion through the apical foramen and has been used in RET. Needles with closed end and side-vents, or EndoVac™ are suggested irrigation devices, together with copious irrigant with a slow rate of infusion, to reduce the risk of extrusion into the periapical tissue. Importantly, the irrigating needle should be positioned 1~2 mm from apex to minimize cytotoxicity to stem cells seeding in the apical tissues. In addition, PUI can be considered in the second appointment in order to promote tissue dissolution and accelerate growth factors releasing from the dentin walls.[58,59] An in vitro study demonstrated that irrigation with PIPS activated EDTA for 40s led to the smear layer removal without undesirable impairment of dentin microhardness. Additionally, this irrigation created more cell-friendly dentin conditioning that was beneficial for SCAPs adhesion and survival.[60]

The use of 17% EDTA resulted in increased SCAP survival expression as well as partially reversing the deleterious effects of NaOCl.[52] EDTA acts to demineralize the dentine and expose the dentine matrix to release growth factors[61-63] In cases where minimal filing has been undertaken, the use of EDTA and removal of the smear layer may expose binding sites for attachment of newly formed tissue to the canal walls as well as stem cell differentiation[61,64] EDTA conditioning of dentine promoted the adhesion, migration and differentiation of dental pulp stem cells towards or onto dentine.[63]Therefore, a final rinse with EDTA before creation of a blood clot is advised. Again, release of growth factors from dentine matrix after EDTA treatment was performed in noninfected root canals.[62,64]

Intra-canal medicaments

Microbial ecology in the canals of traumatized immature permanent teeth with infected necrotic pulp was reported to be similar to that of mature permanent teeth.[65] Biofilms were also formed on the radicular canal walls and bacteria penetrated into the canal dentinal tubules of immature permanent teeth with infected necrotic pulp.[46,66] The use of topical antimicrobial agent to sterilize the infected root canal was first described by Grossman.[67] Later, Hoshino et al.[68] and Sato et al.[69] also used triple antibiotic paste to sterilize infected root canals in vitro.

A variety of medicaments have been used for root canal disinfection in the REPs, including triple antibiotic paste (TAP) with different combinations, double antibiotic paste (DAP) and $Ca(OH)_2$.[32] The TAP mixed with 1:1:1 ciprofloxacin, metronidazole and minocycline has been reported in 51%~80% of RET cases,[32] and its efficacy in disinfecting necrotic root canal systems has been demonstrated.[70,71] However, the effect of TAP on the survival of SCAPs has also been tested in vitro[72,73] and shown that high concentrations (10~100 $mg \cdot mL^{-1}$) of TAP were detrimental to the survival of SCAPs. The AAE has suggested a concentration of 1~5 $mg \cdot mL^{-1}$ TAP in RET to avoid damage of SCAPs.[55] At this concentration range, the antibiotics are prepared as solution formulation and TAP can be delivered into canal system via a syringe.

It should also be noted that TAP can cause tooth discoloration due to the component of minocycline. DAP without minocycline or replacing minocycline with other antibiotic substitution such as clindamycin and cefaclor are possible alternatives. If TAP is used, a dentin bonding agent can be used to seal the pulp chamber before placing medicament, and TAP is recommended to remain below cement-enamel junction (CEJ) level to minimize crown staining. It has also been suggested that one antibiotic, Augmentin may be as effective as triple antibiotic paste in RET.[74] Augmentin has been shown to kill 100% of microorganism isolated from the infected root canal associated with an apical abscess in vitro.[75] Unlike other antibiotics targeting bacterial protein or DNA synthesis, Augmentin inhibits bacterial cell wall synthesis. Human cells do not have cell wall; therefore, Augmentin only affects bacterial cells and not human cells.

$Ca(OH)_2$ is another intracanal medicament used in RET. Calcium hydroxide is recommended as intracanal medication in RET because of its good antimicrobial property.[76-78] Calcium hydroxide has a high pH 12.5–12.8, which is not a favourable environment for most bacteria to survive.[79] In addition, calcium hydroxide can hydrolyse the lipid moiety of gram-negative bacterial lipopolysaccharide (LPS), thus resulting in the release of free hydroxy fatty acids and degradation of LPS.[80] It appears to be less effective than TAP in antibacterial capacity,[81,82] but has several advantages over TAP including no discoloration, lower cytotoxicity to stem cells, greater survival and proliferation of stem cells on the treated dentin, promotion of growth factor release from the treated dentin and easier removal from root canals. It has been speculated that a long-term calcium hydroxide treatment as an intracanal dressing of teeth might increase the risk of root fracture.[4] But, $Ca(OH)_2$ used in RET is less likely to reduce fracture resistance because of the relatively short-term use as intracanal dressing for 1~4 weeks. Due to the above desirable effects, the ESE has recommended $Ca(OH)_2$ as the prior intra- medicament in RET.[56] It was reported that when calcium hydroxide was radio- graphically restricted to the coronal half of the root canal system, the median percentage increase in dentinal wall thickness was 53.8% as compared to a 3.3% increase when it was placed in the apical half of the root canal.[83] However, where calcium hydroxide was placed in the apical canal, it did not affect the percentage increase in root length. A recent study has shown that the attachment of human apical cells to root dentine was greater when treated with calcium hydroxide rather than TAP in vitro.[84] Furthermore, water-based calcium hydroxide slightly increased the amounts of (TGF)-b1 compared with the use of EDTA alone, although this finding was not statistically significant. [62]

Though antibiotic pastes and $Ca(OH)_2$ have been used as intracanal medicaments in RET, there are few studies comparing their effects in RET. A meta-analysis[85] presented that antibiotic pastes contributed to a higher percentage of root wall thickening while $Ca(OH)_2$ induced a higher percentage of apical closure. Recently, propolis paste has been proposed as an intra-canal medicament and shown similar disinfection effect with TAP in animal studies.[86,87] And pre-clinical studies found that novel intra-canal drug delivery system using nano fibers appeared to be an alternative of biocompatible disinfection strategy for REPs.[88,89]

Blood clot formation

The purpose of inducing intra-canal bleeding in RET is to create a blood clot as a scaffold and to promote growth factors and stem cells from apical region into canal lumen for tissue regeneration. It was believed that the stem cells induced into the canal space were from the apical papilla.[90] In clinical practice, inadequate intra-canal bleeding has been identified as a challenge to successful RET.[10,91]Severe destruction of periapical tissues, the resolution of inflammatory reaction after dressing with antibiotic paste and the use of local anesthesia containing epinephrine are the possible reasons for the failure to induce sufficient bleeding.[21,92] The available guidelines of AAE and ESE have proposed that vasoconstrictor-containing local anaesthetics should be avoided at the second appointment to minimize the possibility of inadequate intra-canal bleeding. If bleeding is insufficient, lidocaine, a potent vasodilator without epinephrine could be locally injected before attempting to induce apical bleeding.[93] If induction of periapical bleeding cannot be achieved at the treatment visit, the procedure can be postponed to the following visits until the periapical tissues recover from the severe injury. Platelet-rich plasma (PRP),[29, 94-96] platelet-rich fibrin (PRF)[29, 97-101]or Concentrated growth factors (CGF) can also be a clinical alternative in RETs when intra-canal bleeding induction is unsuccessful. There are conflicting evidences on their outcomes in RET. However, a systematic review of clinical studies of platelet concentrations in revitalization of immature necrotic teeth demonstrated that PRP or PRF was not significantly superior to a blood clot in promoting thickening of the canal walls/ continued root development in RET.[102] Furthermore, PRP was better than PRF or induced bleeding in the healing of periapical lesion, while no significant differences with respect to root lengthening and lateral wall thickening.[103] However, Zhou et al. argued that compared to blood clot alone, the combination of PRF and blood clot did not improve the outcomes of REPs.[104] Furthermore, there is still lack of evidence to prove that PRP or PRF could improve the regeneration of the pulp-dentin complex. Overall, induced bleeding is more commonly suggested as a scaffold in REPs of a non-vital immature permanent tooth.[103]

Case Selection for RET

Regenerative endodontic treatment is indicated in necrotic permanent teeth with incomplete root formation regardless of presence or absence of periradicular lesion. RET is not applicable in cases of immediate replantation after avulsion, cases with extensive loss of coronal structure requiring a post restoration or teeth with endo-perio lesions.

Stage of root development

Immature permanent teeth with necrotic pulp at the stage 1 (less than 1/2 of root formation with open apex), stage 2 (1/2 root formation with open apex) and stage 3 (2/3 of root development with open apex) are suitable for RET because of the short root, thin canal walls and wide-open apex[105] as apexification has no potential for root maturation (thickening of the canal walls and/or continued root development). Immature permanent teeth at stage 4 (nearly completed root formation with open apex) can be managed with either RET or an apical MTA plug and root canal filling because the canal walls have enough thickness and strength. However, RET has been recently performed on teeth with closed apices with pulp and peri radicular disease.[106,107]

Apical Diameter

Apical diameter of immature permanent teeth has been a major concern in RET and is still controversial. In early transplantation studies, it was concluded if apical foramen of the tooth was smaller than 1 mm, revascularization was unpredictable.[108] Newly formed tissue was found in the root canals with the smallest apical diameter ranging between 0.24 mm and 0.53 mm,[109] while Abada et al.[110] showed an increase of the ingrowth tissue in the root canals with increased apical diameter. In a clinical study, it was demonstrated that regenerative procedures were successful with apical diameter as small as 0.5 mm although immature permanent teeth with preoperative apical diameter wider than 1 mm demonstrated greater root maturation.[111] Recently, in a review article it was further shown that apical diameter smaller than 1 mm achieved clinical success after regenerative endodontic treatment. Nevertheless, apical diameters of 0.5–1.0 mm attained the highest clinical success rate.[112]

Age

RET is not indicated in deciduous teeth because of risk of disturbing eruption pattern of succeeding permanent teeth. Literature shows that 90% of patients undergoing RET were below 17 years old.[16]

Various studies reported failure in inducing root development in immature teeth after REPs in patients over 18 years old.[113-6] Better treatment outcome was achieved with greater root development in the younger patient group (9 to 13 years old) compared to the group of 14 to 18 years old.[111] This is possibly attributed to a greater healing capacity or stem cell regenerative potential in younger patients, which is evidenced by studies showing that proliferative and differentiation potential of MSCs decreased with aging.[117-8] Another important factor related to age is the stage of root development. The large diameter of open apex allows the ingrowth of tissue to the root canal space with a rich source of MSCs from the apical papilla. In this regard, the patient's age appears to be an important factor in case selection.

Outcomes

Clinical outcomes

The American Association of Endodontists clinical considerations for regenerative endodontic procedures define success by three measures:[55]

• Primary goal (essential): The elimination of symptoms and the evidence of bony healing

• Secondary goal (desirable): Increased root wall thickness and/or increased root length

• Tertiary goal: positive response to vitality testing

The primary goal of resolution of the signs/symptoms of infection and bone healing are generally achievable[119] although failed cases have been attributed to disinfection protocols and minimal filing. Two recent systematic reviews[11,120] demonstrated that the primary goal of RET could be reliably achieved with high probabilities (91–94% of periapical healing). The majority of the studies reported that RET had the potential to promote thickening of the canal walls and/or continued root development of immature permanent teeth with necrotic pulps (secondary goal). However, these observations are not always predictable after RET of immature permanent teeth with necrotic pulps[11,121-3]

Studies reported that the tertiary goal of return of a positive response to pulp sensibility testing after RET of immature permanent teeth with necrotic pulp was in 50–60% of published cases.[124,125] This does not necessarily indicate that a more organized vital pulp tissue is formed in the canals. It has been demonstrated that a human immature permanent tooth with apical periodontitis after RET regained pulp sensibility.[126] However, histologic and immunohistochemical findings of a human immature permanent tooth with apical periodontitis after regenerative endodontic therapy revealed cementum-like, bone-like and nerve fibres in the canal even though the vital tissue was not pulp tissue.[127] Vital tissues are normally vascularized and innervated and can respond to pulp testing. Therefore, positive response to pulp sensibility testing of immature permanent teeth with necrotic pulp after RET does not necessarily indicate regeneration of the pulp tissue.

The ESE has described a series of success criteria for REPs, including the absence of inflammation, healing of pre-existing bony lesion in the periapical tissue, increased root length and wall thickness, lack of external inflammatory resorption, a positive response to pulp sensibility testing, radiographic detection of new PDL along the inner wall of root canal, and no tooth discoloration.[56]

Histological outcome

In earliest attempts to regenerate the pulp tissue, the canal space was found filled with fibrous connective tissue and cementum,[9,10] fibrous connective tissue and bone,[128] hard and soft connective tissue[129]. In the subsequent animal studies, the tissues formed in the canal space of immature teeth were characterized as bone, cementum and periodontal ligament-like tissue. [61,130,131] In human studies, similar tissues were observed in the disinfected canal space of immature permanent teeth with necrotic pulp/apical periodontitis after RET.[132-4] It was proposed that the apical papilla could survive in apical periodontitis, and the stem cells from the apical papilla might migrate into the disinfected root canal space and differentiate into odontoblasts to produce dentine in RET [18,135]

Significantly higher expression of osteogenic markers on immunohistochemical staining than those in the mineralized tissue,[61] indicating RET may promote repair instead of inducing true regeneration. However, Torabinejad et al.[136] reported the possibility of regeneration of pulp-dentin complex when the apical 1~4 mm of vital pulp remained intact in immature teeth of ferrets. A recent case report has interestingly demonstrated that healthy fibrous connective tissue with blood vessels was found in the canal space and odontoblast-like cells were found along the newly formed dentin wall in the tooth with clinical success for 54 months after REPs.[137] In addition, the immunohistochemical outcomes showed positive staining of vascular and neuronal markers,[137] suggesting a partial regeneration of components of the pulp-dentin complex is possible

Success and Failure of RET

Successful resolution of periapical pathosis was reliably achieved (91%) with RET[11,120] Secondary outcomes of increased root development (80%) and apical closure (76%) were more variable results. Other studies reported that root maturation was not reliable achieved[138,139] so these outcomes may be more variable.

Failed cases can be due to inadequate removal of biofilm possibly due to minimal instrumentation[46] or inadequate disinfection[140,141] reinfection of the root canal system,[122] due to failed restorations allowing coronal leakage. In some cases, root fracture occurred.[132,133,142] One study[143] reported that the prevalence of revascularization-associated intracanal calcification was about 62.1% . This raises the concern how to treat root canal calcification of immature permanent teeth with failed revascularization. It has been suggested that the use of surgical operating microscope, ultrasonic tips and CBCT can help manage the immature permanent teeth with failed RCT[144]

The treatment of immature permanent teeth after failed RET includes root canal treatment,[141] regenerative endodontic retreatment,[145] or apexification.

Crown discolouration

Many studies have shown that discolouration is a significant aesthetic problem following regenerative endodontic treatment particularly for traumatized anterior teeth as appearance and pleasing aesthetics are patient centred outcomes. Discolouration is more often associated with TAP that includes minocycline although discolouration has also been reported with calcium hydroxide.[65] MTA can also cause tooth discolouration.[146] Two systematic reviews have reported discolouration after RET in 40% of cases.[120,138] To minimize the risk of discolouration, Biodentine instead of MTA can be used[147,148,149] Bleaching of discoloured teeth is generally effective to improve the aesthetic outcome.[150]

Recent Advancements

Based on the studies of human and animal regenerative endodontics, regeneration of dental pulp may be achieved by cell transplantation and cell homing approaches.

Stem cell transplantation is a cell-based approach involving the transplantation of exogenous stem cells loaded onto scaffolds into the root canal system to allow regeneration. Pulp/dentin regeneration has been reported in animal studies using exogenously transplanted dental stem cells with tissue engineering concept.[151-54] Nakashima et al clinically achieved pulp regeneration in permanent teeth with irreversible pulpitis through the transplantation of mobilized dental pulp stem cells (MDPSCs).[155] In a randomized controlled clinical trial, stem cells from exfoliated deciduous teeth were implanted into immature permanent teeth with pulp necrosis after trauma, and successfully reconstructed three-dimensional pulp tissue with blood vessels and sensory nerves.[156]These studies indicated that transplantation of stem cells would be a potential approach of whole pulp regeneration. However, this cell- based therapy faces many challenges in clinical translation due to the complexity of procedures, such as pulp extirpation, cell culture, stem/progenitor cell populations sorting, cell expansion, storage and shipping. In addition, potential contamination, difficulty with regulatory approval and the high costs should also be taken into account.

Cell homing has been regarded as cell-free approach to regenerate pulp-dentin complex through chemotaxis of endogenous stem cells into root canal via biological signaling molecules.[157] Kim et al. has firstly reported regeneration of pulp-like structure via the cell-homing approach in a mouse model. The human pulpless root canals were filled with collagen scaffolds loaded with bFGF, VEGF or PDGF as well as basal NGF and BMP7, and then the canals were transplanted subcutaneously into mice dorsum for 3 weeks. New dentin-like hard tissue and vascular pulp-like tissues with innervation and odontoblast layers were observed under microscope.[158] Since then, several in vitro studies illustrated the chemotactic effect of different cytokines including stromal cell-derived factor-1α (SDF-1α), stem cell factor (SCF), bFGF and BMP7 on dental stem/progenitor cells to support the cell homing strategy.[159,160] Yang et al. established an in vivo mice model and found that SDF-1α-loaded scaffolds generated vascularized connective tissues in the canals with fibrous matrix and new dentin.[161] Cell homing strategies omitted in vitro procedures for stem cell isolation and manipulation. Therefore, it might be simpler and easier to do in clinic as compared to the stem cell transplantation. However, there is a lack of knowledge in the type of growth factors to be used for pulp regeneration.

Despite the excellent effects in resolution of apical lesion, the outcomes of pulp regeneration by REPs are still unpredictable. Stem cells transplantation and cell homing are currently proposed as the potential ways to regenerate true pulp tissues with scientific validity. However, prospective clinical trials and histological evaluations are necessary to identify their applications in clinical translation, making them achievable and predictable in dental practice.

Conclusion

Regenerative endodontic therapy has introduced a major biologic shift in the management of immature permanent teeth with necrotic pulp by moving beyond traditional apexification toward procedures that aim to restore a functional intracanal environment and support continued root development. Current evidence shows that RET is highly effective in achieving its primary goals, particularly resolution of symptoms and healing of periapical pathology, while outcomes related to root wall thickening, apical closure, and return of pulp sensibility remain more variable and less predictable. The success of these procedures depends greatly on the balance between effective canal disinfection and preservation of stem cell viability, which highlights the importance of carefully selected intracanal irrigants, medicaments, and scaffold systems. In this regard, local drug delivery is not merely an adjunct but a central element in RET, influencing microbial control, growth factor release, and the biologic conditions required for repair or regeneration. Emerging biomimetic scaffolds, platelet-derived concentrates, antibiotic-loaded nanofibers, and cell-based or cell-homing strategies further strengthen the translational relevance of this field. Nevertheless, true regeneration of a functional pulp–dentin complex has not yet been achieved in a predictable manner, and many reported outcomes still appear to represent repair rather than complete regeneration. Future well-designed clinical trials, standardized protocols, and histologic studies are still needed to improve treatment predictability and to define the most effective biologically driven delivery systems for routine clinical use.

References:

1. Frank AL (1966) Therapy for the divergent pulpless tooth by continued apical formation. Journal of American Dental Association 72, 87–93.
2. Heithersay GS (1975) Calcium hydroxide in the treatment of pulpless teeth with associated pathology. *Journal of the British Endodontic Society* 8, 74–93.
3. Rafter M (2005) Apexification: a review. Dental Traumatology. 21, 1-8
4. Andreasen JO, Farik B, Munksgaard EC (2002) Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. Dental Traumatology 18, 134–7.
5. Chala S, Abougal R, Rida S, Moracco RR (2011) Apexification of immature teeth with calcium hydroxide or mineral trioxide aggregate: systematic review and meta-analysis.
6. Iwaya S, Ikawa M, Kubota M (2001) Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dental Traumatology 17, 185–7.
7. Huang GT-J, Lin LM (2008) Letter to the editor: comments on the use of the term "revascularization". Journal of Endodontics 34, 511.
8. Murray PE, Garcia-Godoy F, Hargreaves KM (2007) Regenerative endodontics: a review of current status and a call for action. Journal of Endodontics 33, 377–90.
9. Nygaard-Ostby B (1961) The role of the blood in endodontic therapy. An experimental histological study. Acta Odontologica Scandinavia 19, 324–53.
10. Nygarrd-Ostby B, Hjortdal O (1971) Tissue formation in the root canal following pulp removal. Scandinavian Journal of Dental Research 79, 333–49.

11. Tong HJ, Rajan S, Bhujel N, Kang J, Duggal M, Nazzal H. Regenerative endodontic therapy in the management of nonvital immature permanent teeth: A systematic review-outcome evaluation and meta-analysis. J Endod 2017;43:1453-64.

12. Almutairi W, Yassen GH, Aminoshariae A, Williams KA, Mickel A. Regenerative endodontics: A systematic analysis of the failed cases. J Endod 2019;45:567-77.

13. Petrino JA, Boda KK, Shambarger S, Bowles WR, McClanahan SB. Challenges in regenerative endodontics: A case series. J Endod 2010;36:536-41.

14. Nazzal H, Duggal MS. Regenerative endodontics: A true paradigm shift or a bandwagon about to be derailed? Eur Arch Paediatr Dent 2017;18:3-15

15. Hargreaves, K. M., Diogenes, A. & Teixeira, F. B. Treatment options: biological basis of regenerative endodontic procedures. J. Endod. 39, S30–S43 (2013).

16. Zou, X. Y. & Yue, L. Biological basis and clinical exploration of regenerative endodontic therapy. Zhonghua Kou Qiang Yi Xue Za Zhi 57, 3–9 (2022).

17. Ruparel, N. B., de Almeida, J. F., Henry, M. A. & Diogenes, A. Characterization of a stem cell of apical papilla cell line: effect of passage on cellular phenotype. J. Endod. 39, 357–363 (2013).

18. Huang, G. T. et al. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J. Endod. 34, 645–651 (2008).

19. Xu, L. et al. The apical region of developing tooth root constitutes a complex and maintains the ability to generate root and periodontium-like tissues. J. Periodontal Res. 44, 275–282 (2009).

20. Sequeira, D. B. et al. Regeneration of pulp-dentin complex using human stem cells of the apical papilla: in vivo interaction with two bioactive materials. Clin. Oral. Investig. 25, 5317–5329 (2021).

21. Kim, S. G., Malek, M., Sigurdsson, A., Lin, L. M. & Kahler, B. Regenerative endodontics: a comprehensive review. Int Endod. J. 51, 1367–1388 (2018).

22. Liao, J., Al Shahrani, M., Al-Habib, M., Tanaka, T. & Huang, G. T. Cells isolated from in amed periapical tissue express mesenchymal stem cell markers and are highly osteogenic. J. Endod. 37, 1217–1224 (2011)

23. Barrientos S, Stojadinovic O, Golinko MS, Brem H, Tomic-Canic M (2008) Growth factors and cytokines in woundhealing. Wound Repair and Regeneration 16, 585–601.

24. Smith, A. J., Duncan, H. F., Diogenes, A., Simon, S. & Cooper, P. R. Exploiting the Bioactive Properties of the Dentin-Pulp Complex in Regenerative Endodontics. J. Endod. 42, 47–56 (2016).

25. Tomson, P. L. et al. Dissolution of bio-active dentine matrix components by mineral trioxide aggregate. J. Dent. 35, 636–642 (2007).

26. Smith, A. J., Lumley, P. J., Tomson, P. L. & Cooper, P. R. Dental regeneration and materials: a partnership. Clin. Oral. Investig. 12, 103–108 (2008).

27. Zeng, Q. et al. Release of Growth Factors into Root Canal by Irrigations in Regenerative Endodontics. J. Endod. 42, 1760–1766 (2016).

28. Kim SG (2017) Biological molecules for the regeneration of the pulp-dentin complex. Dental Clinic of North America 61, 127–41.

29. Narang I, Mittal N, Mishra N (2015) A comparative evaluation of the blood clot, platelet-rich plasma and platelet-rich fibrin in regeneration of necrotic immature permanent teeth: a clinical study. Contemporary Clinical Dentistry 6, 63–8.

30. Andrukhov, O., Behm, C., Blufstein, A. & Rausch-Fan, X. Immunomodulatory properties of dental tissue-derived mesenchymal stem cells: Implication in disease and tissue regeneration. World J. Stem Cells 11, 604–617 (2019).

31. Zhujiang A, Kim SG (2016) Regenerative endodontic treatment of an immature necrotic molar with arrested root development using recombinant human platelet-derived growth factor: a case report. Journal of Endodontics 42, 72–5.

32. Kontakiotis EG, Filippatos CG, Tzanetakis GN, Agrafioti A (2015) Regenerative endodontic therapy: a data analysis of clinical protocols. Journal of Endodontics 41, 146–54.

33. Nagy MM, Tawfik HE, Hashem AAR, Abu-Seida AM (2014) Regenerative potential of immature permanent teeth with necrotic pulps after different regenerative protocols. Journal of Endodontics 40, 192–8.

34. Nakashima M, Akamine A (2005) The application of tissue engineering to regeneration of pulp and dentin in endodontics. Journal of Endodontics 31, 711–8.

35. Cerqueira-Neto, A. et al. Clinical and Radiographic Outcomes of Regenerative Endodontic Procedures in Traumatized Immature Permanent Teeth: Inter-appointment Dressing or Single-Visit? J. Endod. 47, 1598–1608 (2021).

36. Abu Zeid, S. T., Alamoudi, R. A., Alothmani, O. S., Mokeem Saleh, A. A. & Siddiqui, A. Y. A Prospective Study of Long-Term Regenerative Endodontics Outcomes of Necrotic Immature Permanent Teeth: An 8-Year Follow-Up. Healthcare 9, https:// doi.org/10.3390/healthcare9121670 (2021).

37. Maniglia-Ferreira, C., Gurgel Filho, E. D., Gomes, F. A., Reis, S. A. & Pappen, F. G. 12-Year Follow-Up of Regenerative Endodontic Treatment of Immature Permanent Upper Incisors with Acute Abscess. Braz. Dent. J. 31, 680–684 (2020).

38. Galler, K. M., D'Souza, R. N., Hartgerink, J. D. & Schmalz, G. Scaffolds for dental pulp tissue engineering. Adv. Dent. Res 23, 333–339 (2011).

39. Jazayeri, H. E. et al. Polymeric scaffolds for dental pulp tissue engineering: a review. Dent. Mater. 36, e47–e58 (2020).

40. Ulusoy, A. T., Turedi, I., Cimen, M. & Cehreli, Z. C. Evaluation of Blood Clot, Platelet-rich Plasma, Platelet-rich Fibrin, and Platelet Pellet as Scaffolds in Regenerative Endodontic Treatment: A Prospective Randomized Trial. J. Endod. 45, 560–566 (2019).

41. Hong, S., Li, L., Cai, W. & Jiang, B. The potential application of concentrated growth factor in regenerative endodontics. Int Endod. J. 52, 646–655 (2019).

42. Ramachandran, N. et al. A comparison of two pulp revascularization techniques using platelet-rich plasma and whole blood clot. J. Conserv Dent. 23, 637–643 (2020).

43. Nosrat, A. et al. Clinical, Radiographic, and Histologic Outcome of Regenerative Endodontic Treatment in Human Teeth Using a Novel Collagen-hydroxyapatite Scaffold. J. Endod. 45, 136–143 (2019).

44. Alqahtani, Q. et al. Decellularized Swine Dental Pulp Tissue for Regenerative Root Canal Therapy. J. Dent. Res. 97, 1460–1467 (2018).

45. Xi Wei, Maobin Yang, Lin Yue, Dingming Huang, Xuedong Zhou et al. Expert consensus on regenerative endodontic procedures. International Journal of Oral Science (2022) 14:55

46. Lin LM, Shimizu E, Gibbs JL, Loghin S, Ricucci D (2014) Histologic and histobacteriologic observations of failed revascularization/revitalization therapy: a case report. Journal of Endodontics 40, 291–5.

47. Myers WC, Fountain SB (1974) Dental pulp regeneration aided by blood and blood substitutes after experimentally induced periapical infection. Oral Surgery, Oral Medicine and Oral Pathology 37, 441–50.

48. Kim SG (2016) Infection and pulp regeneration. Dentistry Journal 4, 4.

49. Fouad AF (2017) Microbial factors and antimicrobial strategies in dental pulp regeneration. Journal of Endodontics 43,S46–50.

50. de-Jesus-Soares, A. et al. Clinical and Molecular Microbiological Evaluation of Regenerative Endodontic Procedures in Immature Permanent Teeth. J. Endod.m46, 1448–1454 (2020).

51. Trevino EG, Patwardhan AN, Henry MA et al. (2011) Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. Journal of Endodontics 37, 1109–15.

52. Martin DE, De Almeida JFA, Henry MA et al. (2014) Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. Journal of Endodontics 40, 51–5.

53. Hashimoto, K. et al. EDTA Treatment for Sodium Hypochlorite-treated Dentin Recovers Disturbed Attachment and Induces Differentiation of Mouse Dental Papilla Cells. J. Endod. 44, 256–262 (2018).

54. Hancerliogullari, D., Erdemir, A. & Kisa, U. The effect of different irrigation solutions and activation techniques on the expression of growth factors from gallerdentine of extracted premolar teeth. Int Endod. J. 54, 1915–1924 (2021).

55. AAE Clinical Considerations for a Regenerative Procedure[EB/OL]. https:// f3f142zs0k2w1kg84k5p9i1o-wpengine.netdna-ssl.com/specialty/wp-content/uploads/sites/2/2021/08/ClinicalConsiderationsApprovedByREC062921.pdf (2021).

56. Galler, K. M. et al. European Society of Endodontology position statement: Revitalization procedures. Int. Endod. J. 49, 717–723 (2016).

57. Widbiller, M., Althumairy, R. I. & Diogenes, A. Direct and Indirect Effect of Chlorhexidine on Survival of Stem Cells from the Apical Papilla and Its Neutralization. J. Endod. 45, 156–160 (2019).

58. Elnaggar, S. E., El Backly, R. M., Zaazou, A. M., Morsy Elshabrawy, S. & Abdallah, A.A. Effect of different irrigation protocols for applications in regenerative endodontics on mechanical properties of root dentin. Aust. Endod. J. 47, 228–235 (2021).

59. Aksel, H., Albanyan, H., Bosaid, F. & Azim, A. A. Dentin Conditioning Protocol for Regenerative Endodontic Procedures. J. Endod. 46, 1099–1104 (2020).

60. Wu, L. et al. Effect of Optimized Irrigation With Photon-Induced Photoacoustic Streaming on Smear Layer Removal, Dentin Microhardness, Attachment Morphology, and Survival of the Stem Cells of Apical Papilla. Lasers Surg. Med 53, 1105–1112 (2021).

61. Yamauchi N, Nagaoka H, Yamauchi S, Teixeira FB, Miguez P, Yamauchi M (2011) Immunohistological characterization of newly formed tissues after regenerative procedure in immature dog teeth. Journal of Endodontics 37, 1636–41.

62. Galler KM, Buchalla W, Hiller K-D et al. (2015) Influence of root canal disinfectants on growth factor release from dentin. Journal of Endodontics 41, 363–8.

63. Galler KM, Widbiller M, Buchalla W et al. (2016) EDTA conditioning of dentine promotes adhesion, migration and differentiation of dental pulp stem cells. International Endodontic Journal 49, 581–90.

64. Galler KM, D'Souza RN, Federlin M et al. (2011) Dentin conditioning codetermines cell fate in regenerative endodontics. Journal of Endodontics 37, 1536–41.

65. Nagata JY, Soares AJ, Souza-Filho FJ et al. (2014) Microbial evaluation of traumatized teeth treated with triple antibiotic paste or calcium hydroxide with 2% chlorohexidine gel in pulp revascularization. Journal of Endodontics 40, 778–83.

66. Kakoli P, Nandakumar R, Romberg E, Arola D, Fouad AF (2009) The effect of age on bacterial penetration of radicular dentin. Journal of Endodontics 35, 78–81.

67. Grossman LI (1972) Sterilization of infected root canals. Journal of the American Dental Association 85, 900–5.

68. Hoshino E, Kurihara-Ando N, Sato I et al. (1996) In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. International Endodontic Journal 29, 125–30.

69. Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E (1996) Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. International Endodontic Journal 29, 118–24.

70. Wang, H. J., Chen, Y. M. & Chen, K. L. Conservative treatment of immature teeth with apical periodontitis using triple antibiotic paste disinfection. J. Dent. Sci. 11, 196–201 (2016).

71. Windley W, Teixeira F, Levin L, Sigurdsson A, Trope M (2005) Disinfection of immature teeth with a triple antibiotic paste. Journal of Endodontics 31, 439–43.

72. Althumairy, R. I., Teixeira, F. B. & Diogenes, A. Effect of dentin conditioning with intracanal medicaments on survival of stem cells of apical papilla. J. Endod. 40, 521–525 (2014).

73. Ruparel NB, Teixeira FB, Ferraz CC, Diogenes A (2012) Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. Journal of Endodontics 38, 1372–5.

74. Nosrat A, Li KI, Vir K, Hicks ML, Fouad AF (2013) Is pulp regeneration necessary for root maturation. Journal of Endodontics 39, 1291–5.

75. Baumgartner JC, Xia T (2003) Antibiotic susceptibility of bacteria associated with endodontic abscesses. Journal of Endodontics 29, 44–7.

76. Sjogren U, Figdor D, Spangberg L, Sundqvist G (1991) The antimicrobial effect of calcium hydroxide a short-term intracanal dressing. International Endodontic Journal 24, 119–25.

77. Siqueira JF Jr, Lopes HP (1999) Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. International Endodontic Journal 32, 361–9.

78. Mohammadi Z, Dummer PMH (2011) Properties and application of calcium hydroxide in endodontics and dental traumatology. International Endodontic Journal 44, 697–730.

79. Estrela C, Sydney GD, Bammau LL, Felippe JO (1995) Mechanism of action on calcium hydroxyl ions of calcium hydroxide on tissue and bacteria. Brazilian Dental Journal 6, 85–90.

80. Safavi KE, Nichols K (1993) Effect of calcium hydroxide on bacterial lipopolysaccharide. Journal of Endodontics 19, 76–8.

81. Abbaszadegan, A. et al. Antimicrobial and Cytotoxic Activity of Cinnamomum zeylanicum, Calcium Hydroxide, and Triple Antibiotic Paste as Root Canal Dressing Materials. J. Contemp. Dent. Pract. 17, 105–113 (2016).

82. Maniglia-Ferreira, C. et al. In vitro evaluation of the antimicrobial effects of different intracanal medications in necrotic immature teeth. Eur. Arch. Paediatr. Dent. 17, 251–255 (2016).

83. Bose R, Nummikoski P, Hargreaves K (2009) A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. Journal of Endodontics 35, 1343–9.

84. Kitikuson P, Srisuwan T (2016) Attachment ability of human apical papilla cells to root dentin surfaces treated with either 3Mix or calcium hydroxide. Journal of Endodontics 42, 89–94.

85. Baez, V., Corcos, L., Morgillo, F., Imperatrice, L. & Gualtieri, A. F. Meta-analysis of regenerative endodontics outcomes with antibiotics pastes and calcium hydroxide. The apex of the iceberg. J. Oral. Biol. Craniofac. Res. 12, 90–98 (2022)

86. Pagliarin, C. M. et al. Tissue characterization following revascularization of immature dog teeth using different disinfection pastes. Braz. Oral Res. 30, https://doi.org/10.1590/1807-3107BOR-2016.vol30.0074 (2016).

87. El-Tayeb, M. M., Abu-Seida, A. M., El Ashry, S. H. & El-Hady, S. A. Evaluation of antibacterial activity of propolis on regenerative potential of necrotic immature permanent teeth in dogs. BMC Oral. Health 19, 174 (2019).

88. Bottino, M. C. et al. A novel patient-specific three-dimensional drug delivery construct for regenerative endodontics. J. Biomed. Mater. Res B Appl Biomater. 107, 1576–1586 (2019).

89. Karczewski, A. et al. Clindamycin-modified Triple Antibiotic Nano fibers: A Stain-free Antimicrobial Intracanal Drug Delivery System. J. Endod. 44, 155–162 (2018).

90. Lovelace TW, Henry MA, Hargreaves KM, Diogenes A (2011) Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. Journal of Endodontics 37, 133–8.1384

91. Nosrat A, Homayounfar N, Oloomi K (2012) Drawbacks and unfavorable outcomes of regenerative endodontic treatments of necrotic immature teeth: a literature

review and report of a case. Journal of Endodontics 38, 1428–34.

92. Ding, R. Y. et al. Pulp revascularization of immature teeth with apical periodontitis: a clinical study. J. Endod. 35, 745–749 (2009).

93. Ilan Rotstein, D. & John I. Ingle, D. Ingle's Endodontics (PMPH USA, Limited, 2019)

94. Torabinejad M, Turman M (2011) Revitalization of tooth with necrotic pulp and open apx by using platelet-rich plasma: a case report. Journal of Endodontics 37, 1109–15.

95. Jadhav G, Shah N, Logani A (2012) Revascularization with and without platelet-rich plasma in nonvital, immature, anterior teeth: a pilot clinical study. Journal of Endodontics 38, 1581–7.

96. Bezgin T, Yilmaz AD, Celik BN, Kolsuz ME, Sonmez H (2015) Efficacy of platelet-rich plasma as a scaffold in regenerative endodontic treatment. Journal of Endodontics 41, 36–44.

97. Shivashankar VY, Johns DA, Vidyanath S, Kumar MR b(2012) Platelet Rich Fibrin in the revitalization of tooth with necrotic pulp and open apex. Journal of Conservative Dentistry 15, 395–8.

98. Keswani D, Pandey RK (2013) Revascularization of an immature tooth with a necrotic pulp using platelet-rich fibrin: a case report. International Endodontic Journal 46, 1096–104.

99. Mishra N, Narang I, Mittal N (2013) Platelet-rich fibrin- mediated revitalization of immature necrotic tooth. Contemporary Clinical Dentistry 4, 412–5.

100. Ray HL Jr, Marcelino J, Braga R, Horwat R, Lisien M, Khaliq S (2016) Long-term follow up of revascularization using platelet-rich fibrin. Dental Traumatology 32, 80–4.

101. Bakhtiar H, Esmaeili S, Fakhr Tabatabayi S, Ellini MR, Nekoofar MH, Dummer PM (2017) Second-generation platelet concentrate (platelet-rich fibrin) as a scaffold in regenerative endodontics: a case series. Journal of Endodontics 43, 401-8.

102. Lolato A, Bucchi C, Taschieri S, Kabbaney AE, Del Fabbro M (2016) Platelet concentrations for revitalization of immature necrotic teeth: a systematic review of the clinical studies. Platelets 5, 383–92.

103. Shivashankar, V. Y. et al. Comparison of the Effect of PRP, PRF and Induced Bleeding in the Revascularization of Teeth with Necrotic Pulp and Open Apex: A Triple Blind Randomized Clinical Trial. J. Clin. Diagn. Res 11, ZC34–ZC39 (2017).

104. Zhou R, Wang Y, Chen Y et al. (2017) Radiographic, histologic, and biomechanical evaluation of combined application of platelet-rich fibrin with blood clot in regenerative endodontics. Journal of Endodontics 43, 2034–40.

105. Cvek M (1992) Prognosis of luxated non-vital maxillary incisors treated with calcium hydroxide and filled with gutta-percha. A retrospective clinical study. Endodontics and Dental Traumatology 8, 45–55.

106. Youssef, A., Ali, M., ElBolok, A. & Hassan, R. Regenerative endodontic procedures for the treatment of necrotic mature teeth: A preliminary randomized clinical trial. Int Endod. J. 55, 334–346 (2022).

107. Mittal, N., Baranwal, H. C., Kumar, P. & Gupta, S. Assessment of pulp sensibility in the mature necrotic teeth using regenerative endodontic therapy with various scaffolds - Randomised clinical trial. Indian J. Dent. Res 32, 216–220 (2021).

108. Andreasen JO, Paulsen HU, Yu Z, Bayer T, Schwartz O (1990) A long-term study of 370 autotransplanted premolars. Part II. Tooth survival and pulp healing subsequent to transplantation. European Journal of Orthodontics 12,14–24.

109. Laureys WGM, Cuvelier CA, Dermaut LR, De Pauw GAM (2013) The critical apical diameter to obtain regeneration of the pulp tissue after tooth transplantation, replantation, or regenerative endodontic treatment. Journal of Endodontics 39, 759–63.

110. Abada, H. M., Hashem, A. A. R., Abu-Seida, A. M. & Nagy, M. M. The effect of changing apical foramen diameter on regenerative potential of mature teeth with necrotic pulp and apical periodontitis. Clin. Oral. Investig. 26, 1843–1853 (2022).

111. Estefan BS, El Batouty KM, Nagy MM, Diogenes A (2016) Influence of age and apical diameter on the success of endodontic regeneration procedures. Journal of Endodontics 42, 1620–5.

112. Fang Y, Wang X, Zhu J, Su C, Yang Y, Meng L (2018) Influence of apical diameter on the outcome of regenerative 1382 International Endodontic Journal, 51, 1367–1388, 2018

113. Gaviño Orduña, J. F. et al. Use of Platelet-rich Plasma in Endodontic Procedures in Adults: Regeneration or Repair? A Report of 3 Cases with 5 Years of Follow-up. J. Endod. 43, 1294–1301 (2017).

114. Jadhav, G. R., Shah, N. & Logani, A. Platelet-rich plasma supplemented revascularization of an immature tooth associated with a periapical lesion in a 40-year-old man. Case Rep. Dent. 2014, 479584 (2014).

115. Aggarwal, V., Miglani, S. & Singla, M. Conventional apexi cation and revascularization induced maturogenesis of two non-vital, immature teeth in same patient: 24 months follow up of a case. J. Conserv Dent. 15, 68–72 (2012).

116. Ding, R. Y. et al. Pulp revascularization of immature teeth with apical periodontitis: a clinical study. J. Endod. 35, 745–749 (2009).

117. Nakashima, M. & Iohara, K. Mobilized dental pulp stem cells for pulp regeneration: initiation of clinical trial. J. Endod. 40, S26–S32 (2014).

118. Kellner, M. et al. Differences of isolated dental stem cells dependent on donor age and consequences for autologous tooth replacement. Arch. oral. Biol. 59, 559–567 (2014).

119. Chen Y-P, del Mar J-SM, Sheth CC (2015) Is revascularization of immature permanent teeth an effective and reproducible technique? Dental Traumatology 34, 429–36.

120. Torabinejad M, Nosrat A, Verma P, Udochukwu O (2017) Regenerative endodontic treatment or mineral trioxide aggregate apical plug in teeth with necrotic pulps and open apices: a systematic review and meta-analysis. Journal of Endodontics 43, 1806–20.

121. Chen MY-H, Chen K-L, Chen C-A, Tayebaty F, Rosenberg PA, Lin LM (2012) Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. International Endodontic Journal 45, 294–305.

122. Alobaid AS, Cortes LM, Lo J et al. (2014) Radiographic and clinical outcomes of the treatment of immature permanent teeth by revascularization or apexification: a pilot retrospective cohort study. Journal of Endodontics 40, 1063–70.

123. Kahler B, Mistry S, Moule A et al. (2014) Revascularization outcomes: a prospective analysis of sixteen consecutive cases. Journal of Endodontics 40, 333–8.

124. Diogenes A, Ruparel NB (2017) Regenerative endodontic procedures: clinical outcomes. Dental Clinics of North America 61, 111–25.

125. Diogenes A, Henry MA, Teixeira FB, Hargreaves KM (2013) An update on clinical regenerative endodontics. Endodontic Topics 28, 2–23.

126. Banchs F, Trope M (2004) Revascularization of immature permanent tooth with apical periodontitis: new treatment protocol? Journal of Endodontics 30, 196–200.

127. Lei L, Chen Y, Zhou R, Huang X, Cai Z (2015) Histologic and immunohistochemical findings of a human immature permanent tooth with apical periodontitis after regenerative endodontic therapy. Journal of Endodontics 41, 1172–9.

128. Nevins AJ, Finkelstein F, Borden BG, Laporta R (1976) Revitalization of pulpless open apex teeth in rhesus monkeys, using collagen-calcium phosphate gel. Journal of Endodontics 2, 159–65.

129. Thibodeau B, Teixeira F, Yamauchi M, Caplan D, Trope M (2007) Pulp revascularization of immature dog teeth with apical periodontitis. Journal of Endodontics 33, 680–9.

130. da Silva LAB, Nelson-Filho P, da Silva RAB et al. (2010) Revascularization and periapical repair after endodontic treatment using apical negative pressure irrigation versus conventional irrigation plus triantibiotic intracanal dressing in dog's teeth with apical periodontitis. Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics 109, 779–87.

131. Wang X, Thibodeau B, Trope M, Lin LM, Huang GT (2010) Histologic characterization of regenerated tissues in canal space after the revitalization/revascularization procedure of immature dog teeth with apical periodontitis. Journal of Endodontics 36, 56–63.

132. Martin G, Ricucci D, Gibbs JL, Lin LM (2013) Histological findings of revascularized/revitalized immature permanent molar with apical periodontitis using platelet-rich plasma. Journal of Endodontics 39, 138–44.

133. Shimizu E, Ricucci D, Albert J et al. (2013) Clinical, radiographic, and histological observation of a human immature permanent teeth with chronic apical abscess after revitalization treatment. Journal of Endodontics 39, 1078–83.

134. Becerra P, Ricucci D, Loghin S, Gibbs JL, Lin LM (2014) Histological study of a human immature permanent premolar with chronic apical abscess after revascularization/revitalization. Journal of Endodontics 40, 133–9.

135. Sonoyama W, Liu Y, Yamaza T et al. (2008) Characterization of apical papilla and its residing stem cells from human immature permanent teeth– a pilot study. Journal of Endodontics 34, 166–71.

136. Torabinejad, M. et al. Effect of Residual Dental Pulp Tissue on Regeneration of Dentin-pulp Complex: An In Vivo Investigation. J. Endod. 44, 1796–1801 (2018).

137. Austah, O. et al. Comprehensive Characterization of 2 Immature Teeth Treated with Regenerative Endodontic Procedures. J. Endod. 44, 1802–1811 (2018).

138. Linsuwanont P, Sinpitaksakul P, Lertsakchai T (2017) Evaluation of root maturation after revitalization in immature permanent teeth with nonvital pulps by cone beam computed tomography and conventional radiographs. International Endodontic Journal 50, 836–46.

139. Silujjai J, Linsuwanont P (2017) Treatment outcomes of apexification or revascularization in nonvital immature permanent teeth: a retrospective study. Journal of Endodontics 43, 238–45.

140. Yadav P, Pruthi PJ, Naval RR, Talwar S, Verma M (2015) Novel use of platelet-rich fibrin matrix and MTA as an apical barrier in the management of a failed revascularization case. Dental Traumatology 31, 328–31.

141. Zizka R, Buchta T, Voborna I, Harvan L, Sedy J (2016) Root maturation in teeth treated by unsuccessful revitalization: 2 case reports. Journal of Endodontics 42, 724–9.

142. Saoud TM, Mistry S, Kahler B, Sigurdsson A, Lin LM (2016) Regenerative endodontic procedures for traumatized teeth after horizontal root fracture, avulsion, and perforating root resorption. Journal of Endodontics 42, 1476–82.

143. Song M, Cao Y, Shin SJ et al. (2017) Revascularization-associated intracanal calcification: assessment of prevalence and contributing factors. Journal of Endodontics 43, 2025–33.

144. de Toubes KMS, de Oliveira PAD, Machado SN, Pelosi V, Nunes E, Silveira FF (2017) Clinical approach to pulp canal obliteration: a case series. Iranian Endodontic Journal 12, 527–33.

145. Chaniotis A (2017) Treatment options for failing regenerative endodontic procedures: report of 3 cases. Journal of Endodontics 43, 1472–8.

146. Parirokh M, Torabinejad M (2010) Mineral trioxide aggregate: a comprehensive literature review-part III: clinical applications, drawback, and mechanism of action. Journal of Endodontics 36, 400–13.

147. Marconyak LJ Jr, Kirkpatrick TC, Roberts HW et al. (2016) A comparison of coronal tooth discoloration elicited by various endodontic reparative materials. Journal of Endodontics 42, 470–3.

148. Shokouhinejad N, Nekoofar MH, Pirmoazen S, Shamshiri AR, Dummer PM (2016) Evaluation and comparison of occurrence of tooth discoloration after th application of various calcium silicate-based cements: an ex vivo study. Journal of Endodontics 42, 140–4.

149. Yoldas ß SE, Bani M, Atabek D, Bodur H (2016) Comparison of the potential discoloration effect of Bioaggregate, Bio dentine, and white mineral trioxide aggregate on bovine teeth: in vitro research. Journal of Endodontics 42, 1815–8.

150. Kirchhoff AL, Raldi DP, Salles AC, Cunha RS, Mello I (2015) Tooth discolouration and internal bleaching after the use of triple antibiotic paste. International Endodontic Journal 48, 1181–7.

151. Iohara K, Imabayashi K, Ishizaka R et al. (2011) Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stroma cell-derived factor-1. Tissue Engineering: Part A 17, 1911–20.

152. Huang GT, Yamaza T, Shea LD et al. (2010) Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Engineering Part A 16, 605–15.

153. Cordeiro MM, Dong Z, Kaneko T et al. (2008) Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. Journal of Endodontics 34, 962–9.

154. Rosa V, Zhang Z, Grande RH, N€ or JE (2013) Dental pulp tissue engineering in full-length human root canals. Journal of Dental Research 92, 970–5.

155. Nakashima M, Iohara K, Murakami M et al. (2017) Pulp regeneration by transplantation of dental pulp stem cells in pulpitis: a pilot clinical study. Stem Cell Research & Therapy 9, 61.

156. Xuan, K. et al. Deciduous autologous tooth stem cells regenerate dental pulp after implantation into injured teeth. Sci. Transl. Med. 10, https://doi.org/10.1126/scitranslmed.aaf3227 (2018).

157. He, L. et al. Regenerative Endodontics by Cell Homing. Dent. Clin. N. Am. 61, 143–159 (2017).

158. Kim JY, Xin X, Moiolo EK et al. (2010) Regeneration of dentin-pulp-like tissue by chemotaxis-induced cell homing. Tissue Engineering Part A 16, 3023–31.

159. Liu, J. Y., Chen, X., Yue, L., Huang, G. T. & Zou, X. Y. CXC Chemokine Receptor 4 Is Expressed Paravascularly in Apical Papilla and Coordinates with Stromal Cell- derived Factor-1α during Transmigration of Stem Cells from Apical Papilla. J. Endod. 41, 1430–1436 (2015).

160. Suzuki, T. et al. Induced migration of dental pulp stem cells for in vivo pulp regeneration. J. Dent. Res. 90, 1013–1018 (2011).

161. Yang, J. W. et al. Autophagy in SDF-1α-mediated DPSC migration and pulp regeneration. Biomaterials 44, 11–23 (2015).