

A REVIEW OF REGENERATIVE ENDODONTICS: CURRENT PROTOCOLS AND FUTURE DIRECTIONS

Rejeneratif Endodonti Üzerine Bir Derleme: Güncel Protokoller ve Geleceğe Yönelik Öneriler

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ABSTRACT

This review outlines the biological basis and clinical protocols currently used in regenerative endodontic procedures (REPs) and discuss future directions in pulp regeneration approaches. The treatment of immature teeth with REPs has been described as a 'paradigm shift' as there is the potential for further root maturation. Clinically, REPs involve disinfection of the root canal system without damaging the endogenous stem cell potential present in the apical papilla and other tissues. These stem cells are introduced into the root canal space by inducing a blood clot followed by placement of an intracanal barrier to prevent microleakage. The biological concept of REPs involves the triad of stem cells, scaffold and signalling molecules. Currently, repair rather than true regeneration of the 'pulp-dentine complex' is achieved and further root maturation is variable. However, many clinicians consider the treatment of teeth with REPs as the optimal treatment approach for immature teeth with pulp necrosis.

Keywords: Regenerative endodontic protocols; immature teeth; pulp necrosis; root maturation; triple antibiotic paste; calcium hydroxide

ÖZ

Bu derlemede rejeneratif endodontik tedavinin biyolojik temelleri ve güncel klinik protokolleri anlatılmakta ve pulpa rejenerasyonu yaklaşımlarının geleceği tartışılmaktadır. Kök ucu kapanmamış dişlerin rejeneratif endodontik tedavi ile kök gelişiminin devam etme potansiyeli köklü bir değişim yaratmıştır. Klinik olarak rejeneratif endodontik tedavi apikal papilla ve diğer dokularda bulunan endojen kök hücrelere zarar vermeden kök kanalı sisteminin dezenfeksiyonunu içerir. Bu kök hücrelerin kan pıhtısı oluşturulması ile kök kanalı içine taşınmasını takiben mikrosızıntıyı önleme amacıyla kanal içine bariyer malzemesi yerleştirilir. Rejeneratif endodontik tedavinin biyolojik prensibi kök hücreler, doku iskeleleri ve sinyal molekülleri triadından oluşur. Günümüzde, "pulpa-dentin kompleksi" nin gerçek rejenerasyonundan ziyade tamir gerçekleşmektedir ve kök gelişiminin tamamlanması ise değişkenlik göstermektedir. Ancak, birçok klinisyen pulpası nekroze kök gelişimi tamamlanmamış dişlerde en uygun tedavinin rejeneratif endodontik yaklaşımlarla sağlanabileceğini düşünmektedir.

Anahtar kelimeler: Rejeneratif endodontik tedavi protokolleri; kök gelişimi tamamlanmamış dişler; pulpa nekrozu; kök gelişimi; üçlü antibiyotik pat; kalsiyum hidroksit

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Introduction

Regenerative endodontics is an exciting and developing field in the treatment of immature teeth with infected root canals that has been described as a “paradigm shift” in the management of these teeth and can result in continued root maturation and apical closure (1-3). Traditional approaches of calcium hydroxide apexification and apical barrier techniques with mineral trioxide aggregate (MTA) have been used in the treatment of immature teeth with pulp necrosis though generally there is no further root development so the roots remain thin and fragile with a higher risk of fracture and tooth loss. Recently, it has been suggested that regenerative endodontic protocols (REPs) that utilize endogenous stem cells that are introduced in the canal by lacerating the periapical tissues to fill the canal with blood should be used for the treatment of immature teeth with pulp necrosis (4). Regenerative endodontics has been defined as “biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex” (4). The clinical considerations for regenerative endodontic protocols are (1) disinfection of the root canal system (2), provision of a scaffold which often involves laceration of the periapical tissue to induce a blood clot and introduce stem cell activity within the root canal (3), and an adequate coronal seal to prevent reinfection (5-10). The assumption is that regenerative endodontic protocols which result in continued root maturation mean the teeth and roots are not as inherently weak and susceptible to fracture as the traditional techniques of Ca(OH)₂ apexification and MTA barrier placements. Studies which have compared REPs with the traditional approaches of calcium hydroxide apexification and MTA apical barrier techniques have shown comparable outcomes (11, 12). However, another study that compared the traditional and regenerative protocols showed revascularization was associated with significantly greater increases in root length and thickness compared with calcium hydroxide apexification and MTA barrier placement as well as higher overall survival rates (13).

Terminology

A number of terms have been adopted in the literature with regenerative endodontics, revascularization and revitalization being the most commonly used. The term ‘revascularization’ is well

established in the endodontic literature and relates to the reestablishment of vascularity in the pulp space after traumatic injuries that sever the blood supply to the pulp of immature teeth (14, 15). Earlier reports on this new technique showed renewed root maturation in infected immature teeth and described the introduction of a blood clot into the root canal as “revascularization” (16, 17). Regenerative endodontics implies that further root maturation results in reestablishment of the pulp dentine complex. Many studies show that this is not the case with a variety of tissues such as dentine, cementum, periodontal ligament, bone, osteoid and possibly pulp being the tissue found in treated teeth with regenerative endodontic protocols, suggesting ‘repair’ rather than ‘regeneration’ (18-20).

‘Revitalization’ has been suggested as it describes non-specific vital tissue rather than just blood vessels as implied by the term ‘revascularization’ (21).

The term regenerative endodontic procedures (REPs) has been widely adopted and refers to all procedures that aim to attain organized repair of the dental pulp and include future therapies yet to evolve in the field of regenerative endodontics (6).

Regeneration or repair

Tissue injury caused by infection or trauma after appropriate treatment will result in healing by either regeneration or repair (22). Regeneration is defined as reconstitution of damaged tissue by tissue similar to original tissue and restoration of biological functions (22, 23). Repair is replacement of damaged tissue by tissue different from original tissue and loss of biological functions (22, 23). The dental pulp has a limited potential of regeneration (24). In regenerative endodontic procedures, many growth factors embedded in the dentin matrix are released into the canal space after EDTA treatment (25). These growth factors have been shown to be able to signal pulp stem cells to differentiate into odontoblast-like cells and produce reparative dentin (26-29). However, the mesenchymal stem cells (30) introduced into the canal space during regenerative endodontic procedures do not appear to be able to differentiate into odontoblast-like cells and produce the dentin-pulp complex in many animal and human studies.

Histologic characterization of tissues formed in the canal space

Many histological studies of regenerative

endodontic procedures of immature teeth with necrotic pulps and apical periodontitis have been investigated in animal models and humans and show that the tissues formed in the canal space were mineralized tissue similar to cementum and bone, and fibrous connective tissue resembling periodontal ligament (18, 20, 31-34).

Histologically, regenerative endodontic procedures of human immature permanent teeth with necrotic pulp are considered a reparative and not a regenerative process (35, 36). Radiographic thickening of the canal walls, and continued root maturation of immature permanent teeth with necrotic pulps after regenerative endodontic procedures should not be regarded as a regeneration of dentin-pulp complex without histological confirmation. If the primary goal of regenerative endodontic procedures is elimination of clinical symptom/sign and resolution of apical periodontitis (37), then repair, although not an ideal wound healing, is not a treatment failure (35, 36).

History

The promise and potential of regenerative endodontic therapies in necrotic teeth was first explored by Nygaard-Östby in 1961 who investigated the potential for repair when bleeding was induced by over-instrumentation beyond the apex prior to partial root filling of the canal with limited success (38). Forty years later, in 2001 Iwaya *et al.* (17) reported a case using a procedure termed ‘revascularization’, on an infected necrotic immature premolar tooth that showed continued root maturation and thickening of root canal walls with mineralized tissue. Subsequent case reports also demonstrated the efficacy of this technique primarily in premolar teeth when using a triple-antibiotic paste (16, 39, 40) or calcium hydroxide (1, 41). Further reports demonstrated successful outcomes in traumatized central incisor teeth (21, 42-44).

A number of authors have described regenerative endodontic techniques as a “paradigm shift” in the treatment of immature teeth with necrotic pulps (1-3).

Clinical considerations for regenerative endodontic procedures

Young patient

Whilst REPs have been utilised on mature teeth (45-47), the vast majority of reported cases are undertaken on young patients with immature

infected teeth where cessation of root maturation has occurred as a consequence of pulp necrosis. The American Association of Endodontists (AAE) clinical considerations for regenerative endodontic procedures define success by three measures (37): 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 (48) Whilst the primary goal is an objective for all endodontic treatments, it is the secondary goal of increased width and/or length that is pertinent in the immature tooth. The assumption is that further root maturation often assessed in conjunction with apical closure of the immature root apex may minimise the incidence of root fracture. Another assumption is in the tertiary goal that a return of neural capacity may indicate a more organized vital pulp tissue (37). In 2013, a review reported many cases demonstrated a positive response to pulp sensibility testing (6).

Minimal or no instrumentation of the dentinal walls

REPs advocate minimal or no filing of the canal (49). However, in a histologic and histobacteriologic study of a failed REP treatment most bacteria were noted in the apical and not the coronal portion of the canal where a biofilm had formed on the canal walls and penetrated into the dentinal tubules. The authors concluded that on the basis of the histobacteriologic findings that some degree of mechanical debridement may also be required to disrupt biofilm on the canal walls for continued root maturation to occur (49).

Disinfection of the root canal system

Disinfection of the root canal system is thought to be critical to the success of REPs as infection prevents regeneration, repair and stem cell activity (49, 50). Chemical disinfection of the root canal system is not solely dependent on bacteriocidal/ bacteriostatic properties of the agents as these irrigants/medicaments should not damage the survival and proliferative capacity of the patient’s stem cells (6). As few case reports have been performed in a single visit (51-53), the protocol describes a two-visit approach with the use of irrigants and an intra-canal medicament (to be discussed in the next subsection). The guidelines suggest copious irrigation with 20 ml sodium hypochlorite (NaOCl) using an irrigation system that minimizes 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 (1.5% sodium hypochlorite (20 mL/canal, 5 min) and then irrigated with saline or EDTA (20 mL/canal, 5 min) with the irrigating needle positioned about 1 mm from the root end to minimize cytotoxicity to stem cells in the apical tissues (37). Growth factors contained in the dentine matrix are released by endodontic irrigants (25, 54, 55). Lower concentrations of NaOCl are now recommended as higher concentration significantly decreases the survival of stem cells of the apical papilla (SCAP) (56, 57). A concentration of 1.5% NaOCl had minimal destructive effects on SCAP (56). Furthermore the use of 17% EDTA resulted in increased SCAP survival expression as well as partially reversing the deleterious effects of NaOCl (56). EDTA acts to demineralise the dentine and expose the dentine matrix which acts to release growth factors from the dentine matrix (25, 34, 55). EDTA conditioning of dentin promoted the adhesion, migration and differentiation of dental pulp stem cells towards or onto dentin (55). Exposure of the dentin matrix by EDTA also appeared to increase the adherence of newly formed mineralized tissue to the root walls (34). Therefore, a final rinse with EDTA before creation of a blood clot is advised (25, 55).

Placement of an intra-canal medicament

The first report of revascularization in an infected immature tooth in 2001 used a double antibiotic paste of metronidazole and ciprofloxacin (17). The next report used a combination of metronidazole, ciprofloxacin and minocycline often referred to as ‘3mix’ or ‘triple antibiotic paste’ (TAP) (6). The rationale for the use of TAP was a number of studies of endodontic infections in deciduous teeth where a combination of metronidazole, ciprofloxacin and minocycline at a concentration of 100 µg/mL of each drug completely eliminated cultivatable bacteria from infected root canals *in vitro* and *in vivo* (58, 59). Triple antibiotic pastes using this combination of antibiotics have been shown to reliably disinfect dentine in infected root canals with diffusion of the drugs throughout the entire root dentine (60, 61). The AAE protocol recommends TAPs at concentrations no greater than 0.1 mg/mL (37). At this concentration, TAP is conducive with stem cell survival and proliferation (19) and also effective in eliminating microorganisms within the root canal (58, 59). AAE guidelines also

recommended calcium hydroxide (37). Calcium hydroxide has been shown to be conducive to SCAP survival and proliferation (62). Furthermore a study has shown that SCAPs had the highest survival when cultured on dentine exposed to calcium hydroxide when compared to dentine exposed to concentrations of 1 mg/mL or higher TAP (63). Clinically, an analysis of case reports that used calcium hydroxide as an intracanal medicament showed further root maturation (64). Thus the concentrations of both irrigants and medicaments are important to find the balance between disinfection of the canal, alteration of the dentine matrix for the release of growth factors and survival and proliferation of SCAP.

Creation of a blood clot or protein scaffold in canal

After disinfection of the canal and resolution of symptoms, REPs usually involve lacerating the periapical tissues to initiate bleeding or the use of platelet-rich plasma (PRP) or platelet-rich fibrin (PRF). An important study demonstrated that the evoked-bleeding step in regenerative procedures triggers a significant accumulation of undifferentiated stem cells into the canal space (30). Contemporary regenerative endodontics follows principles of bioengineering with the foundation dependent on the interaction of stem cells, scaffolds and growth factors (65).

Effective coronal seal

Once a blood clot or scaffold is in place within the canal, a coronal barrier is placed to prevent coronal leakage of microorganisms. Current protocols recommend that when a blood clot is formed, a premeasured piece of Collaplug (Zimmer Dental Inc, Warsaw, IN) should be carefully placed on top of the blood clot to serve as an internal matrix for the placement of approximately 3 mm of white MTA (Dentsply, Tulsa, OK) followed by a 3–4 mm layer of glass ionomer layer (eg, Fuji IX; GC America, Alsip, IL, or other) is placed over the MTA. A bonded reinforced composite resin restoration (e.g., Z-100; 3M, St Paul, MN, or other) is then placed over the glass ionomer (37). MTA is a biocompatible material with bioactive properties that resists bacterial contamination (66). Biodentine® (Septodont, Lancasted, PA, USA) can be used as an alternative calcium silicate-based cement (67).

Clinical regenerative endodontic procedures

As aforementioned, regenerative endodontic procedures for immature permanent teeth with non-infected and infected necrotic pulps in humans varies considerably in terms of concentrations of sodium

hypochlorite irrigant and triple antibiotic paste in all published studies. There is no standard protocol of REP. The American Association of Endodontists suggests a Clinical Considerations for a Regenerative Procedure for its members (37).

Table 1. Regenerative endodontic procedures (37).

| First appointment |
|--|
| Local anesthesia, dental dam isolation and access. |
| Copious, gentle irrigation with 20 ml NaOCl using an irrigation system that minimizes the possibility of extrusion of irrigants into the periapical space (e.g., needle with closed end and side-vent, or EndoVac™). Lower concentrations of NaOCl are advised (1.5% NaOCl (20 ml/canal, 5 min) and then irrigated with saline (20 ml/canal, 5 min), with irrigation needle positioned about 1 mm from root end, to minimize cytotoxicity to stem cells in the apical tissues. |
| Dry canals with paper points. |
| Place calcium hydroxide or low concentration of triple antibiotic paste. If the triple antibiotic paste is used: 1) consider sealing pulp chamber with a dentin bonding agent (to minimize risk of staining) and 2) mix 1:1:1 ciprofloxacin: metronidazole: minocycline to a final concentration of 0.1 mg/ml. |
| Deliver into canal system via syringe. |
| If triple antibiotic is used, ensure that it remains below CEJ (minimize crown staining). |
| Seal with 3-4 mm of a temporary material such as Cavit, IRM, glass-ionomer or another temporary material. Dismiss patient for 1-4 weeks. |
| Second appointment (1-4 weeks after 1st visit) |
| Assess response to initial treatment. If there are signs/symptoms of persistent infection, consider additional treatment with antimicrobial, or alternative antimicrobial. |
| Anesthesia with 3% mepivacaine without vasoconstrictor, dental dam isolation. |
| Copious, gentle irrigation with 20 ml of 17% EDTA. |
| Dry with paper points. |
| Create bleeding into canal system by over-instrumenting (endo file, endo explore) (induce by rotating a pre-curved K-file at 2 mm past the apical foramen with the goal of having the entire canal filled with blood to the level of cemento-enamel junction). |
| Stop bleeding at a level that allows for 3-4 mm of restorative material. |
| Place a resorbable matrix such as CollaPlug, Collacote, CollaTape or other material over the blood clot if necessary and white MTA/CaOH as capping material. |
| A 3-4 mm layer of glass ionomer (e.g., Fuji IILCTM, GC America, Alsip, IL) is flowed gently over the capping material and light-cured for 40 s. MTA has been associated with discoloration. Alternatives to MTA should be considered in teeth where there is an esthetic concern like Biodentine® (Septodont, Lancasted, PA, USA). |
| * Anterior and premolar teeth – Consider use of Collatape/Collaplug and restoring with 3 mm of RMGI followed by bonding a filled composite to the beveled enamel margin. |
| * Molar teeth or teeth with PFM crown – Consider use of Collatape/Collaplug and restoring with 3 mm of MTA, followed by RMGI or alloy. |
| Follow-up |
| Clinical and radiographic exam |
| * No pain, soft tissue swelling or sinus tract (often observed between first and second appointments). |
| * Resolution of apical radiolucency (often observed 6-12 months after treatment). |
| * Increased width of root walls (this is generally observed before apparent increase in root length and often occur 12-24 months after treatment). |
| * Increased root length. |
| * Pulp vitality test. |



Figure 1. A mandibular premolar tooth where the evaginatus has fractured causing pulp necrosis and chronic abscess.

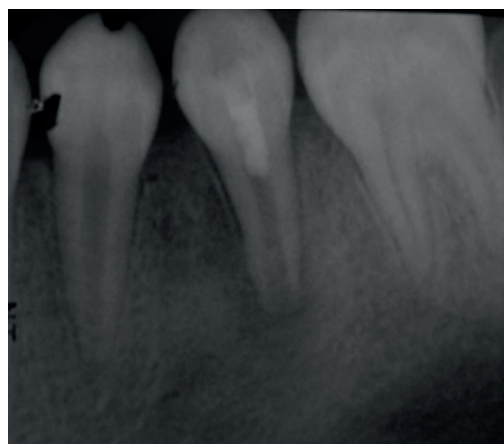


Figure 4. A radiograph showing an intracanal barrier of MTA placed on the blood clot. The tooth was restored with glass ionomer cement and composite resin.

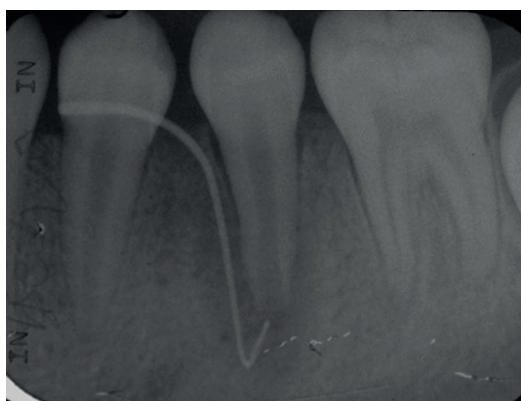


Figure 2. A GP marker tracks to the periapical tissues of an immature root.

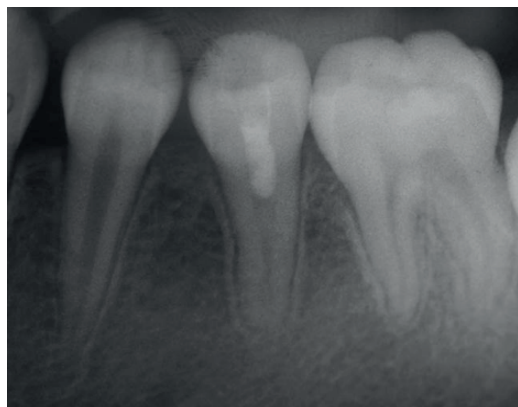


Figure 5. A radiograph taken at an 18 month review showing resolution of the apical periodontitis as well as further root maturation indicated by increased width of the root dentine walls and apical closure.



Figure 3. A blood clot was induced into the canal to the level of the cemento-enamel junction after a disinfection protocol four weeks earlier that employed 1% sodium hypochlorite and the canal was then medicated with a triple antibiotic paste consisting of metronidazole, ciprofloxacin and amoxicillin.

Outcome assessment

In 2014, there were 51 publications of clinical studies that employed regenerative endodontic protocols with 255 teeth treated. There were 2 high-level cohort studies (LOE 2), 8 case series (LOE 4) and 41 case reports (LOE 5) (68).

Is revascularization an effective and reproducible technique?

A recent systematic review reported that 97 of 101 teeth (96%) treated with REPs 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 no further root maturation. Apical closure was detected in 55.4% of teeth with increased root length (76.2%) and increased root width (79.2%) reported more frequently. These outcomes were independent of clinical variables such as operator and material selection or differences in protocols (69).

Radiographic responses of teeth treated with REPs

Chen *et al.* (70) described 5 types of responses for teeth treated with REPs. Type 1 where there is increased thickening of the canal walls and continued root maturation. Type 2 where there is no significant continuation of root development with the root apex becoming blunt and closed. Type 3 where there is continued root development with the apical foramen remaining open. Type 4 where there is severe calcification (obliteration) of the canal space. Type 5 where there is a hard tissue barrier formed in the canal between the coronal MTA plug and the root apex (70).

Discoloration

Many studies have shown that discoloration is a significant problem following regenerative endodontic treatment (71). This is of particular concern for traumatized anterior teeth as appearance and pleasing aesthetics are patient centered outcomes. Discoloration is more often associated with TAP that includes minocycline though discoloration has also been reported with calcium hydroxide. MTA which is the most commonly used material as an intacanal barrier has also been shown to discolor teeth (72). Therefore patients should be advised that discoloration of the tooth is often associated with REPs as stated in the AAE guidelines (37).

Follow-up

Most studies have undertaken 6-month radiographic reviews for 12-18 months and longer to make assessment of periapical healing and for further root maturation.

What does the future hold?

Currently, there are two approaches to pulp tissue regeneration in regenerative endodontics, namely cell-free and cell-based. Both approaches are based on the concept of tissue engineering applying stem cells, bioactive growth/differentiation factors and biomimetic scaffold. Clinical regenerative endodontic procedures

can be considered a cell-free approach. The cell-based approach requires isolation and ex vivo expansion of stem cells seeded in the scaffold and then transplanted into the canal space (73-79). Technically, the cell-free approach is simpler than 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 and can be from apical papilla, periodontal ligament or bone marrow. The cell-based approach employs pulp tissue specific stem cells, such as dental pulp stem cells (DPSCs) (80), stem cells from exfoliated deciduous teeth (81), and stem cells from apical papilla (82).

These stem cells have been shown to be capable of differentiating into odontoblast-like cells and produce dentine-like mineralized tissue (80-82). Therefore, this approach has more promise to result in true regeneration. Nevertheless, several problems are associated with cell-based approach and have to be resolved, such as limited availability of stem cell source, isolation and ex vivo expansion of stem cells, good manufacturing practice facilities, contamination, stem cell bank, cost, regulatory issues and clinician's ability to perform stem cell transplantation (83, 84). Basically, both cell-free and cell-based approaches of pulp tissue regeneration are still in the preclinical stage of experiments (84). However, from future prospect pulp tissue regeneration appears to be an attainable goal, based on the concept of stem cell-based pulp tissue engineering (85).

Conclusion

Regenerative endodontics presents a new era in biological and clinical endodontics. Currently, this biologically based treatment is being recognized as the first treatment choice for immature teeth with pulp necrosis based on the success of many published cases in the literature. Our understanding of the clinical protocols has evolved to eliminate pulp infection and to also allow for stem cell potential to be induced in the canal and for the release of growth factors fossilized in the dentine walls. While repair rather than true regeneration is achieved with current protocols, it is hoped that further research in the area of stem cell-based pulp engineering will allow for true regeneration and improved treatment outcomes.

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Conflict of interest

None declared.

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