

REVASCULARIZATION IN MATURE PERMANENT TEETH WITH NECROTIC PULP AND APICAL PERIODONTITIS: CASE SERIES

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ABSTRACT

INTRODUCTION: Revascularization procedures have been limited to immature teeth but application to mature teeth should be clinically investigated as an alternative to conventional root canal treatment. The translation of regenerative endodontic procedures into treating mature teeth is challenging and depends on efficient root canal disinfection, proper size of the apical foramen, the availability and delivery of stem cells into the root canal system.

OBJECTIVES: Evaluation of revascularization in mature teeth after enlargement of the apical foramen to size 35 K-file.

MATERIALS AND METHODS: Three necrotic incisors with mature roots and apical periodontitis in three patients ranging from 20–30 years old were treated by revascularization procedures. Access opening was performed. The canal was mechanically instrumented to the radiographic apex. The apical foramen was enlarged with a size 35 K-file. Triple antibiotic paste (0.1 mg/ml) was applied in the canal using a syringe. After three weeks, the medication was removed with 20 ml of 1.5% sodium hypochlorite followed by final irrigation with 20 ml of 17% EDTA. Bleeding was induced in the root canal and mineral trioxide aggregate (MTA) was placed approximately 3-4 mm below the CEJ. The tooth was restored with a layer of glass ionomer followed by composite resin. The patient was scheduled for follow up and evaluation of healing after 3 months, 6 months and 9 months. Resolution of apical periodontitis and regression of clinical signs and symptoms were observed during the follow up periods.

RESULTS: After a follow-up period of 9 months, the three teeth demonstrated radiographic evidence of periapical healing with absence of clinical signs and symptoms.

CONCLUSIONS: The present cases demonstrated a favorable outcome of the revascularization procedure in mature necrotic incisors with chronic apical periodontitis.

KEYWORDS: Mature teeth; necrotic pulp; apical periodontitis; periapical healing; pulp tissue regeneration.

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INTRODUCTION

Regenerative endodontic therapy has been defined as biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex (1). Because of the encouraging results of regenerative procedures in young immature permanent teeth, regenerative endodontic procedures has been tried for use in adult teeth with closed apices in a series of cases reported by Shah et al. based on the rationale of elimination of clinical signs/ symptoms and resolution of apical periodontitis (2). The major difference in regenerative endodontic procedures for mature teeth with infected, necrotic pulps is that complete mechanical debridement is required to help eliminate root canal infection and remove necrotic tissue (3). The importance of pulp vitality in mature tooth can be understood from the biological rationale of endodontics which is to prevent or treat apical periodontitis (4). Conventional root canal therapy leads to the loss of reparative capacity, brittleness and the loss of a protective mechanism against noxious stimuli (5). Additionally, the removal of the pulp and the subsequent loss of moisture inside the root may alter the light-transmitting properties of root-filled teeth leading to change of the translucency of the tooth (6).

Revascularization in mature teeth will encounter more challenges than in immature teeth. Less stem cells and narrower apical pathway for stem cell migration will be the major limitations (7). Potential sources of stem cells in mature teeth include bone marrow, periodontal ligament, stem cells from periapical inflamed tissues and may be some surviving dental pulp stem cells (8,9). Chrepa et al (10) in their study proved that the intracanal bleeding evoked by the over instrumentation of periapical tissues elicits the influx of undifferentiated mesenchymal stem cells (MSCs) into the root canal systems of adult patients with mature teeth and apical lesions.

Induction of periapical bleeding into the canal space brings fibrin scaffold, mesenchymal stem cells, and blood-derived bioactive growth factors into the canal space. In addition, growth factors embedded in the dentin matrix are also released into the canal space after demineralization of dentin with 17% Ethylenediaminetetraacetic acid (EDTA) (11). In regenerative endodontic therapy, although the pulp replacement tissues are not true pulp tissue, they are vital tissues inherited with innate and adaptive immune defense mechanisms and innervated by sensory nerve fibers to detect and protect themselves from foreign invaders such as bacteria (3).

The size of the apical foramen used for successful revascularization and in-growth of new tissue has not been accurately determined. Laureys et al (12) in their histological study proved that an apical foramen as small as 0.32 mm did not prevent in-growth of new tissue. If the apical foramen is too small in size, it will impact not only the migration of endogenous cells but also the neovascularization and re-innervation during regeneration (13).

Performing apical widening of the cemental canal and apical foramen removes a greater amount of contaminated cementum promoting a more favorable condition for healing and promote adequate nutrient diffusion and oxygen supply for the initial viability of the cells, as the cells that are more than 200 μm away from the point of maximum oxygen diffusion are prone for anoxia and necrosis (14,15). Studies suggest that the regenerative endodontic therapy provides another treatment option for mature permanent teeth with necrotic pulps (3). The purpose of this case series is to evaluate using regenerative endodontic therapy for human mature permanent teeth with necrotic pulps and apical periodontitis in terms of elimination of clinical signs/symptoms and resolution of apical periodontitis.

CASE SERIES

The study was performed after the approval of research ethics committee, Faculty of Dentistry, Alexandria University. The patients received both oral and written information about the study protocol and signed their informed consent for agreeing to participate in this study.

Case 1

A 24-year-old man presented to the Faculty of Dentistry, Alexandria University with the chief complaint of pain in the maxillary right anterior region. The patient gave a history of trauma to his maxillary right central incisor seven months ago. Composite filling was done to the tooth at the time of trauma and it was fractured six months later. The patient did not have any significant medical history contradictory to regenerative endodontic therapy. In extraoral examination neither swelling nor sinus tract was noted. There were no palpable lymph nodes in the head and neck. Extraoral examination showed no significant signs of pathosis. Intraoral Examination showed that the patient's oral hygiene was acceptable and that the tooth #8 had an uncomplicated crown fracture. The tooth was tender to percussion and periapical palpation. There was no response to either thermal or electric pulp tests, whereas the contralateral and neighboring teeth responded positively to both tests. No sinus tract was observed and the tooth did not show any discoloration (Fig. 1A). Periodontal probing of the tooth was within normal limits, with normal physiological mobility. Standardized pre-operative periapical radiographs were taken using the paralleling technique with an Endoray film holder and a putty occlusal index for each tooth to ensure standardization during the different radiographic exposures to help in assessing healing of the periapical lesion in follow up periods. Radiographically, there was a periradicular rarefaction around the apex of tooth #8 and tooth #7 (Fig. 1B). Tooth #7 responded normally to pulp tests. On the basis of the results of the clinical and radiographic examinations, the pulpal and periradicular diagnosis for tooth #8 was pulp necrosis with periapical periodontitis. Regenerative endodontic

treatment of the affected tooth was considered. After comprehensive discussion of the risks and possible outcomes of this treatment and the treatment plan in case of failure, consent of the patient was obtained. The treatment was initiated at the same visit. The tooth was anesthetized and isolated using rubber dam. Upon access, a purulent discharge exuded from the point of penetration. Access cavity was completed and working length (WL) was determined to the radiographic apex with an electronic apex locator and a periapical radiograph. The canal was cleaned and shaped to the radiographic apex using step back technique to remove necrotic tissues. The apical foramen was enlarged with a size 35 K-file and the canal was copiously irrigated with 20 ml of 1.5% sodium hypochlorite. The canal was dried with paper points. Access cavity was acid etched using 37% phosphoric acid gel. The etchant was left to react on the enamel for 15 seconds and on the dentin for 10 seconds. The etchant was removed with thorough rinsing and the access cavity was lightly dried with air. Excite adhesive (Ivoclar, Vivadent) was applied to the entire surface of the access cavity to seal the pulp chamber walls before application of triple antibiotic paste to prevent coronal discoloration. A gentle stream of dry air was applied to disperse the dentin bonding agent into a thin, uniform, shiny appearing surface. The adhesive was light-cured for 20 seconds. The canal was medicated with 0.1 mg/ml triple antibiotic (Metronidazole: Minocycline: Ciprofloxacin=1: 1: 1). Triple antibiotic was placed to the root apex using a syringe ensuring that it remains below CEJ. The access cavity was temporized with intermediate restorative material (IRM-Dentsply). The patient was dismissed for three weeks. At the following appointment, response to initial treatment was assessed and there were no signs or symptoms of persistent infection like sinus tract or excessive exudates and the tooth was asymptomatic. Local anesthesia with 3% mepivacaine without vasoconstrictor was injected and rubber dam was applied. The temporary restoration was removed. The medication was removed with 20 ml of 1.5% sodium hypochlorite followed by final irrigation with 20 ml of 17% EDTA to release growth factors from the dentin. EDTA was left in the canal for about 1 minute and the canal was dried with paper points. Bleeding was induced in the canal by inserting file #30 past the apical foramen by 2 mm and then rotating it with the goal of having the entire canal filled with blood to the level of the cemento-enamel junction. A pre-curved K-file #15 was also used in the same manner to help in induction of bleeding. Bleeding was stopped at a level that allows for 3–4 mm of MTA (MTA Angelus) which was placed over blood clot with the hand plugger and adapted softly to dentinal walls via a moist cotton pellet. Access cavity was sealed with intermediate restorative material (IRM-Dentsply). One day after the second visit, the tooth was restored with glass ionomer cement followed by composite resin. Checking of occlusion was performed to prevent traumatic occlusion. The patient was scheduled for follow up and evaluation of healing after 3 months, 6 months and 9 months. At the 3-month follow-up, the periapical lesion had slightly decreased in size (Fig. 1C). At the 6-month and 9 months follow-ups, there was a further decrease in size of radiolucency (Fig. 1D, E). The patient presented to the clinic 30 months after the treatment was completed. He was asymptomatic and there was a significant shrinkage of the periapical lesion. A calcified bridge was evident under

MTA in the coronal third of the root canal. (Fig. 1F). Throughout the follow up visits, the patient was asymptomatic, the tooth was not sensitive to percussion and palpation, there was no sinus tract nor swelling related to the tooth. The tooth had probing depths within normal limit and normal physiological mobility. The tooth did not respond to pulp tests with cold, heat, and electric pulp test at the 3-month, 6-months, 9-months and 30 months follow-up visits. The tooth remained with no discoloration after 30 months of treatment. (Fig.1G)



Figure 1. (A) Pre-treatment intraoral photograph of the maxillary right central incisor showing fracture of the tooth #8. (B) Pre-operative periapical radiograph of the tooth. Note the periapical radiolucency. (C) Periapical radiograph obtained at the 3 months follow-up. (D, E) Periapical radiographs obtained at the 6 and 9 months follow-up. Periapical radiolucency has decreased in size. (F) Periapical radiograph obtained after 30 months demonstrating a marked decrease of the size of the periradicular radiolucency and formation of a calcified bridge (G) Appearance of the tooth after 30 months with no discoloration.

Case 2

A 25-years-old male patient was referred to post graduate clinic with complaint of pain upon biting on the tooth #9. The medical history was noncontributory. The dental history revealed that the patient had a traumatic injury to his maxillary anterior teeth 2 years ago. Subsequently to the trauma, a dentist performed composite filling on tooth #9. Intraoral examination showed that small hard swelling was localized labially in the periapical region of tooth #9. There was a composite filling restoring the fractured part of the crown (Fig. 2A). The tooth did not respond to thermal tests or electric pulp tester and it was sensitive to percussion and palpation. Periodontal probing of the tooth was within normal limits. Radiographic examination showed complete root formation and periradicular radiolucency related to tooth #9 extending to the distal aspect of the root in the apical third (Fig. 2B). On the basis of the results of the clinical and radiographic examinations, the diagnosis of the tooth was pulpal necrosis with periapical periodontitis. The patient was informed of regenerative endodontic treatment and consent was obtained. The intraoral swelling was hard and localized and not very painful to palpation. Therefore, an incision and draining were not performed. The patient was treated with the same regenerative procedures as in case 1, and he was recalled at 3, 6 and 9 months after treatment to evaluate the outcome. In clinical examination, the tooth was asymptomatic and functional with a normal periodontal condition. The tooth was not tender to percussion or palpation and the swelling disappeared. The tooth did not

respond to pulp tests with cold, heat and electric pulp test at the 3 months, 6-month and the 9-month follow-up periods. Radiographically, osseous healing of the periapical lesion was evident throughout the follow up visits (Fig. 2 C-E). The appearance of the tooth after 9 months showed no obvious change in shade or color (Fig. 2F). d after fish oil treatment.



Figure 2. (A) Pre-treatment intraoral photograph of the maxillary left central incisor. (B) Pre-operative periapical radiograph of the tooth. Note the periradicular radiolucency related to tooth #9 extending to the distal aspect of the root in the apical third.(C) Periapical radiograph obtained at the 3 months follow-up (D) Periapical radiograph obtained at the 6 months follow-up. Periapical radiolucency has decreased in size (E) Periapical radiograph obtained after 9 months demonstrating a further decrease of the periradicular radiolucency (F) The appearance of the tooth after 9 months with no obvious change in shade or color.

Case 3

A 20-year-old man presented to the faculty of dentistry with fractured lower incisor tooth. The patient’s medical history was noncontributory. The dental history revealed that the patient had a traumatic injury to the tooth since one year. Extraoral examination revealed that there was no swelling or palpable lymph nodes in the head and neck. Intraoral examination showed that tooth #24 was fractured (Fig. 3A). The tooth was tender to percussion and palpation. Periapical radiography showed a periapical radiolucent lesion related to the tooth apex. (Fig. 3B) Periodontal probing of the tooth was within normal limits. Based on the dental history and clinical radiographic findings, tooth #24 were diagnosed as having necrotic pulp with apical periodontitis. Treatment options including nonsurgical root canal treatment and regenerative endodontic treatment were presented to the patient. The patient opted for regenerative endodontic treatment. Tooth #24 was treated with the same revascularization procedures as the previous cases, The patient was recalled at 3, 6 and 9 months after treatment. At the 3-month follow-up, the periapical lesion had slightly decreased in size (Fig. 3C). At the 6-month and 9 months follow-up periods, the periapical lesion showed further decrease in size. (Fig.3 D, E) The tooth did not respond to pulp tests with cold, heat and electric pulp test at the 3 months, 6-month and the 9-month follow-up periods. After 9 month the tooth was functional with normal probing pocket depth and there was no change in tooth shade. (Fig.3F)



Figure 3. (A) Pre-operative intraoral photograph of the mandibular left central incisor. (B) Pre-operative periapical radiograph showing periapical radiolucency related to tooth #24 (C) Periapical radiograph obtained at the 3 months follow-up. (D) Periapical radiograph obtained at the 6 months follow-up. Periapical radiolucency has decreased in size (E) Periapical radiograph obtained after 9 months demonstrating a further decrease in the size of the perirapical radiolucency. (F) Intraoral photograph after 9 months.

DISCUSSION

Pulpal regeneration of an infected immature tooth was once thought to be impossible. The first successful revascularization case was published by Iwaya et al in 2001 (16), followed by Banchs and Trope in 2004 (17). Ever since then, there have been numerous case reports and case series about revascularization of necrotic permanent teeth with open apices. Now, the scientists are debating if using regeneration-based procedures in root canals of fully mature teeth can be used to regain pulp vitality.

The root canals in this case series were instrumented to the radiographic apex in order to remove necrotic tissues and bacteria from the canal. Lin et al (18) published a failed clinical case of regenerative endodontic therapy and stated that the main reason of the failure was the bacterial biofilms that remained firmly attached to the apical canal walls because of the lack of mechanical debridement. The impact of mechanical debridement of canal walls on the control of root canal infection as well as the possible jeopardy of the vitality of apical and periapical tissues still remain questionable.

While high concentration sodium hypochlorite denatures the growth factors embedded in the dentin, EDTA stabilizes them and increases their bioavailability (5,19).

Recent studies recommended the use of EDTA because of its chelating action on the root canal wall. Through decalcification, growth factors are released from the root canal dentin and they promote differentiation of cells into odontoblast-like cells (20,21).

Therefore, 1.5% sodium hypochlorite was used to preserve any vital cells remaining on the canal walls and to minimize cytotoxicity to the stem cells in the apical tissues, followed by 17% EDTA as a final rinse.

Recent studies have revealed that triple antibiotic paste, a common medicament in revascularization procedures, is cytotoxic and leads to tooth discoloration when used at high concentrations (22,23,24).

Reyhani et al (25) proved that the low concentration triple antibiotic paste is effective in disinfecting the canal. In the present case series staining prevention and preservation of stem cells required the reduction of the amount of minocycline in the TAP to 0.1 mg/ml which is not cytotoxic to the stem cells. Dentinal tubules within the chamber was sealed with dentine bonding agent. The triple antibiotic medication was injected below CEJ to minimize crown staining and this is according to the American Association of Endodontists clinical considerations for a regenerative procedure (26). Kim et al (27) in their study found that the dentin bonding agent reduced the intensity of the discoloration but did not prevent it. In the present case series, the three treated teeth did not show any discoloration throughout the follow up periods.

The size of the apical foramen appears to be a major concern in regenerative endodontic therapy. In human regenerative endodontic therapy studies of mature permanent teeth with necrotic pulps and apical periodontitis, Shah and associates (2) enlarged the apical foramen to #30 K-file, while Paryani and Kim to #60 K-file (7). Fang et al (28) conducted a review of the published clinical studies on the regenerative endodontic treatment of teeth with pulp necrosis to determine the smallest diameter of apical foramen required to obtain proper pulp revascularization. The review suggested that teeth with an apical diameter less than 0.5 mm achieved 90% clinical success. In the three presented cases the apical foramen was enlarged to size #35 which allowed proper induction of bleeding and provided stem cells needed for regeneration. That was proved radiographically by the formation of a calcified bridge in one of the cases, which means that there is a vital tissue inside the root canal. Moreover, the decreased radiolucency confirmed the success of the treatment.

One of the challenges in the management of the cases was the induction of bleeding. It took several attempts to induce bleeding into the canal space inspite of using anesthetic solution that did not contain a vasoconstrictor.

The antibiotic paste contained 1 mg of each of the three antibiotics in a total volume of 10 mL. The pharmaceutical carriers propylene glycol and polyethelene glycol (Macrogol) were used. Propylene glycol is often used as a solvent in pharmaceutical preparations whereas polyethelene glycol is used as vehicles in pharmaceutical preparations and both are chemically inert. These ingredients allow increased solubility and delivery of the TAP into the canal (29,30). We cannot rule out any effect the triple antibiotic preparation may have had on bleeding reduction. However, Ding et al (31) discussed that a possible reason might be the resolution of inflammatory reaction after dressing with the antibiotic mixture, making it more difficult to induce bleeding.

The importance of a bacteria-tight coronal seal for successful revascularization is well-documented (32). Sealing ability and biocompatibility of MTA are shown in several studies (33). In the first case a calcified bridge was evident under MTA in the coronal third of the root canal after 30 months of treatment. An animal study on revascularization showed that a cemental bridge has formed beneath MTA in most cases, which might be the result of cementogenic and osteogenic properties of MTA (34). Completion of these bridges over time might create a biological seal beneath MTA. The definitive contributing

factors for revascularization associated intra canal calcification are currently unknown. Prior study by Seo et al (35) showed that MSCs from different tissue sources retain their innate differentiation potential that reflects the tissue of their origin. Because bleeding from periapex would carry periodontal ligament stem cells and bone marrow stem cells from alveolar bone, induced bleeding as part of revascularization would recruit cells with cementogenic and osteogenic differentiation capacities into the root canal space (36).

CONCLUSION

The treated cases with mature roots and chronic apical periodontitis demonstrated a favorable outcome of the revascularization procedure both clinically and radiographically. In conclusion, the treatment objectives and goals of nonsurgical root canal therapy and regenerative endodontic therapy are the same which is elimination of clinical signs/symptoms and healing of apical periodontitis. It may be preferable to fill the disinfected root canals with the host's own vital tissue rather than with nonvital foreign material.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

- Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: A review of current status and a call for action. *J Endod.* 2007; 33: 377-90.
- Shah N. and Logani A. SealBio: A novel, non-obturation endodontic treatment based on concept of regeneration. *J Conserv Dent.* 2012; 15: 328-32.
- Saoud TM, Ricucci D, Lin LM, Gaengler P. Regeneration and Repair in Endodontics—A Special Issue of the Regenerative Endodontics—A New Era in Clinical Endodontics. *Dent. J.* 2016; 4: 1-15.
- Trope M. Regenerative Potential of Dental Pulp. *J Endod.* 2008; 34: 13-17.
- Gupta P, Sneha S, Shetty H. Regenerative Endodontics: An Evidence Based Review. *J Cont Med A Dent.* 2015; 3: 12-19.
- Ahmed HM, Abbott PV. Discoloration potential of endodontic procedures and materials: a review. *Int Endod J.* 2012; 45: 883-97.
- Paryani K, Kim SG. Regenerative endodontic treatment of permanent teeth after completion of root development: A Report of 2 Cases. *J Endod.* 2013; 39: 929-34.
- Diogenes A, Henry MA, Teixeira FB, Hargreaves KM. An update on clinical regenerative endodontics. *Endod Topics.* 2013; 28: 2-23.
- Liao J, Al Shahrani M, Al-Habib M, Tanaka T, Huang GT. Cells isolated from inflamed periapical tissue express mesenchymal stem cell markers and are highly osteogenic. *J Endod.* 2011; 37:1217-24.
- Chrepa V, Henry MA, Daniel J, and Diogenes A. Delivery of Apical Mesenchymal Stem Cells into Root Canals of Mature Teeth. *J. Dent. Res.* 2015; 94: 1-7.
- Galler KM, Buchalla KA, Federlin M, Eidt A, Schiefersteiner M, Schmalz G. Influence of root canal disinfections on growth factor release from dentin. *J Endod.* 2015; 41: 363-68.
- Laureys WG, Cuvelier CA, Dermaut LR, De Pauw GA. The critical apical diameter to obtain regeneration of the pulp tissue after tooth transplantation, replantation, or regenerative endodontic treatment. *J Endod.* 2013; 39: 759-63.
- Yang J, Yuan G, Chen Z. Pulp Regeneration: Current Approaches and Future Challenges. *Front Physiol.* 2016; 7: 1-8.
- Vemuri S, Rkotha S, Raghunath RG, Kandregula C.R. Root canal revascularization via blood clotting in regenerative endodontics: Essentials and expectations. *J Dr NTR Univ Health Sci.* 2013; 2: 235-38.
- Borlina SC, de Souza V, Holland R, Murata SS, Gomes-Filho JE, Dezan Junior E, et al. Influence of apical foramen widening and sealer on the healing of chronic periapical lesions induced in dogs' teeth. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;109: 932-40
- Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. *Dent Traumatol.* 2001; 17:185-7.
- Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? *J Endod.* 2004; 30:196-200.
- Lin LM, Shimizu E, Gibbs JL. Histologic and histobacteriologic observations of failed revascularization/revitalization therapy: a case report. *J Endod.* 2014; 40: 291-295.
- Fouad AF. Microbial factors and antimicrobial strategies in dental pulp regeneration. *J Endod.* 2017; 43:46-50.
- Galler KM, Souza RN, Federlin M, Cavender AC, Hartgerink J.D., Hecker S. Dentin Conditioning Codetermines Cell Fate in Regenerative Endodontics. *J Endod.* 2011; 37:1536-41.
- Park HB, Na Lee B, Hwang YC, Hwang IM, Oh WM, Chang HS. Treatment of non-vital immature teeth with amoxicillin-containing triple antibiotic paste resulting in apexification. *Restor Dent Endod.* 2015; 40:322-27.
- Ruparel NB, Teixeira FB, Ferraz CC, Diogenes A. Direct Effect of Intracanal Medicaments on Survival of Stem Cells of the Apical Papilla. *J Endod.* 2012; 38:1-4.
- Sabrah AH, Yassen GH, Wai CL, Goebel WS, Gregory RL, Platt JF. The effect of diluted triple and double antibiotic pastes on dental pulp stem cells and established *Enterococcus faecalis* biofilm. *Clin Oral Invest.* 2015; 19:2059-66.
- Althumairy RI, Teixeira FB, Diogenes A. Effect of Dentin Conditioning with Intracanal Medicaments on Survival of Stem Cells of Apical Papilla. *J Endod.* 2014; 40: 521-25.
- Reyhani MF, Rahimi S, Fathi Z, Shakouie S, Milani AS, Barhaghi MH, Shokri J. Evaluation of Antimicrobial Effects of Different Concentrations of Triple Antibiotic Paste on Mature Biofilm of *Enterococcus faecalis*. *JODDD.* 2015; 9: 138-143.
- AAE Clinical Considerations for a Regenerative Procedure. Revised 6-8-2016. (Internet). Available at: <http://www.aae.org/>. Accessed on 10-10-2017.
- Kim JH, Kim Y, Shin SJ, Park JW. Tooth Discoloration of Immature Permanent Incisor Associated with Triple Antibiotic Therapy: A Case Report. *J Endod.* 2010; 36:1086-91.
- Fang Y, Wang X, Zhu J, Su C, Yang Y, Meng L. Influence of apical diameter on the outcome of regenerative endodontic treatment in teeth with pulp necrosis: a review. *J Endod* 2017; 1-18.

29. Cruz EV, Kota K, Huque J, Iwaku M, Hoshino E. Penetration of propylene glycol into dentine. *Int Endod J*. 2002; 35: 330-6.
30. Petrino JA, Boda K, Shambarger S. Challenges in Regenerative Endodontics: A Case Series. *J Endod*. 2010; 36:536- 41.
31. Ding RY, Cheung GS, Chen J, Yin YZ, and Wang Q. Pulp Revascularization of immature teeth with apical periodontitis: A Clinical Study. *J Endod*. 2009; 35:745-9.
32. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? *J Endod*. 2004; 30:196-200.
33. Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review—part II: leakage and biocompatibility investigations. *J Endod* 2010; 36: 190-202.
34. Wang X, Thibodeau B, Trope M, Lin L, Huang G. Histologic characterization of regenerated tissue in canal space after the revitalization/revascularization procedure of immature dog teeth with apical periodontitis. *J Endod*. 2010; 36:56-63.
35. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. *The Lancet*. 2004; 364: 149–155.
36. Song M, Cao Y, Shin SJ, Shon WJ, Chugal N, Kim RH et al. Revascularization-associated intracanal calcification: Assessment of prevalence and contributing factors. *J Endod* .2017;43:2025–33