Case Report

Modified Revascularization in Human Teeth Using an Intracanal Formation of Treated Dentin Matrix: A Report of Two Cases

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Abstract

Ethylenediaminetetraacetic acid (EDTA)-treated dentin matrix (TDM) is an enriched source of bioactive molecules. Therefore, it was hypothesized that fabrication of autogenous TDM on root dentinal walls of necrotic immature permanent teeth may allow more predictable outcome of revascularization treatments. Two young patients with permanent nonvital immature teeth were chosen for revascularization treatment. After appropriate disinfection of root canal system, TDM was fabricated on root dentinal walls using different dilutions of EDTA. Then, bleeding was induced in canals and calcium-enriched mixture (CEM) cement was placed over the blood clots. In all follow-up periods, both cases were asymptomatic and radiographic findings have shown a continued root development. Revascularization is a valuable treatment for nonvital immature teeth, allows continuation of root development. Modification of root regeneration through a TDM protocol may seem more predictable treatment and improve maturogenesis than traditional therapy.

Keywords: *Calcium-enriched mixture, dentin-pulp regeneration, open apex, revascularization, treated dentin matrix*

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Introduction

Endodontic management of immature permanent teeth with necrotic pulps and open apices has been challenging due to insufficient dentinal walls and open apices.[1,2] Conventionally, such teeth have been treated by the apexification technique using calcium hydroxide or artificial apical barrier methods which involve multiple visits and cannot predictably stimulate root development.[3-5] More recently, revascularization/revitalization is a known alternative practical approach in endodontic treatment. This therapeutic approach not only helps close the wide-open apex but also increases the root thickness and length.[2,6-8] However, there are still debates and many efforts to find out new approaches or modifications to improve the outcome of conventional root regeneration treatment.[9]

Recently, several studies showed the positive effect of treated dentin matrix (TDM) as a source of bioactive molecules in differentiation of odontoblasts and pulp-dentine regeneration.[10] Demineralization of human dentin by ethylenediaminetetraacetic acid (EDTA) can lead to release of its dentine bioactive molecules, inducing nearby stem cells to differentiate into odontoblast-like cells.[11-13]

To date, there is no report evaluating the positive contribution of intracanal formation of TDM on revascularization therapy. In fact, the aim of the present report of two cases is to evaluate the outcome of revascularization protocol modified by fabrication of an autogenous TDM on root dentinal walls of necrotic immature teeth with open apices.

Case Reports

Case 1

An 8-year-old boy was referred to Endodontic Clinic Department of Islamic Azad University of Tehran for management of an immature permanent anterior tooth with pulp necrosis secondary to trauma and severe pain on chewing. In his dental history, the root canal of tooth #9 had already been opened and instrumented. On clinical examination, tooth number #9 had complicated crown fracture and severe sensitivity to percussion and palpation

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but mobility and probing measurement were in normal limit. The pulp responses to cold, heat, and electronic pulp tester (EPT) tests were negative, whereas the contralateral and adjacent teeth were normally responded. No swelling or sinus tracts were detected. In periapical initial radiograph, incomplete root apical formation was observed, along with periapical radiolucency [Figure 1]. According to clinical and radiographic findings, a common diagnosis of pulp necrosis and acute apical periodontitis was determined. Based on wide-open apex, a modified regenerative treatment was planed.

An informed written consent was obtained from his parents after complete explanation of the risks and possible failures and the alternative treatment modalities.

Revascularization method

A two-visit regenerative endodontic procedure was performed. On initial visit, the access cavity was reopened after administration of local anesthesia by 3% mepivacaine plain (Septodont, Cedex, France) without vasoconstrictor and rubber dam isolation. Following working length determination through an ISO #45 K-file and periapical radiograph, the root canal was gently irrigated with 20 ml of 2.5% sodium hypochlorite (NaOCl) without any mechanical instrumentation. To prevent the destruction of thin dentinal walls and progenitor cells which exist in the apical tissues, a passive decontamination was performed using NaOCl without conventional mechanical instrumentation.[2]

Final rinse with 10 ml normal saline was performed to remove the remaining NaOCl. After irrigation, a double antibiotic paste (prepared from the precursors of ciprofloxacin 250 mg and metronidazole 250 mg in 1:1 ratio)[14] was mixed with sterile distilled water in a 3:1 ratio as manufacturer's instruction. Then, the mixture was placed in the canal though lentulo spiral and taped down the root canal with a moist large paper cone. The access cavity was sealed with e-Temp (DiaDent Group International, Korea), and the patient was recalled 4 weeks later.

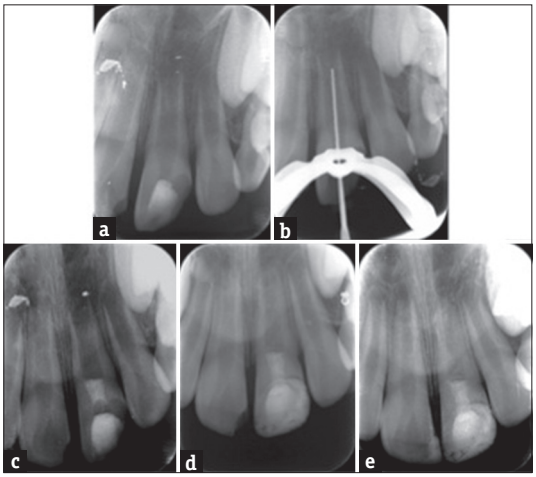

Figure 1: (a) Preoperative intraoral periapical radiograph of left maxillary central incisor. (b) Initial radiography for working length determination. (c) Postoperative radiograph after root canal disinfection, treated dentin matrix fabrication, bleeding induction, placement of calcium-enriched mixture cement over the blood clot and temporary restored with resin-modified glass-ionomer cement over a wet cotton pellet. (d) Follow-up radiograph at 3-month postoperation. A dentinal bridge was formed underneath calcium-enriched mixture. (e) Follow-up radiograph at 12-month postoperation showed continued maturation and apical closure

On second visit, the tooth was asymptomatic. The tooth was anesthetized with 3% mepivacaine plain (Septodont, Cedex, France) without vasoconstrictor, isolated by rubber dam, and reopened. The double antibiotic paste was removed with copious irrigation of 20 ml of 2.5% NaOCl and final rinse with 20 ml normal saline; thereafter, the root canal was irrigated by different dilutions of EDTA as a TDM protocol preparation (root canals were dried with paper points and filled with 1 ml of 17% EDTA for 5-min, 10% EDTA for 5-min and 5% EDTA for 10-min, respectively, and distilled water at regular intervals) and a final rinse with 10 ml sterile saline.[11] Apical bleeding was induced by gentle apical patency into apical tissue with ISO#35 K-files, until an obvious blood hemorrhage was achieved approximately at 2-3 mm below the cementoenamel junction (CEJ). A sterile cotton pellet was inserted into the canal and held there for 10 min to allow blood clot formation. Next, calcium-enriched mixture (CEM) cement (CEM-Bionique Dent, Iran) was mixed on a glass-mixing pad according to the manufacturer's instructions and applied gently on the blood clot just below the CEJ at a 5–6 mm thickness using a micro spoon excavator and large paper points. Finally, a wet cotton pellet was placed over the CEM cement, and the access cavity was temporarily restored with resin-modified glass-ionomer cement (RMGIC) base material, Vitrabond (3M, ESPE, St. Paul, MN, USA). The tooth was restored with final resin bonded composite restoration 4 days later after removing cotton pellet and RMGIC. The patients' parent was instructed to refer to our dental clinic in case of any pain or discomfort, tenderness to chewing pressures, mobility, and swelling.

Clinical and radiographic findings

At 6-month follow-up, the tooth was clinically asymptomatic and showed complete radiographic healing of periradicular radiolucency and a thin layer of dentinal bridge formed on CEM plug [Figure 1]. At 12 months, radiographic examination showed thickening of the lateral dentinal walls, elongation of the root, and advanced apical closure with formation of the apical barrier [Figure 1]. During recall examinations, the tooth was not responsive to cold test; however, responded positively to EPT test at maximum output power.

Case 2

A 7-year-old boy was referred to Endodontic Department Clinic of Islamic Azad University of Tehran by a general practitioner for endodontic management of an immature permanent molar with pulp necrosis secondary to caries.

The tooth #29 had been instrumented and dressed with $Ca(OH)_2$ by his referral dentist 3 weeks ago. On clinical examination, the tooth was asymptomatic and no responses to percussion and palpation. Periodontal examination revealed no mobility and normal probing depth (<3 mm). Responses to cold, heat, and EPT tests were negative, whereas the contralateral teeth were normally responsive. No signs of swelling and sinus tract were observed.

Radiographic examination revealed incomplete root formation and periapical radiolucencies [Figure 2]. All clinical and radiographic findings indicated pulp necrosis along with chronic apical periodontitis. According to the immature root formation with wide-open apices, endodontic revascularization treatment was planned.

An informed written consent was obtained from his parents after complete explanation of the risks and possible failures and the alternative treatment modalities.

Revascularization technique

Since the patient and his parents lived overseas and could not attend again earlier than 6 months, the tooth was treated by one-visit endodontic revascularization protocol. The tooth was anesthetized with 3% mepivacaine plain (Septodont, Cedex, France) without vasoconstrictor, isolated with rubber dam, and reopened. Remnant of $Ca(OH)_2$ paste was removed by gentle filing and copious normal saline irrigation. Then, the root canals were gently irrigated by 20 ml of full strength (5.25%) NaOCl followed by 10 ml sterile normal saline, then 10 ml 2% chlorhexidine (CHX) and a final rinse with sterile normal saline.

The chemically cleaned root canals were filled with different dilutions of EDTA based on a TDM protocol preparation (root canals dried with paper points and filled with 1 ml of 17% EDTA for 5-min, 10% EDTA for 5-min and 5% EDTA for 10-min, respectively, and distilled water at regular intervals) and final irrigation with 10 ml sterile saline and dried with paper points.[11] Apical bleeding was induced by gentle apical patency with #35 K-file. Blood hemorrhage was established 1–2 mm below the orifice levels. After 5 min and formation of the blood clot, CEM cement (CEM-Bionique Dent, Iran) was mixed on a glass-mixing pad based on the manufacturer's instructions and was placed and back-filled over blood clot to the level of the CEJ with a 4–5 mm thickness using a micro spoon excavator and large paper points. Finally, a well-moistened cotton pellet was temporary placed over the CEM Cement for 15 min. After initial set of CEM cement, the access cavity was permanently restored with amalgam restoration over a RMGIC base material, Vitrebond (3M, ESPE, St. Paul, MN, USA) at the same visit.

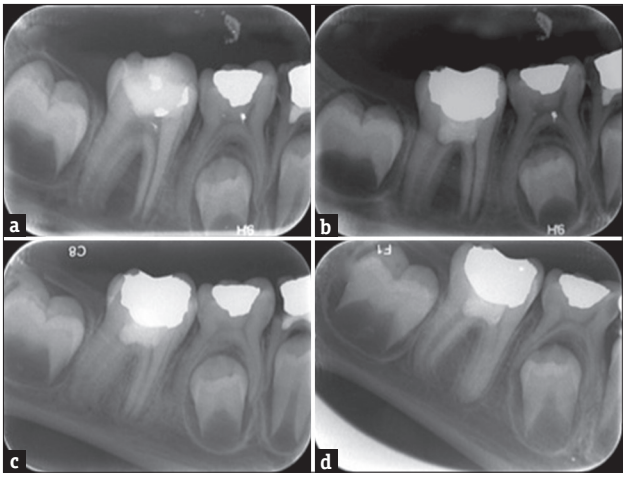

Figure 2: (a) Preoperative radiograph of right mandibular first molar with immature apices and necrotic pulp and apical radiolucencies. (b) Postoperative radiograph after root canal disinfection, treated dentin matrix fabrication, bleeding induction, and placement of calcium-enriched mixture cement over the blood clot. (c) Follow-up radiograph 6-month postoperation. (d) Follow-up radiograph 12-month postoperation

Clinical and radiographic findings

After 6 months, the tooth was symptom-free and complete radiographic healing of periradicular radiolucencies was observed. At 12-month follow-up, radiographic examination showed visible increase in the roots canal wall thickness [Figure 2]. The roots showed complete apical closure. During 6- and 12-month recall examinations, the tooth was not responsive to EPT and cold tests.

Discussion

In these two cases, revascularization treatment protocol or maturogenesis performed due to its advantages over more traditional apical plug treatments.[15] One of the concerns of this regeneration treatment is the nature of the tissue formed in root canal space and its cellular composition which are not yet identified clearly.[6] Recent animal studies showed this tissue consists of loose connective tissue similar to periodontal ligament and thickening of root dentinal wall is secondary to apposition of a cementum-like tissue.[16] On the other hand, traditional revascularization treatment has not always been successful.[11] Therefore, an attempt to find modern reliable methods or materials to regenerate newly-formed tissues similar to dentin-pulp complex with more expectable successful outcome is still an ideal goal.

In the present case reports, all root regenerated teeth were clinically asymptomatic and complete resolution of periapical lesions were successfully observed at the 6-month follow-ups. Apical closure, thickening of root canal wall, and continued root lengthening were detectable after 12 months. These findings are similar to the findings of a prospective cohort study on the outcome of twenty revascularized teeth which have been treated by a standard clinical protocol introduced by Banchs and Trope.[17]

Several *in vitro* and *in vivo* studies have reported the regenerative potential of TDM as a biocompatible scaffold containing growth factors (dentin matrix protein-1, bone morphogenetic protein-2, transforming growth factor-ß, fibroblast growth factor-2, platelet derived growth factor

and like growth factor) for attachment, proliferation and differentiation of dental pulp cells, and differentiate stem cells into odontoblast-like cells.[10-12] Hence, we hypothesized that an autogenous TDM fabricated on root dentinal walls of necrotic immature teeth using intracanal placement of EDTA, may promote root regeneration during revascularization treatment.[18]

Li *et al*.[11] demonstrated a method for fabrication of human dentine matrix and we utilized the same concept, except for mechanical cleaning of root dentin surface by ultrasonic cleaner.

Calcium-enriched mixture cement was a successful alternative for MTA in revascularization of necrotic immature permanent molars.[19]

A single-visit technique for revascularization of necrotic mandibular premolar through irrigation with NaOCl 6% followed by CHX 2% and without using triple antibiotic as intracanal medicament was introduced with successful results.[20] Thus, we decide to use same method for disinfection of root canal system in Case 2.

One of the possible side effects of this treatment protocol could be dentin erosion. An *in vitro* study showed this side effect occurs significantly when 17% EDTA presented in contact to the root canal dentin for 10 min,[21] however in the present study, we used 17% EDTA for 5 min, 10% EDTA for 5 min and 5% EDTA for 10 min. Moreover, Yamauchi *et al*. showed that EDTA had no negative effect in tissue engineering of immature dogs 'teeth and even increased the strength of root canals by the formation of newly formed hard dentin-like tissues.[22]

In contrast to previous case reports of dental pulp revascularizations, in which the thickening and maturation of the root canal wall took longer (24–48 months) to be completed, we found promising result in this regard within 12 months. TDM formation in root dentinal walls, induces more rapid differentiation of odontoblast/cementoblasts-like cells or at least promotes these cells to secret more dentin/cementum-like tissues, compared to traditional revascularization method, and accelerates treatment results including apical formation and dentinal wall thickening.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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