Drawbacks and Unfavorable Outcomes of Regenerative Endodontic Treatments of Necrotic Immature Teeth: A Literature Review and Report of a Case

Ali Nosrat, DDS, MS,* Negar Homayounfar, DDS, MS,† and Kaveh Oloomi, DDS, MS‡

Abstract

Introduction: Endodontic treatment of immature necrotic teeth is challenging. Recently a biologically based treatment called regenerative endodontic treatment was introduced. Although regenerative endodontic treatment causes root development, there are several drawbacks and unfavorable outcomes that should be addressed. This article describes regenerative endodontic treatment of 2 maxillary central incisors with poor root development outcomes. Methods: A healthy 14-year-old female patient was referred. The patient had history of an impact trauma 6 years before the first visit. Clinical and radiographic examinations revealed that maxillary central incisors were immature and necrotic with symptomatic apical periodontitis. After local anesthesia, rubber dam isolation, and access cavity preparation each tooth was irrigated with 20 mL of NaOCl 5.25% and received triple antibiotic dressing (ciprofloxacin, metronidazole, minocycline) for 4 weeks. In the next visit, after eliminating antibiotic dressing, bleeding was induced inside the canals, and then the coronal thirds of the canals were sealed with mineral trioxide aggregate. A week later, both teeth were permanently restored. Results: In clinical and radiographic follow-ups, both teeth were functional, periapical lesions were healed, and the apices formed. However, the roots were not developed. After 6 years, because of moderate discoloration and caries, both teeth received root canal therapy and were permanently restored with casting dowel core and full crown restorations. Conclusions: Criteria for case selection in regenerative endodontic treatments should be determined. (J Endod 2012;38:1428–1434)

Key Words

Dental trauma, immature teeth, MTA, regenerative endodontics, root development, triple antibiotic paste, unfavorable outcome

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reatment of necrotic immature teeth is very challenging in endodontics. Immature roots are weak, short, and more susceptible to fracture. It is difficult to perform chemomechanical debridement and create an effective apical seal by using conventional endodontic treatment methods (1). Historically, multiple-visit apexification was the treatment of choice (2). Although this method was successful (3), it had several disadvantages including long-term treatment, increased root dentin brittleness, and increased risk of root fracture because of long-term presence of calcium hydroxide inside the root canal space (4). Apical barrier technique was introduced as a replacement for apexification with calcium hydroxide (5). In the apical barrier technique a barrier material is placed at the apex to facilitate obturation procedure. Considering its sealing ability (6), biocompatibility (7), hard-tissue induction potential (8), and the ability to set in the presence of moisture (9), mineral trioxide aggregate (MTA) is the material of choice for the apical barrier technique. Clinical studies have shown high success rates of this method (10). However, none of the aforementioned methods can promote root development.

Recently, a biologically based treatment called regenerative endodontic treatment was introduced (11). This approach is based on the presence of osteo/odonto progenitor stem cells in the apical papilla that are resistant to the infection and necrosis caused by proximity to periodontal blood supply (12). In this treatment, the ideal goal is to prepare an appropriate environment inside the root canal space (ie, absence of bacteria and necrotic pulp tissue, presence of a scaffold and a tight coronal seal) that promotes repopulation of these stem cells, regeneration of pulp tissue, and continuation of root development (13). The treatment procedure begins with chemical disinfection by copious irrigation of the root canal space with NaOCl (14), combination of NaOCl/chlorhexidine (15, 16) or NaOCl/hydrogen peroxide (17), followed by placement of an intracanal medicament at the first visit. Several medicaments like triple antibiotic mixture (metronidazole, ciprofloxacin, and minocycline) (18), calcium hydroxide (19), and formocresol (20) have been used successfully. At the next visit, which should be at least 1 week after the initial session (21) or more (14), in the absence of clinical signs of inflammation, the clinician removes the intracanal medicament and induces bleeding inside the root canal space by irritating the periradicular tissue. After clot formation, the clinician seals the root canal space by placing an MTA plug over the blood clot (18). There are several case studies that demonstrate successful clinical and radiographic outcome for this treatment approach in single-rooted (15, 22) and molar teeth (23, 24).

However, there are several drawbacks and unfavorable outcomes that are not addressed yet. The purpose of this study was to present these drawbacks and their
probable etiologies based on available case reports/series and animal studies. These problems are as follows.

**Discoloration**

Discoloration of the tooth after regenerative endodontic treatments, as revealed by Kim et al (25), is a problem mostly related to the use of minocycline in the triple antibiotic paste. They demonstrated that the main reason for tooth discoloration after treatment was the contact of minocycline in the triple antibiotic paste with coronal dentinal walls during treatment procedure. A recent study suggested sealing dentinal walls of the access cavity by using dentin bonding agent and composite resin before placement of triple antibiotic paste inside the canal (26). On the other hand, Kim et al examined the performance of this prevention technique for tooth discoloration. In this study teeth treated with dentin bonding were evaluated with naked eye and then with colorimeter. In the eye assessment teeth did not have any change in color, but in the colorimeter assessment they had. They concluded that using dentin bonding agents before placement of the triple antibiotic paste might not completely prevent tooth discoloration. A practical way to prevent discoloration is replacing minocycline with an antibiotic that does not stain teeth. Thibodeau and Trope (14) reported a successful regenerative endodontic treatment of a maxillary central incisor by using cefaclor instead of minocycline in the antibiotic mixture. Some efforts have been made to omit intracanal medication from the treatment procedure. Shin et al (16) presented a successful single-visit technique of regenerative endodontic treatment. The technique used in this study consisted of irrigation of the coronal portion of the root canal space with NaOCl and chlorhexidine gluconate and then MTA placement without bleeding induction. A recent animal study on regenerative endodontic treatment introduced a new technique for root canal disinfection (27). They used NaOCl 2.5% and apical negative pressure produced by EndoVac (Discus Dental, Culver City, CA) system without antibiotic medication. Histologic evidence of successful treatment in this study demonstrated that the introduced method of root canal disinfection is promising for immature necrotic teeth, and the use of triple antibiotic paste may not be necessary. Both studies (16, 27) introduced novel methods to shorten the treatment period and also prevent tooth discoloration by omitting the intracanal medication process.

Although the main reason for discoloration after regenerative endodontic treatments is minocycline (25), several studies revealed that gray MTA (26) and white MTA (15) can cause discoloration after treatment. Discoloration after treatment of teeth that were treated with calcium hydroxide might be related to presence of MTA in cervical portion of the root canal space (28) (2 of 20 cases). A recent report on pulp capping in anterior teeth revealed that presence of white MTA in the crown can cause considerable discoloration (29).

**Treatment Period**

The required time for disinfection of the root canal space with triple antibiotic paste or calcium hydroxide and increased number of clinical sessions (compared with one-visit MTA apical barrier technique) are other drawbacks of regenerative endodontic treatment. Elimination of antibiotic paste and bleeding induction should be done in the absence of clinical signs of inflammation (including pain, swelling, drainage from root canal, and presence of a sinus tract) (18). The time spent on antibiotic disinfection in clinical studies done from 2004 varies between one (21) to eleven (14) weeks. However, MTA apical barrier technique also should be done in more than one session in the presence of clinical signs of inflammation (5).

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**Challenging Histologic Outcomes of Animal Studies**

A few histologic and immunohistologic studies have been performed on the outcome of regenerative endodontic treatments in dogs’ teeth (27, 30–33). Histologic evidence of hard-tissue deposition on the root canal walls (43.9%), apical closure (54.9%), and formation of vital tissue in root canal space (29.3%) was demonstrated by Thibodeau et al (33) in a dog model. They also evaluated the effect of blood clot and a soluble collagen scaffold on the outcome. Histologic outcome of the treatment was not different in the presence or absence of blood clot inside the root canal space. In addition, presence of soluble collagen scaffold did not improve the results. A histologic study by da Silva et al (27) revealed that the generated tissue inside the root canal space after regenerative endodontic treatment was basically ingrowth of periodontal connective tissue instead of pulpal connective tissue. In another study, histologic evaluation of the tissues produced inside the root canal after regenerative endodontic treatment of dogs’ immature necrotic teeth revealed 3 types of tissues: cementum-like tissue that was responsible for increase in root length and thickness, bone-like tissue and periodontal ligament (PDL)–like tissue inside the canal space (30). There was only one case with partially survived pulp tissue in which the presence of odontoblasts lining was seen. Formation of cemental bridges in different levels inside the canal was also demonstrated, which might be related to hard-tissue induction potential of MTA (30). In an effort to improve the root thickening and lengthening of treated immature necrotic teeth, Yamauchi et al (31) designed and evaluated a tissue engineering protocol including use of insoluble collagen sponge as a scaffold and ethylenediaminetetraacetic acid (EDTA) 17% as a demineralizing agent that could expose dentin matrix and possibly promote differentiation of mesenchymal cells and formation of mineralized tissues. Outcomes revealed that the use of cross-linked collagen significantly increased formation of mineralized tissues, and use of EDTA 17% significantly increased attachment of newly formed mineralized tissues to the dentinal canal walls. Two forms of hard tissue were detected: dentin-associated mineralized tissues (DAMT) that were adhered to or detached from dentinal walls, devoid of vasculature and cells; and bony islands (BI) that were in the inner lumen independent of dentinal walls and contained many embedded blood vessels, cells, and bone narrow-like tissues. In a separate study, further histologic assessments and immunohistochemical analyses were performed on DAMT and BI by the same group (32). Outcomes showed that DAMT was clearly different from dentin and bone and, to some extent, from cementum. Although lack of vasculature and immunostaining patterns in DAMT resembled cementum, the organization and maturation of collagen fibers were significantly different (32). Immunoreactivity of dentin sialoprotein (DSP) and bone sialoprotein (BSP) in BI was similar to alveolar bone. No odontoblastic cell layer, dentin-like structure, and pulp-like tissue were detected (32). All in all, histologic findings of animal studies show that the tissue formed inside the canal is not pulp and therefore does not function like pulp tissue. It means that this treatment procedure does not result in pulp regeneration in dogs’ teeth. However, the histologic outcomes of treatment of human necrotic immature teeth might differ from that of dogs’ teeth. Although the presence of stem cells has been demonstrated in apical papilla of human teeth (12) and in the blood clot formed inside the root canal space after disinfection with triple antibiotic paste (34), none of the aforementioned studies have characterized these cells in dog’s apical papilla or in the blood clot formed inside the canals. On the other hand, if the tissue formed in immature human teeth after regenerative endodontic treatment is the pulp tissue, it should function as a normal pulp. That means in cases with poor root development after
Poor Root Development

Ideal root development pattern in immature teeth includes increase in root length, increase in root wall thickness, and formation of the root apex. In some studies, the outcome of regenerative endodontic treatments of necrotic immature teeth was lower than ideal, including absence of increase in root length (15, 23), absence of increase in root wall thickness (15, 28), or lack of formation of tooth apex (28). Formation of a hard-tissue barrier inside the canal between the coronal MTA plug and the root apex (28) is another reported unfavorable outcome. A recent study revealed that root development potential of immature necrotic teeth is related to the vitality of Hertwig epithelial root sheath (28). Therefore, there might be a correlation between dental history and quality of root development; the longer the duration of pulp necrosis, the lower the quality of root development after regenerative endodontic treatments. Such an association could be found in studies that reported decreased or no root development. In a case series study by Petrino et al. (15), a case of 2 traumatized necrotic immature maxillary central incisors (case 1) had a history of impact trauma 6 years before initial visit. One year after treatment, the apex of the left central incisor was blunt and closed, and the root length did not increase. The right central incisor did not show any sign of root development. Lenzi and Trope (35) reported regenerative endodontic treatment in 2 traumatized necrotic immature maxillary central incisors. The patient had a history of impact trauma 2.5 months earlier. On the basis of the severity of immaturity, they found that left central incisor had been traumatized long before the right one. Twenty-one months after treatment, the left central incisor did not indicate any sign of root development, and just a radiopaque hard-tissue barrier formed at the apex. Meanwhile, the right one showed signs of successful treatment and full root development. The authors discussed the possibility that longstanding infection might destroy the cells capable of pulp regeneration. However, on the basis of successful outcomes of regenerative endodontic treatments in cases with long-lasting apical periodontitis, they concluded that this might not be the reason. We reviewed the available history of all reported cases of successful regenerative endodontic treatment with continued root development done from 2004 to establish a probable relationship between dental history and the quality of root development (Table 1). This information shows that history of pulp necrosis in cases with successful treatment is no longer than 6 months (mean, ~47.8 days), which is much shorter than 6 years. Therefore, there might be a relationship between the duration of pulp necrosis and outcome of treatment. However, this information shows the time between initiation of patient’s symptoms and treatment or the time between traumatic injury and treatment. There is a possibility that pulp necrosis occurs before initiation of patient’s symptoms (in cases with pain or swelling) or after traumatic impacts (Table 1). In addition, there were several reports of successful regenerative endodontic treatment with continued root development in which the dental history of the patients did not show the duration of chief complaint or the approximate time of the pulp necrosis before treatment (14, 19, 21, 23–25, 28). This subject deserves further studies to determine case selection criteria based on patient’s dental history.

A retrospective evaluation of radiographic outcomes discovered that regenerative endodontic treatment with triple antibiotic dressing increased root wall thickness significantly more than either calcium hydroxide or formocresol (38). In addition, this study revealed that in cases disinfected with calcium hydroxide, the radiographic location of calcium hydroxide inside the root canal space influenced the root development. When calcium hydroxide was radiographically limited to coronal half of the root canal space, the increase in root wall thickness was greater than when it was placed beyond coronal half.

Insufficient Bleeding

Some authors have reported failure to induce bleeding (21, 23, 24). A recent study revealed that mesenchymal stem cells are delivered into root canal space after bleeding induction in human teeth, a phenomenon that did not happen in the absence of blood clot inside the disinfected root canal space (34). In addition, it is assumed that the blood clot formed inside the root canal space after disinfection contains platelet-derived growth factors and serves as a protein-rich scaffold (13). An animal study demonstrated that root canals that had a blood clot formation inside them after disinfection had better radiographic outcome compared with those without blood clot (35). To facilitate bleeding after root canal disinfection, using local anesthetics without vasoconstrictors is recommended (15). However, lack of bleeding or insufficient bleeding after using plain local anesthetics is reported (15, 23, 24), which has been shown to be related to poor root development in some cases (25). On the other hand, several cases with sufficient bleeding and lack of root development have been reported (15, 35). One study assumed that there was a possibility that blood clot broke down and left the root canal space without scaffold into which the new vital tissue could
grow (35). In addition, there are several reports of successful regenerative endodontic treatment and continued root development without bleeding induction (16, 19, 22). A recent case report suggested use of platelet-rich plasma instead of blood clot inside the root canal space (36). Interestingly, the histologic evaluation of the soft tissue produced inside the canal revealed presence of pulp-like tissue (37). Therefore, this subject is controversial and should be studied more.

**Root Canal Calcification/Obliteration**

Root canal calcification/obliteration is another problem after regenerative endodontic treatment of necrotic immature teeth that is reported in cases disinfected by calcium hydroxide (19, 22, 28). In a case series study by Chen et al (28) complete root canal calcification/obliteration happened in 4 of 20 cases within an average follow-up time of 16 months. Although it is stated that the maximum interval of calcium hydroxide therapy was 4 weeks in this study, there is not any information about the duration of calcium hydroxide therapy in each case. A retrospective study by Chueh et al (19) on 23 immature necrotic teeth treated with calcium hydroxide revealed complete root canal obliteration in 2 cases within 17 and 59 months after initial treatment. The duration of calcium hydroxide therapy was 9 and 6 months, respectively. In addition, other 21 teeth showed partial obliteration of the root canal space in comparison with adjacent normal teeth. In a study by Chueh and Huang (22) on 4 cases of regenerative endodontic treatment, complete root canal calcification occurred in one case within 34 months (18.5 months of calcium hydroxide therapy), and severe narrowing of the root canal space occurred in another case within 5 years. However, there were 3 studies by Cotti et al (17) (1 central incisor), Cehreli et al (24) (6 molars), and Cehreli et al (39) (2 central incisors) in which calcium hydroxide was used as intracanal medicament, and no root canal calcification/obliteration was reported. The duration of calcium hydroxide therapy in these 3 studies was 2, 3, and 3 weeks, respectively, which was much shorter than the same period in aforementioned studies. In addition, Cehreli et al (24) followed their cases for 9–10 months. The complete root canal calcification/obliteration has occurred at least within an average of 16 months after initial treatment in aforementioned studies. Therefore, the follow-up period of the study by Cehreli et al (24) might not be enough for a conclusion in this regard. On the other hand, there is no report of complete root canal calcification/obliteration in cases disinfected by triple antibiotic paste. Although complete root canal calcification/obliteration is not mentioned as a failure in cases that have undergone regenerative endodontic treatment, it can cause serious challenges in case the involved tooth needs root canal therapy.

There are other issues that should be addressed. Randomized clinical trials comparing the long-term success of this new treatment with traditional ones, especially MTA apical plug techniques, have not been performed. In addition, the criteria for case selection and criteria of success/failure have not been determined. Besides, there is a study reporting the continuation of symptoms after disinfection procedure with NaOCl 5.25% and triple antibiotic dressing (2 patients out of 12) that caused changes in treatment planning (21).

**Case Report**

A healthy 14-year-old female patient was referred to the Endodontic Department of the Dental School, Tehran University of Medical Sciences in 2005. As stated by her legal guardians, the patient’s chief complaints were repeated swelling and pain in the anterior maxilla within past few months before initial visit and crown fracture of the anterior maxillary teeth. They reported a history of impact trauma to the anterior maxillary teeth 6 years before initial visit. Clinical examinations revealed extensive caries of tooth #8, complicated crown fracture of tooth #8, and uncomplicated fracture of tooth #9. Both teeth showed normal mobility. Cold test by using Endo-Frost cold spray (Roeko; Coltene Whaledent, Langenau, Germany) did not elicit any response in maxillary anterior teeth. They reported a history of impact trauma to the anterior maxillary teeth 6 years before initial visit. Clinical examinations revealed extensive caries of tooth #8, complicated crown fracture of tooth #8, and uncomplicated fracture of tooth #9. Both teeth showed normal mobility. Cold test by using Endo-Frost cold spray (Roeko; Coltene Whaledent, Langenau, Germany) did not elicit any response in maxillary anterior teeth. Whereas maxillary lateral incisors responded normally to the test. Both

![Figure 1](https://example.com/image1)

(A) Preoperative periapical radiograph of teeth #8 and #9. Note periapical radiolucent lesions and immaturity of roots. (B) After regenerative endodontic treatment and permanent coronal restoration. (C) 6-year follow-up. The periapical lesions healed, and root apices formed without increase in length and thickness of the roots. Note the mesial coronal caries in tooth #9. (D) Immediately after root canal therapy. Note several sealer pathways at apex of tooth #9. (E) After casting dowel core and full crown restoration of both teeth.
maxillary central incisors were sensitive to percussion and palpation. There was no palpable periapical swelling on the maxillary central incisors at the time of examination. Both maxillary central incisors were immature with visible periapical radiolucent lesions in the radiographs (Figs. 1A and 2A). Considering the history of impact trauma and outcome of the clinical and radiographic examinations, the concluding diagnosis for both central incisors was pulp necrosis with symptomatic periapical periodontitis. Taking into consideration that both teeth were immature and necrotic, the treatment of choice was regenerative endodontic treatment. After explaining the treatment procedure, risks, and benefits to the patient’s legal guardians, a written consent was obtained.

After local anesthesia with 3% plain mepivacaine (Septodont, Cedex, Switzerland) and rubber dam isolation, caries in tooth #8 was removed, and access cavities on teeth #8 and #9 were prepared by using a diamond-coated fissure bur (Diatech, Heerbrugg, Switzerland) and a high-speed handpiece. Each root canal was passively irrigated with 20 mL NaOCl 5.25% without instrumentation. Canals were gently dried with paper points (Ariadent, Tehran, Iran). Equal powdered proportions of metronidazole (ParsDaru, Tehran, Iran), ciprofloxacin (AminDaru, Tehran, Iran), and minocycline (Razak, Tehran, Iran) were mixed with saline, the creamy paste was placed inside the canals by using counterclockwise motion of a size 50 K-file (Dentsply Maillefer, Tulsa, OK), and the teeth were restored temporarily with Cavite (Asia Chemi Teb Co, Tehran, Iran). Four weeks later, the patient was asymptomatic, and none of the maxillary central incisors were sensitive to percussion and palpation. Under rubber dam isolation and local anesthesia with 3% plain mepivacaine, the temporary restorations were removed, and the antibiotic paste was eliminated by using copious irrigation with NaOCl 5.25%. A sterile size 40 K-file was overextended beyond the root length several times in both teeth to irritate periradicular tissues and initiate bleeding. The blood could be seen on the apical part of the file, but not inside the canals. After 10 minutes, MTA powder (ProRoot tooth-colored MTA; Dentsply Tulsa Dental, Tulsa, OK) and distilled water were mixed according to manufacturer’s instructions. By using a sterile amalgam carrier, approximately 3 mm of MTA was placed in the coronal third of the canals. MTA was gently adapted to the dental walls with a moistened cotton pellet. The access cavities were filled with saline, a moistened cotton pellet was placed inside the access cavities, and both teeth were temporarily restored. A week later the patient was referred for permanent restoration of the teeth (Figs. 1B and 2B).

The patient was recalled yearly for clinical and radiographic follow-ups. At follow-up sessions both maxillary central incisors were asymptomatic and functional. No recurrence of swelling was reported by the patient. The teeth were not sensitive to percussion and palpation. The response to the cold test was negative in all follow-up sessions. In radiographic examinations the radiolucent lesions healed, and the apices formed. However, there was no increase in the length and thickness of the roots (Fig. 1C).

Six years after initial treatment the patient complained about the appearance of her maxillary central incisors. Clinical examinations revealed moderate discoloration of both teeth and deep mesial caries in maxillary left central incisor (Figs. 1C and 2C and D). In consultation with Department of Prosthodontics, because of the amount of remaining tooth structure, severity of discoloration, patient demands and expectations, and to get the ideal esthetic and better prognosis, full crown restoration for both teeth was suggested. Therefore, the patient was scheduled for root canal therapy of both central incisors. The same clinician performed the root canal therapy. After local anesthesia and rubber dam isolation, restorative materials and caries were removed, and access cavities were prepared by using a diamond-coated bur and high-speed handpiece. The MTA barriers were hard and intact. They were removed by using a Cavitron ultrasonic scaler (Dentsply International, York, PA) with copious water irrigation. Both root canals were empty, and no vital tissue or bleeding was seen. Root canal preparation was performed by using hand instrumentation with K-files sizes 45, 50, 55, and 60, with copious NaOCl 1.25% irrigation between instruments. The canals were dried with paper points. The apical thirds of the root canals were obturated with

**Figure 2.** (A) Preoperative photograph of the central maxillary incisors of a 14-year-old girl. The patient had a history of impact trauma. Note the fracture of the incisal edges in both teeth and the caries lesion in the right central maxillary incisor. (B) View after revascularization and permanent coronal restoration. (C) Facial view of both teeth 6 years after initial visit. Note the mesial caries in the left central maxillary incisor and the discoloration of both teeth. (D) Palatal view 6 years after initial visit. Note the discoloration.
warm vertical compaction of size 70 gutta-percha (Ariadent, Tehran, Iran) and sealer (Pulp Canal Sealer, Kerr, MI) by using preheated pluggers (M-series; Dentsply Maillefer). The radiograph after obturation revealed several pathways of sealer extrusion in the apical area of the left maxillary central incisor (Fig. 1D). After root canal therapy both teeth were temporarily restored, and the patient was referred to the prosthodontist for coronal restorations. Finally, both teeth were restored with casting dowel core and full crown (Fig. 1E).

Discussion

A recent clinical study on the responses of necrotic immature permanent teeth to regenerative endodontic treatment revealed that there would be 5 types of root development pattern based on survival of Hertwig epithelial root sheath (28). As stated in this study, type 2 is “no significant continuation of root development with the root apex becoming blunt and closed”. In the presented case, after treatment, root apices of both central incisors formed without any significant increase in the root length and root wall thickness. Although the disinfection method used in this study was different, this finding was almost comparable with type 2 developmental pattern described by Chen et al (28) (Fig. 1). However, all developmental patterns described in that study showed signs of root wall thickening, which was not seen in the presented case. Therefore, the radiographic findings of this case were similar to findings of an apexification treatment, the same as that reported by Lenzi and Trope (35). In the present case, passage of a long time (6 years) without any treatment after traumatic impact might be related to damaged Hertwig epithelial root sheath and, subsequently, decreased root development potential.

Root canal disinfection in regenerative endodontic treatments is a challenge. Although in the traditional endodontic treatments, lowering of the bacterial loads and prevention of bacterial access to the periapical tissues might be conducive to healing, in pulp regeneration a higher level of disinfection is required (40). Bacterial cells are smaller in diameter compared with the pulpal end of dentinal tubules and therefore can penetrate deep into the dentinal tubules of infected teeth (41). Moreover, in the younger teeth bacteria penetrate through more dentinal tubules and advance deeper in comparison with older ones (42). Although NaOCl is an effective irrigant to reduce bacterial loads, it cannot render the infected root canal space bacteria free (43), which would be an ideal condition for pulp regeneration. Two in vitro studies by Hoshino et al (44) and Sato et al (45) revealed that the mixture of ciprofloxacin, metronidazole, and minocycline was effective against endodontic pathogens and was able to deeply disinfect the infected dentinal tubules. Animal studies by Windley et al (46) and Coheanca et al (47) revealed that irrigation with NaOCl followed by triple antibiotic paste dressing renders 70%–78% of the canals culture negative. On the other hand, culturing bacteria after antibiotic dressing is a problematic issue. Bacteria may remain viable but uncontrollable, and also it is difficult to inactivate antibiotics in the culture medium (48). Meanwhile, several successful cases have been reported that used the similar disinfection regimen (14, 23), the same as we used in the presented case. The effect of disinfectants on the root canal dentinal walls is another issue that should be addressed. Ring et al (49) demonstrated that irrigation with NaOCl or chlorhexidine can interfere with the attachment of dental pulp stem cells to dentinal walls.

Lenzi and Trope (35) demonstrated that healing of the periapical lesion and production of hard-tissue barrier in the apex after regenerative endodontic treatment can be seen even in the absence of vital tissue inside the root canal space. They concluded that disinfection protocol in regenerative endodontic treatments (NaOCl irrigation followed by 1 month of triple antibiotic dressing) renders the root canal space sterile or has a long-lasting effect suppressing the regrowth of bacteria over time. The follow-up time in that study was 21 months. The presented case showed that empty root canal spaces remained bacteria free for 6 years, which confirms the conclusion of Lenzi and Trope.

Tight coronal seal is an important component of successful regenerative endodontic treatment (13, 18). Most of the studies have used a double seal consisting of MTA and a permanent resin restoration over the blood clot (15, 18, 26), similar to what was done in the presented case. The calcium ion released from MTA reacts with environmental phosphorus. The reaction leads to formation of hydroxyapatite crystals on the surface of MTA (50) and MTA–dentin interface (51). It is hypothesized that this bioactive reaction is responsible for MTA’s sealing ability and biocompatibility (50, 51). Sealing ability of MTA against bacterial leakage is well-documented (6). The presented case shows that MTA might maintain its sealing ability even after the seal of the coronal permanent restoration is lost. However, presence or absence of leakage around MTA barriers could not be shown by clinical or radiographic examinations.

The importance of presence of blood clot inside the root canal space as a protein-rich scaffold that contains platelet-derived growth-factors and mesenchymal stem cells has been shown before (33, 34). In addition, it has been demonstrated that insufficient bleeding might be related to poor root development after regenerative endodontic treatment (23). Therefore, the absence of vital tissue inside the root canal spaces of both central incisors and lack of root development in this case might be partly related to insufficient bleeding and lack of scaffold inside the root canals. As stated in previous studies, bleeding induction is part of the treatment procedure and should be encouraged in cases without bleeding (15). Therefore, the presented case should be considered as a deviation of the accepted technique. However, absence of blood inside the canal space was confirmed by naked eye in the presented case. Using magnification in such cases helps the clinician to evaluate the situation more accurately.

A recent case report showed that the tissue formed inside the root canal space of human teeth after regenerative endodontic treatment is a pulp-like tissue (37). However, the information about the hard tissue produced on dentinal walls and the cells responsible for hard-tissue production is still lacking. An animal study revealed that a cementum-like tissue was deposited on the root canal dentinal walls after regenerative endodontic treatment. This tissue was irregular and was assumed to be responsible for root development (30). In the present case, the radiographic view of several sealer extrusion pathways in the apical region of the left maxillary central incisor might indicate that the hard tissue formed in the apical region is porous and irregular. In addition, although the treatment procedure was the same for both teeth, radiographic view of the apex formed in the right central incisor was more uniform, and there were not any sealer extrusion pathways.

A case series study on regenerative endodontic treatment revealed that a mean follow-up period of 8 months for evaluation of osseous healing and 16 months for evaluation of root development are necessary (19). There are several studies on this new treatment that have followed their cases more than 16 months (18, 22, 26), but also there are lots of studies with shorter follow-up periods (15, 24, 25). Except for one study in which calcium hydroxide was used for root canal disinfection (22), none of the prospective clinical studies followed the cases beyond the time of completion of the root development, and there is no information about what happened after completion of apical closure. The presented case is unique in this regard.

The present review and poor outcomes of the reported case show the importance of determining criteria for case selection in regenerative endodontic treatments. The ideal goal of this new treatment is pulp
regeneration inside the root canal space and, consequently, root development. Healing of the periradicular disease has occurred in all cases with complete treatment, which shows the predictability of the disinfection protocols. Randomized clinical trials that compare long-term outcomes of this new approach with the traditional ones, specifically MTA apical barrier technique, are recommended.

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