Revascularization: A Treatment for Permanent Teeth with Necrotic Pulp and Incomplete Root Development

Ronald Wigler, DMD,* Arieh Y. Kaufman, DMD,* Shaul Lin, DMD,*† Nelly Steinbock, DMD,* Hagai Hazan-Molina, DMD,‡ and Calvin D. Torneck, DDS, MS§

Abstract

Introduction: Endodontic treatment of immature permanent teeth with necrotic pulp, with or without apical pathosis, poses several clinical challenges. There is a risk of inducing a dentin wall fracture or extending gutta-percha into the periapical tissue during compaction of the root canal filling. Although the use of calcium hydroxide apexification techniques or the placement of mineral trioxide aggregate as an apical stop has the potential to minimize apical extrusion of filling material, they do little in adding strength to the dentin walls. It is a well-established fact that in reimplanted avulsed immature teeth, revascularization of the pulp followed by continued root development can occur under ideal circumstances. At one time it was believed that revascularization was not possible in immature permanent teeth that were infected. Methods: An in-depth search of the literature was undertaken to review articles concerned with regenerative procedures and revascularization and to glean recommendations regarding the indications, preferred medications, and methods of treatment currently practiced. Results: Disinfection of the root canal and stimulation of residual stem cells can induce formation of new hard tissue on the existing dentin wall and continued root development. Conclusions: Although the outcome of revascularization procedures remains somewhat unpredictable and the clinical management of these teeth is challenging, when successful, they are an improvement to treatment protocols that leave the roots short and the walls of the root canal thin and prone to fracture. They also leave the door open to other methods of treatment in addition to extraction, when they fail to achieve the desired result. (J Endod 2013;39:319–326)

Key Words

Apexification, apexogenesis, immature permanent tooth, maturogenesis, mineral trioxide aggregate, regeneration, revascularization, revitalization, triple antibiotics

Unlike fully developed teeth, pulp necrosis of an immature permanent tooth with apical inflammation does not preclude the presence of residual pulp progenitor cells in the apical third of the root canal (1–4). It does, however, create a situation where achieving the goals of conventional root canal treatment is not only difficult, but even when these are met, it leaves the root short, weak, and prone to fracture (5). Although some of the technical difficulties associated with conventional root canal treatment can be overcome when a calcium hydroxide [Ca(OH)₂] apexification approach (6) or an apical plug of mineral trioxide aggregate (MTA) is used (7), the risk of future root fracture and tooth mobility because of a poor root-crown ratio still remains.

In the retrospective clinical study by Cvek (5), the frequency of cervical root fractures was markedly higher in endodontically treated immature teeth than in mature teeth and ranged in incidence from 28%–77%, in accordance with the stage of root development. This finding emphasized the importance of preserving pulp vitality of the immature teeth involved in dental trauma or deep caries.

Conventional root canal preparation of immature permanent teeth with necrotic pulp and apical pathosis presents several treatment challenges. Mechanical cleaning with instruments that remove dentin is contraindicated, because it may further weaken the already thin root canal walls. Obturation of the root canal without extending the root canal filling into periapical tissues is clinically challenging, even for the experienced clinician; the large apical opening that at times can have a divergent configuration does not provide the mechanical stop necessary to confine the filling material to the root canal. In 1966 Alfred L. Frank (6) published an article describing a clinical technique aimed at inducing apical closure. By using repeated Ca(OH)₂ dressings during a 3- to 6-month period, he demonstrated that it was possible not only to induce healing of the apical lesion but also to induce closure of the root apex with calcified tissue (apexification). In some of the teeth in his case series, there was also continued formation of the root. In a subsequent series of articles by Torneck et al (1–4), these events appeared to be related not only to the ingress of a new population of cells but also to the stimulation of residual papilla and root sheath cells that survived the apical infection.

Later, Cvek (8) reported on the outcome of 55 nonvital permanent incisors treated by apexification and noted that in 50 incisors there were healing and apical closure but no continued root formation 14–21 months posttreatment. He reported that the healing rate was dependent on the width of the apical foramen and the diameter of the periapical lesion. He concluded that the long-term outcome of apical closure and periapical healing had a high predictability rate.

However, apexification with Ca(OH)₂ has several disadvantages. It requires multiple visits during a long period of time (6–24 months; average, 1 year ± 7 months) (9, 10), it depends on the parents’ commitment to ensure the child’s dental visits are

From the *Endodontics and Dental Traumatology Department, Graduate School of Dentistry, Rambam Health Care Campus, Haifa, Israel; †B. Rappaport-Faculty of Medicine, Technion Israel Institute of Technology, Haifa, Israel; ‡Orthodontic and Craniofacial Department, Graduate School of Dentistry, Rambam Health Care Campus, Haifa, Israel; and §Discipline of Endodontics, University of Toronto, Toronto, Ontario, Canada.

Address request for reprints to Dr Ronald Wigler, Department of Endodontics and Dental Traumatology, School of Graduate Dentistry, Rambam Health Care Campus, P.O.B 9602, Haifa 31096, Israel. E-mail address: dr.wigler@gmail.com 0099-2399/$ - see front matter Copyright © 2013 American Association of Endodontists. http://dx.doi.org/10.1016/j.joen.2012.11.014

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kept, and it undermines the mechanical strength of dentin because of a prolonged exposure to Ca(OH)$_2$.\(^{(10)}\)

An alternative Ca(OH)$_2$ apexification was suggested by Torabinejad and Chivian\(^{(7)}\). They suggested that cleaning the root canal and sealing the open apex with MTA in 1 or 2 visits could minimize the risk of root canal overfilling and promote apical repair. Simon et al\(^{(11)}\) assessed the outcome of this technique in only 1 appointment in teeth with open apices and apical lesions and concluded that it was a reasonable and predictable treatment alternative to Ca(OH)$_2$ apexification. Although this procedure offered a favorable healing outcome and required only 1 appointment\(^{(12)}\), it still did little to improve on 2 shortcomings of the Ca(OH)$_2$ apexification technique, namely the predisposition to root fracture and the failure to stimulate root development\(^{(5)}\). These shortcomings prompted clinicians to continue the search for a procedure that promoted post-treatment pulp regeneration, dentin formation, and root development\(^{(13)}\).

Nygard Ostby\(^{(14)}\), a pioneer of regenerative endodontic procedures in the early 1960s, showed that new vascularized tissue could be induced in the apical third of the root canal of endodontically treated mature teeth with necrotic pulps and apical lesions. This was accomplished by the creation of a blood clot in the apical third of a cleaned and disinfected root canal by using an apically extended root canal file just before root canal filling. He proposed that through formation of a clot (scaffold), a vasculature could be established to support growth of new tissue into the unfilled portion of the root canal. He provided histologic evidence in support of his concept that was taken surgically from teeth that had been treated in this manner.

Revascularization with continued root development and continued deposition of hard tissue in the root canal has also been shown to occur over time when immature teeth were reimplanted after intentional or traumatically related avulsion\(^{(15,16)}\). Extraoral time and degree of root maturation were shown to be important factors in the clinical success of this procedure\(^{(16,17)}\). It became apparent that the larger the foramen, the greater is the opportunity for ingrowth of a new blood supply and the reestablishment of new tissue. It was also apparent that the shorter the extraoral time, the lesser is the risk of infection and hence the greater the chance for cells to retain their vitality\(^{(16,17)}\). It appeared that the “devascularized” pulp acted as a matrix into which the new blood vessels and tissue could grow\(^{(17,18)}\). Skoglund and Trost\(^{(17)}\) investigated changes that occurred in the root canal of replanted and autotransplanted immature teeth and reported that during the first 6 months there was an ingrowth of vascularized, cell-rich connective tissue throughout the entire root canal. After 6 months most teeth displayed a marked reduction in the number of cells and blood vessels and a newly formed atubular hard tissue. In some teeth, a pulp with a functioning odontoblastic layer was present\(^{(17)}\).

In 2001, Iwaya et al\(^{(19)}\) described a procedure, which they termed revascularization, that was undertaken on a necrotic immature mandibular second premolar with a chronic apical abscess. After 30 months they noted thickening of the root canal walls by mineralized tissue and continued root development.

Subsequent to this case report, Banchs and Trope\(^{(18)}\) described a revascularization procedure for the treatment of a necrotic immature mandibular second premolar with an open apex and a large apical lesion. They stated that many thought that regeneration of pulp tissue in a necrotic infected tooth with apical periodontitis is impossible. Nonetheless, because it had been radiographically proven that regeneration was possible in a re-implanted tooth, the same could be accomplished in an infected tooth if a favorable environment was established. After accessing the root canal, they irrigated it with sodium hypochlorite (NaOCl) and chlorhexidine gluconate (CHX) and sealed in a combination of 3 antibiotics in an attempt to disinfect it and stimulate periapical repair. After 24 months, they found that the root development in the treated tooth was progressing in a manner similar to that of adjacent and contralateral teeth. On the basis of these findings, they proposed a clinical protocol for revascularization of infected immature teeth that they believed would stimulate pulp regeneration and promote apical closure\(^{(18)}\). Although the predictability of this procedure and the true nature of the tissue that developed in the root canal posttreatment were unknown, they believed that the benefits of the procedure, when successful, made it one worth the attempt. These sentiments were supported by Murray et al\(^{(20)}\), who also added that the procedure was technically simple, inexpensive, and adapted to currently available instruments and medicaments.

The drugs required for root canal disinfection can be obtained from any pharmacy and can be easily introduced into the root canal by using readily available instruments\(^{(21)}\). Furthermore, if the revascularization fails, other more traditional treatment options remain available\(^{(21)}\). Whether the newly regenerated tissue is truly pulp or only pulp-like is of little consequence, as long as the root is strengthened by the deposition of new mineralized tissue in the root canal and continued development of the root occurs\(^{(18)}\).

At present, the use of the term revascularization is debatable. Trope\(^{(22)}\) claimed that the term revascularization was chosen because the nature of the tissue formed posttreatment was unpredictable, and the only certainty was the presence of a blood supply; hence it was “revascularized.” Huang and Lin\(^{(23)}\) challenged the term revascularization as applied to endodontic procedures and believed it was more applicable to events that followed dental trauma. They gave 7 reasons why this term was inappropriate for procedures designed to stimulate tooth maturation and suggested the term induced or guided tissue generation and regeneration. More recently, Lenzi and Trope\(^{(24)}\) suggested the term revitalization as being more appropriate because it is descriptive of the nonspecific vital tissue that forms in the root canal. In 2003, Weisleder and Benitez\(^{(25)}\) suggested the term maturogenesis for a direct pulp-capping procedure of a tooth with deep caries that resulted in the complete development of the tooth. They claimed maturogenesis best describes the physiologic development of the root that occurs rather than development restricted to the apical segment. Patel and Cohenca\(^{(26)}\) also agreed that the term maturogenesis was equated with physiological root development and not simply apical development.

In 2008, Hargreaves et al\(^{(27)}\) used the term maturogenesis to describe “continued root development” in contrast to apexogenesis, which they describe as “apical closure.” They too challenged the use of the term revascularization for regenerative endodontic procedures, claiming that the goal of treatment was to regenerate a pulp-dentin complex with functional properties that are capable of supporting continued root development, while resolving apical periodontitis. Huang and Lin\(^{(23)}\) also have suggested the use of this term when a nontraditional approach is used in the treatment of nonvital immature permanent teeth with apical pathosis. Because this new approach in the management of immature teeth with apical lesions has been described in different terms by different investigators, it has caused confusion among clinicians. We suggest that the term apexogenesis be used for procedures designed to encourage continued apical development in teeth with some vital tissue in the root canal, and the term maturogenesis be used for procedures that promote continued root development in infected immature permanent teeth, rather than revascularization or revitalization. Hopefully, universally accepted terms for these procedures will eventually be considered and resolved by the American Association of Endodontists.

Regenerative endodontic procedures are biologically based procedures designed to restore function to a damaged and nonfunctioning...
pulp by stimulation of existing stem and progenitor cells present in the root canal and/or the introduction and stimulation of new stem and dental pulp progenitor cells into the root canal under conditions that are favorable to their differentiation and reestablishment of function. Although this possibility has been explored under experimental aseptic conditions (20, 28–31), it has yet to be translated into a practical and reproducible clinical technique. To date, most of the protocols used in the conservative management of infected immature teeth rely principally on the stimulation of existing cells in the pulp space and periapical tissues to promote their recovery, multiplication, differentiation, and reestablishment of function. This is achieved through endodontic debridement procedures and a combination of medicaments that reduce infection to promote healing. Although the cellular events that participate in this process are, as yet, not fully understood, some evidence to help explain why and how this occurs is available clinically (32–36).

The cells suspected of giving rise to new hard tissue and root formation seen after debridement and disinfection of the root canal in immature teeth appear to be surviving perivascular stem cells found in niches located in the apical papilla (34, 37, 38). Huang (33) and Shah et al (35) have also introduced the possibility that stem and progenitor cells from the periodontal ligament may also play a role by entering the root canal when bleeding occurs. This appears to explain why cementum formation is sometimes seen on the root canal walls and over the apical opening.

Hertwig’s epithelial root sheath (HERS) is a biccular layer that evolves from the fusion of the inner and outer enamel epithelium during odontogenesis. It is the structure responsible for the development of the root and the differentiation of odontoblasts in the dental papilla (32). It also plays a role in the differentiation of cementoblasts and the formation of cementum (32). Subsequent to disruption of the root sheath after the root dentin has formed, some epithelium persists in the form of epithelial rest cells of Malassez and participates in the repair and continued maintenance of the cementum (39, 40).

Andreasen et al (41) have suggested that even if only a portion of HERS survives a traumatic event, a regenerative potential exists that allows continued function of the root sheath.

Another way that healing may occur has been suggested by Shah et al (35). They proposed that when bleeding occurs, mesenchymal stem cells from the bone marrow and periodontal ligament may be transplanted into the root canal. These cells might form bone or a dentin-like tissue (35). The blood clot that forms is in itself a rich source of growth factors that may play an important role in the regeneration process. These growth factors have the potential to stimulate differentiation, growth, and maturation of fibroblasts, odontoblasts, and cementoblasts.

In a clinical study, Lovelace et al (37) demonstrated that initiating bleeding into the apical part of the root canal resulted in the delivery of mesenchymal stem cells to the site. Although the exact source of these cells was not identified, it was suggested that they originated from tissues adjacent to the apex of the root and not from the systemic circulation. They believed the clot acted as the scaffold for the participation of these stem cells in the regenerative response.

According to Huang (33), it is also possible that some vital pulp cells survive the trauma or bacterial infection and are present in the root canal despite the absence of a response to vitality testing. Under favorable and sterile conditions, these cells can participate in the regenerative process (33–36).

According to the American Association of Endodontists statement on regenerative procedures (42), currently there are no evidence-based guidelines to support a protocol that provides the most favorable outcome in the treatment of infected immature permanent teeth.

The presence of an apical radiolucency and the absence of a response to thermal or electrical challenges can no longer be used as the sole determining factor in evaluating tissue vitality. In both of these situations, residual vital pulp and/or apical papilla cells may still be present. Although it is at times difficult or impossible to clinically determine the presence of surviving viable cells or to assess the ability of these cells to survive and differentiate, a factor that appears to be an indicator of that potential is the duration of the infection. Hypothetically, the longer an infection exists, the lower the probability that pulp and stem cells required for regeneration will survive. In addition, the longer the infection exists, the greater is the likelihood that bacteria present in the dentinal tubules and irregular recesses of the root canal can be eradicated (33).

Recently, Garcia-Godoy and Murray (43) published recommendations concerning regenerative endodontic procedures in permanent immature traumatized teeth. They claimed that because of lack of long-term evidence in support of the use of regenerative endodontic procedures in these teeth, revascularization/revitalization procedures should not be undertaken until nonsurgical orthograde endodontic treatment, root canal obturation, apexogenesis, apicification, or pulpotomy treatment have been attempted and failed. This recommendation is in sharp contrast to recommendations made by other researchers and clinicians (13, 18, 19, 21, 24, 32–35, 44, 45). Jeeruphan et al (45) evaluated radiographic and clinical outcomes of 61 immature teeth treated with Ca(OH)2 apicification (n = 22), MTA apicification (n = 19), or revascularization procedure (n = 20) and found that the percentage changes in root width and length were significantly greater in the revascularization group (28.2% and 14.9%) when compared with the MTA apicification group (0.0% and 6.1%) and Ca(OH)2 apicification group (1.5% and 0.4%). Moreover, the survival rate of teeth in the revascularization group (100%) and MTA apicification group (95.5%) was greater than the survival rate observed in the Ca(OH)2 group (77.2%). They concluded that revascularization protocols offered a favorable outcome for resolving the infection and promoting root development in the management of infected immature permanent teeth.

On the basis of these studies and case reports, this article will attempt to review the guidelines for revascularization that have been recommended for the treatment of infected immature permanent teeth, with or without apical pathosis.

**Appointment #1**

An assessment of the patient should be performed, including the state of tooth development, extent and history of the endodontic infection, and the restorability of the crown, before the procedure is undertaken. These factors are important in ensuring that a predictable outcome can be achieved.

Immature permanent teeth with necrotic pulp, with or without apical pathosis, and an incomplete developed root with an apical opening that measures 1 mm or larger are considered suitable candidates for treatment, providing the crown, when damaged, is restorable (16).

An informed consent must be signed by the patients’ parents/guardians, who must be informed that this is a relatively new procedure with no standardized guidelines. Furthermore, they must be told that follow-up appointments are obligatory to assess the outcome of initial treatment and to discuss other treatment options if this treatment should fail to meet expected goals, ie, reduction or resolution of apical lesion when present, continued root development with reduction in the size of the apical foramen, and deposition of additional hard tissue on the root canal walls.

After obtaining consent, the tooth should be anesthetized, a rubber dam placed, the tooth and working field disinfected, and straight line
access made to allow the necrotic tissue in the pulp chamber to be removed after initial irrigation of the root canal. The canal should be inspected by using dental magnification to confirm or refute the presence of residual vital tissue and the level to which it may be present in the root canal. This is the first phase in determining the type of treatment that will follow (revascularization or apexogenesis).

**Suggested Revascularization Guidelines for Cases without Signs of Vital Tissue**

**Debridement and Disinfection.** Removal of necrotic pulp tissue and the disinfection of the root canal are essential prerequisites for a favorable response to this type of treatment. Mechanical cleaning is contraindicated because it may weaken the thin dentinal root walls (5), as well as remove vital tissue remnants that might be present in the apical part of the canal (19). A K-file, or alternatively a gutta-percha cone, should be introduced into the canal to establish a working length (32, 46).

In cases when inserting a file or gutta-percha cone into the canal, a little resistance caused by the presence of tissue is felt and/or although anesthetized, the patient reports a sensation of pain, residual vital tissue should be suspected (47), and an apexogenesis procedure should be performed.

Removal of necrotic tissue from the root canal is accomplished by gently irrigating the root canal with a minimum of 20 mL 2.5% NaOCl dispensed through a syringe and a 20-gauge needle (18, 19, 21, 44, 46–49). NaOCl is a potent antimicrobial agent and effectively dissolves necrotic and organic tissue (50). Its solvent potential is dependent on its concentration and the frequency of fluid exchange (51, 52). Although higher concentrations are potentially toxic to periapical tissue (53), Trevino et al (38) found that the survival rate of human stem cells of the apical papilla (SCAP) exposed to 6% NaOCl, followed by 17% EDTA and then 6% NaOCl again, was 74%. Concentrations of NaOCl ranging from 1.25%–6% have also been used and have reportedly yielded favorable results. It appears then that the concentration of NaOCl can be adjusted if other precautions inherent to the use of NaOCl are followed (13, 18, 19, 21, 32, 44, 46).

When irrigating with NaOCl, the needle should be introduced into the root canal to a point 2 mm short of the apical foramen (13, 18, 19, 21, 32, 44, 46), and the NaOCl is slowly expressed from the syringe to extend it to a point 1 mm short of the root apex. The use of this antibiotic combination has been supported by Banchs and Trope (18).

**Root Canal Medication.** After the root canal has been irrigated, it should be carefully dried with large, sterile paper points. The root canal can then be medicated with 1 of 2 dressings, each leading to a possible different outcome (Table 1).

**Antibiotic Combination.** An intracanal antibiotic dressing can be placed into the root canal to a depth 2 mm short of the root apex and to allow room for reestablishment of a new vasculature and formation of new hard tissue on the root canal walls (58). Hoshino et al (59) introduced a triple antibiotic combination of ciprofloxacin, metronidazole, and minocycline that they claimed was sufficiently potent to eradicate bacteria from the dentin of the infected root and promote healing of the apical tissues. The medicament is made by mixing equal doses of the 3 antibiotics with sterile saline to a paste-like consistency (42, 46). Reynolds et al (46) used a mixture of 250 mg each of ciprofloxacin, metronidazole, and minocycline with sterile water.

Before mixing, it is important to ensure that the metronidazole and ciprofloxacin tablets are ground into a fine powder to give the paste a cream-like consistency. Minocycline, which is available in capsule form, only needs to be opened and added to the mixture. The paste can be inserted into the root canal with a lentulo spiral or with a syringe-type carrier. Once placed into the root canal, it should be tapped down the canal gently with a moist cotton pellet to extend it to a point 1 mm short of the root apex. The use of this antibiotic combination has been supported by Banchs and Trope (18).

In a preclinical study in dogs, Windley et al (60) showed a 99% reduction in mean colony-forming units (CFUs), with approximately 75% of the root canal showing no cultivable microorganisms after the triple antibiotic mixture was applied. This reflected a high efficacy.

Sato et al (61) investigated the antisepic properties of several antibiotic combinations in vitro and found that a combination of ciprofloxacin, metronidazole, and cefaclor was equally effective.

### Table 1. Distribution of Regeneration Case Reports and Studies According to Medication [antibiotics combination or Ca(OH)₂]

<table>
<thead>
<tr>
<th>Article (reference)</th>
<th>Number of cases</th>
<th>Hoshino’s triple antibiotic combination: ciprofloxacin, metronidazole, and minocycline</th>
<th>Hoshino’s triple antibiotic combination: ciprofloxacin, metronidazole, and cefaclor</th>
<th>Two antibiotics combination: metronidazole and ciprofloxacin</th>
<th>Ca(OH)₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iwaya et al, 2001 (19)</td>
<td>1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Banchs and Trope, 2004 (18)</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Chueh et al, 2006 (44)</td>
<td>4</td>
<td></td>
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<tr>
<td>Thibodeau and Trope, 2007 (21)</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Jung et al, 2008 (47)</td>
<td>9</td>
<td></td>
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<tr>
<td>Reynolds et al, 2009 (46)</td>
<td>1</td>
<td></td>
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<tr>
<td>Cehreli et al, 2011 (67)</td>
<td>6</td>
<td></td>
<td></td>
<td>+</td>
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<tr>
<td>Iwaya et al, 2011 (80)</td>
<td>1</td>
<td></td>
<td></td>
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<tr>
<td>Nosrat et al, 2011 (73)</td>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>Lenzì and Trope, 2012 (24)</td>
<td>2</td>
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<td>Chen et al, 2012 (32)</td>
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<td>Jeeruphan et al, 2012 (45)</td>
<td>20</td>
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<tr>
<td>Aggarwal et al, 2012 (72)</td>
<td>1</td>
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</table>
In a subsequent case report, Thibodeau and Trope (21) reported substituting cefaclor for minocycline in the Hoshino triple antibiotic formula to avoid dentin discoloration, a problem that often accompanies the intracanal use of minocycline (59, 62, 63). If the Hoshino antibiotic combination is used, Reynolds et al (46) have suggested that the discolored effect of the minocycline can be minimized by coating the dentinal tubules in the pulp chamber with a bonding agent, then placing a root canal projector (CJM Engineering Inc, Santa Barbara, CA) into the chamber, and filling the space between the projector and the dentin with a flowable composite resin. After the resin sets, the projector can be removed, and the triple antibiotics paste can be placed into the canal in a backfill manner to the level of the cementoenamel junction (CEJ).

When discoloration occurs after using the triple antibiotic paste, internal bleaching can be performed during the follow-up examinations when evidence of maturation of the tooth has been observed (62). Cefaclor instead of minocycline can also be substituted in the paste to avoid discoloration (62).

There are concerns other than tooth discoloration (46, 59) that are associated with intracanal use of an antibiotic or antibiotic combination. First, there is the fear of promoting antibiotic resistance in some root canal bacteria (33). Recent reports have shown that this is already developing in bacteria recovered from endodontic infections (64). Second, there is a risk of precipitating an allergic reaction in a sensitive patient or inducing sensitivity in a patient who has never been sensitive (46). These concerns highlight the need for a full and comprehensive medical and dental history of the patient before treatment, regardless of the method of administering the antibiotic during the course of treatment. Finally, because the preservation of residual cells is critical to a favorable outcome of the treatment, it is important that any antimicrobial medicament including antibiotics or antibiotic combinations be biocompatible. Although several studies have identified the Hoshino combination of antibiotics as biocompatible (65), another has warned that it could be potentially cytotoxic (66). Gomes-Filho et al (65) evaluated the effect of triple antibiotics on rat subcutaneous tissue at different time periods and concluded that it is biocompatible. On the other hand, Wang et al (66) believed that highly concentrated antibiotic paste might be toxic to live tissue. Discrepancies such as these highlight the need to undertake additional clinical research to better understand the biological effects of the drug concentration used and their optimal period of application.

**Calcium Hydroxide.** Ca(OH)\(_2\) has been advocated as a root canal disinfectant and for stimulation of hard tissue repair (apexification) at the apex of infected immature teeth (41). Its method of use has now been modified to comply with the demands of treatment designed to stimulate new hard tissue deposition on the root canal walls and continued growth of the root. Its use is advisable when sensitivity to one of the antibiotics used in Hoshino or modified Hoshino paste has been reported. The protocol was highlighted in a 20-tooth case series report by Chen et al (32). In this case series, the root canals were irrigated with copious amounts of NaOCl and then medicated with an aqueous Ca(OH)\(_2\) paste that was placed into the coronal half of the root canal. Bose et al (58) showed that by using a Ca(OH)\(_2\) paste in this manner, in time, dentinal wall thickness could be increased by 53.8%. This was significantly greater than the 3.3% increase achieved when the paste was placed apical to that point.

Cehreli et al (67) demonstrated that regenerative endodontic treatment of multirotted immature necrotic teeth by using Ca(OH)\(_2\) in the coronal third of the root canal was a viable alternative to conventional apexification treatment. All teeth in their study demonstrated absence of clinical symptoms, radiographic evidence of periapical healing, progressive thickening of dentinal walls, and continued apical development.

A freshly mixed aqueous paste of Ca(OH)\(_2\) has a pH of approximately 12.5 and is potentially toxic to bacterial and human cells. However, several favorable biological properties have been attributed to it when used clinically. It is antimicrobial, it has the ability to dissolve necrotic tissue in the root canal, and it can induce apical closure by hard tissue formation (10, 68). It also acts as a physicochemical barrier, which precludes the proliferation of residual microorganisms and prevents the reinnervation of the root canal from the oral cavity (68). For these reasons Ca(OH)\(_2\) has been used as a preferred agent in apexification (44). The demands of apexification are very different from those of the maturation procedure. The latter requires preservation of vital tissue and a stimulation of odontoblast-like and HERS cells (44). The use of Ca(OH)\(_2\) in revascularization is therefore not without criticism. Some authors claim that because of its high pH, it can destroy cells vital to the repair process (18, 33). Others fear it may induce an uncontrolled calcification of the canal space that would prevent the ingrowth of soft tissue with an odontogenic potential (33). In contrast, clinicians who advocate its use believe that by restricting its placement to the coronal third of the root canal, its beneficial properties can be used and its toxicity limited (32, 44, 58).

Ca(OH)\(_2\) should not be placed into the root canal with a lentulo spiral. Instead, it should be placed to the coronal portion of the root canal with a syringe-type carrier and then tamped down gently with a moist cotton pellet to the junction of the coronal and middle thirds of the root length. This can be confirmed by x-ray.

**Temporary Restoration.** Preventing coronal leakage of bacteria into the cleaned and medicated root canal is a primary prerequisite for successful revascularization. It is for this reason that a double coronal restoration is recommended. This is done by placing a sterile cotton pellet over the root canal medicament and then covering the pellet with Cavit cement (3M ESPE, St Paul, MN). The Cavit is, in turn, covered with glass ionomer cement that affords the seal greater resistance to the occlusal forces and wear during the long interval that can occur between appointments (69).

It is advisable to use non-eugenol temporary cements. Eugenol-containing cements, such as intermediate restorative material, can contaminate the preparation, thus inhibiting the polymerization of certain resin composites subsequently used as permanent restorative filling material (70).

**Medication Period.** No agreement exists concerning the preferred medication or the optimal period for leaving medication in the root canal. Different clinicians have used different periods that have ranged from 7 days to several weeks (18, 19, 21, 32, 46, 47).

**Appointment #2**

Before proceeding with the next phase of treatment, it is important to ensure that all clinical signs and symptoms have abated. If clinical signs or symptoms persist, the procedures performed in the first appointment should be repeated. If they continue to persist over several appointments, an apexification procedure should be considered (35).

When proceeding with the second appointment, the tooth should be anesthetized before the rubber dam is placed. An anesthetic without vasoconstrictor should be chosen to prevent constriction of the blood vessels in the apical region or a limited flow of blood when bleeding is mechanically induced (62). After careful removal of the temporary restoration the medicament should be removed by gently irrigating.
the root canal by using a minimum of 20 mL 2.5% NaOCl. The irrigation should be repeated until no medicament is evident in the canal.

From that point on, the irrigation protocol is similar to that used during the first appointment with one exception, the substitution of 10 mL 17% EDTA instead of CHX as a final rinsing solution (31, 71). Recent studies advocate the use of EDTA at this time and claim that as a chelating agent, it can decalcify the surface of the root canal dentin and expose its collagen fibers (71). Collagen possesses adhesion motifs for the adhesion of new cells, whereas the decalcification of the dentin releases bound growth factors that can attract new cells and promote their differentiation into cells with odontoblast-like properties (20, 71). Both are potentially valuable assets in the regenerative procedure.

The use of EDTA as a final rinse was promoted by Yamauchi et al (31), who concluded after their animal study that EDTA had no negative effect and helped in the formation of a calcified tissue that led to strengthening of the root walls. This protocol was also proposed by Trevino et al (38), who showed that irrigation with 17% EDTA or a combination of 17% EDTA and 6% NaOCl was compatible with stem cell survival, whereas irrigation protocols that included 2% CHX were not. It was feared that because of its substantivity, CHX could interfere with the binding of SCAP cells to the extracellular dental matrix (38).

**Scaffold**

Scaffolds are used in regenerative procedures to provide a framework through which cells and a vasculature can grow (72). Scaffolds can also be infused with a variety of factors that promote cell growth and cell differentiation. They can be constructed from synthetic materials such as polyglycol or from indigenous materials such as acellular, unmineralized tissue matrices or just collagen (31, 47).

In replanted avulsed and extracted teeth, the retained avascular pulp is used as the scaffold for the ingrowth of new pulp tissue (15–17, 69). Its role has led to a clinically acceptable level of success in retaining these teeth and promoting continued root development. A protocol for using a stable blood clot that can act as a scaffold in the revascularization of infected immature teeth has been suggested by numerous researchers (14, 18, 21, 37, 46, 47, 67, 72, 73). The assumption is that by inducing bleeding into the disinfectan canal, a stable blood clot can be established that will not only serve as a scaffold but also provide factors that stimulate their cell growth and differentiation of these cells into odontoblast-like cells (13, 34, 37, 41, 46, 51, 62).

The suggested protocol begins with the introduction of a sterile #20 K-file into the apical tissues 2 mm past the apical foramen to initiate bleeding into the root canal (18, 21, 46, 47, 74). Bleeding should be controlled so that it does not extend beyond a point approximately 3 mm apical to the CEJ. This is done by applying intracanal pressure with a sterile saline soaked cotton pellet until a clot is formed. Estimated mean time for the establishment of a stable blood clot is 15 minutes (18, 21, 46, 47). The clot can be carefully touched with the reverse end of a large sterile paper point to confirm its stability. Once stability is confirmed, the clot should be carefully covered with MTA cement that is back-filled to the level of the CEJ. It is important to note that revascularization and the generation of new tissue will not occur in this area, which predisposes the tooth to fracture in this area. However, to date, there have been no clinical reports of this happening (18, 21, 35, 47). It also should be noted that when the blood clot is not stable, it can break down and allow the MTA to be pushed farther down the root canal. Although not necessarily detrimental to a favorable outcome, its apical displacement can interfere with the depth of new tissue that grows into the root canal (24, 31).

After its initial set, a wet cotton pellet should be placed over the MTA and the access opening sealed with a temporary restoration. MTA is biocompatible, tri-mineral cement that mixes with water to a flowable consistency. It can set in the presence of blood and, once set, is highly resistant to penetration by bacteria (55, 75). MTA is currently marketed in 2 forms, gray and white (WMTA). WMTA was developed sometime after the introduction of the gray type to address problems of tooth discoloration, which at times occurred after the use of the gray type in the crown of the tooth (55). However, a recent report on pulp capping revealed that WMTA in the crown also can result in discoloration (76). It is important to note that there are now several types of tri-mineral cements available for use in revascularization and that they have different setting and biocompatibility characteristics. Nosrat et al (73) reported 2 successful cases of revascularization in necrotic immature molars by using a calcium-enriched mixture cement. However, recommendations for the use of a mineral cement in this protocol are limited to MTA because of the large number of studies that have been published over the years in support of its use in cases such as these (13, 18, 19, 32–35, 44, 46, 47).

Recently published studies reflect the attempts that have been made to explore novel methods of providing a scaffold within the root canal space to support the growth of new tissue. One has been the use of a collagen, with and without an induced blood clot (31, 47). In a study by Yamauchi et al (31), a histomorphometric analysis of canines treated with a revascularization protocol showed significantly more mineralized tissue formation in the root canal when a blood clot was used in combination with a cross-linked collagen scaffold. In another case series by Jung et al (47), the procedure failed in one of the teeth when bleeding into the root canal could not be induced. When a clot was formed in combination with Collatape (Sulzer Dental Inc, Plainsboro, NJ), however, there was complete resolution of the apical radiolucencies and continued apical closure after 17 months.

Several studies have suggested that the use of a polymer scaffold is the most promising means of inducing replacement tissues through tissue engineering (77, 78). Gottlieb et al (78) investigated the ultrastructural appearance of tissue-engineered pulp constructs implanted within cleaned and shaped teeth. Their results support the concept that it is possible to implant tissue-engineered pulp constructs such as stem cells from human exfoliated deciduous teeth into endodontically treated teeth. Future regenerative endodontic treatment could very well involve the use of similar laboratory-created constructs for regenerative procedures. Although pulp constructs hold great promise, they should be considered experimental and as yet unproven for clinical use.

**Appointment #3**

The third appointment is principally scheduled to remove the cotton pellet, confirm the set of the MTA, and place a permanent restoration into the access opening. It is possible to avoid a third appointment by waiting for the MTA to set during the second appointment (21).

**Apexogenesis Guidelines: Suggested Treatment in Cases of Confirmed Residual Vital Tissue**

Perform an apexogenesis procedure if vital pulp remnants have been confirmed. The root canal should be disinfected with copious amounts of NaOCl flowed into the root canal by a syringe carried to a depth 1 mm short of the level of the vital tissue. The root canal should then be gently filled with a mixture of antibiotics or Ca(OH)2 to the vital tissue, and the access opening should be temporarily sealed (19). The medication should remain in the root canal for up to 1 month (43, 47).

At the second appointment, it is important to ensure that there has been resolution of signs and symptoms. If clinical signs and/or symptoms
perspectives, the first appointment guidelines should be repeated. If the clinical symptoms still persist after treatment repetition, other procedures should be considered. If no signs and symptoms are present, the medication should be irrigated out, the root canal dried, and MTA carefully placed over the vital tissue in the root canal below the level of the CEJ. A moist sterile cotton pellet is placed over the MTA, and the access is sealed with a temporary restoration (47). The next appointment is principally scheduled to remove the cotton pellet, confirm the set of the MTA, and restore the access opening with a permanent restoration. It is possible to avoid this appointment by waiting until the MTA has set during the second appointment.

Ca(OH)2 can be used in lieu of MTA; a thin layer of Ca(OH)2 should be carefully placed directly over the vital tissue in the root canal, and the access cavity should be temporarily sealed by using a double restoration (19, 44). Similar to the Ca(OH)2 apicification, the Ca(OH)2 is used to initiate hard tissue formation. Radiographs should be taken at 3-month intervals to check whether a hard tissue barrier has formed and whether the Ca(OH)2 has been washed out of the canal. If washout is not evident, the Ca(OH)2 can be left intact for another 3 months. Dressings may be repeated every 3 months until a calcified barrier develops over the vital tissue. This can be confirmed radiographically and clinically. Once formed, the tooth should be isolated, Ca(OH)2 should be flushed out, and a permanent restoration should be placed (44).

Follow-up and Treatment Outcome

No standard follow-up protocol exists for revascularization procedures. Different clinicians have advised different follow-up periods in their case reports, with some lasting as long as 5 years posttreatment (13, 18, 19, 32–35, 44, 46, 47). In the majority of the cases, improvement or resolution of the apical lesion can be expected in approximately 6 months and root elongation and apical closure, with thickening of the root canal walls, within 12–24 months postoperatively (13, 18, 19, 32–35, 44, 46, 47). Most clinicians suggest that during the first year, 3-month recalls should be scheduled, followed by 6-month recalls unless clinical symptoms develop.

In the last decade, many successful maturation case reports and case series have been published; most were performed on incisors and premolars of children 8–14 years of age (13, 18, 19, 32–35, 44, 46, 47). Successful revascularization case reports in older ages have also been published (35, 72). There is no universal protocol described in the literature, but most depend on the same principles: (1) chemical disinfection of the canal without instrumentation, (2) production of a suitable environment for a scaffold to support tissue ingrowth; and (3) a tight bacterial seal of the access opening to prevent the ingress of bacteria.

Long-term studies are warranted to assess subsequent outcomes such as the redevelopment of apical periodontitis and the incidence of pulp canal obliteration (13). Unless accompanied by signs and/or symptoms of infection, it is advisable that no further treatment beyond maturation be undertaken because most of these cases will remain functional and disease free for many years (21, 79).

Conclusion

Over the years, there have been significant changes in the clinical management of infected immature permanent teeth. Since the 1960s and 1970s when Ostby (14) and Torneck et al (1–4) first demonstrated the capacity of the infected dental pulp to survive and continue to function, albeit in a diminished and altered manner, and the early 2000s when Iwaya et al (19) and Banchs and Trope (18) first published revascularization clinical reports, the approach to clinical management of infected immature teeth has changed. Although the new approach to treatment can at times be challenging and the outcome of revascularization procedures still remains somewhat unpredictable, they represent an improvement over older treatment protocols that have left the roots short and the walls of the root canal thin and prone to fracture. They also leave the door open to other methods of treatment besides extraction, when they fail to achieve the desired result.

Like all dental procedures, careful case selection and full disclosure to the patient (and parent) regarding the goals and limitations of the treatment are essential to make this form of mainstream treatment as an acceptable alternative in the clinical management of infected immature teeth. We have every expectation that continued research on regenerative procedures will provide new answers and new directions in the years to come and that tissue engineering will be the dental treatment of choice in the not too distant future.

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References

Review Article