 Doi: 10.21608/adjalexu.2022.155616.1306

SINGLE VISIT REGENERATIVE ENDODONTIC TREATMENT (RET) IN TRAUMATIZED NECROTIC IMMATURE PERMANENT TEETH: A REPORT OF THREE CASES

Pervine H. Sharaf[1*] *MSc,* Raef A. Sherif [2] *PhD,* Ashraf M. Zaazou [3,2], *PhD*

Soad F. Hafez [4] *PhD,* Rania M. El Backly [5] *PhD*

ABSTRACT

OBJECTIVE: This report is intended to describe three cases treated with single-visit regenerative endodontic therapy in three traumatized immature necrotic permanent maxillary central incisors with periapical lesions.

MATERIALS AND METHODS: Three asymptomatic patients presented with traumatized immature necrotic permanent maxillary central incisors with periapical lesions (>7mm) requiring tooth restoration. Teeth responded negatively to percussion, palpation and to both thermal and electric pulp testing. Local anesthesia without vasoconstrictor was given and access cavities were prepared. A single visit regeneration protocol was applied including an irrigation sequence with 20 ml of 2.5% sodium hypochlorite, sterile saline (10 mL), 20 ml of 17% Ethylenediaminetetraacetic acid (EDTA), and finally sterile saline (10 mL). A # 20 K-file was used 2mm beyond the apical foramen to evoke bleeding. Biodentine (Septodont, France) was used as the cervical plug. Glass ionomer was flowed over the capping material then composite resin restoration was accomplished. After 12 months, clinical and radiographic outcomes were assessed using cone beam computed tomography (CBCT) and periapical radiographs.

RESULTS: At the end of follow up, two cases were asymptomatic. One case presented with a sinus tract and chronic apical abscess. CBCT analysis revealed thinning of the apical diameter, root thickening and lengthening. The periapical radiolucency decreased in two cases and increased in one. The teeth presented no response to thermal or electric pulp testing.

CONCLUSION: Single-visit regenerative endodontic therapy may present questionable clinical and radiographic outcomes for treatment of necrotic immature permanent teeth. Further clinical studies are needed to validate its efficacy.

KEY WORDS: Case report, CBCT, Immature traumatized permanent, Necrotic, Single visit regeneration

RUNNING TITLE: Single visit regeneration in immature teeth.

1 PhD student, Endodontics, Conservative Dentistry Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt. Endodontic specialist, Ministry of health, Alexandria, Egypt.
2 Professor of Endodontics, Conservative Dentistry Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt.
3 Professor of Endodontics, Faculty of Dentistry, King Abdel Aziz University, Jeddah, KSA.
4 Professor of Medical Microbiology and Immunology, Medical Microbiology and Immunology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt.
5 Associate professor of Endodontics, Conservative Dentistry Department, Faculty of Dentistry, Alexandria University, Alexandria, Egypt.

Corresponding author:

E-mail: pervinehsharaf@gmail.com

INTRODUCTION

The presence of open apex in immature necrotic permanent teeth presents one of the difficulties faced by endodontists. Regenerative endodontic treatment (RET) is currently the favored management. Apexification which was the treatment option for these cases, achieved only apical closure. Continuous root development is the major goal offered by (RET). Healing of apical periodontitis, sinus tract resolution, and vitality responses observed in certain cases, present other clinical successes of (RET) (1). Regenerative endodontic treatment adopts the cell homing strategy; which is the recruitment of

endogenous cells to the affected tissue via signaling molecules (2). Stem cells of apical papilla are the main type in immature teeth regeneration. Platelets are an important ally in this modality of treatment enhancing healing through merging intricate chain of mechanisms via their mediators as cytokines, transforming growth factors, platelet growth factors, and vascular endothelial growth factors. Yet, infection is a major challenge to successful RET as it has been shown to be the cause of failed regenerative endodontic treatment (RET) in 79 % of unsuccessful cases (3).

In RET, canal disinfection is based mainly on irrigation solutions and intracanal medicament dressing, with slight dentinal wall filing to evade damaging the present stem cells (4). The Clinical Considerations for a Regenerative Procedure by the American Association of Endodontics and the Statement for Revitalisation Procedures by the European Society of Endodontology recommend the use of intracanal medicaments as triple antibiotic paste or calcium hydroxide following irrigation in a multiple visit regeneration procedure (5,6). However, the use of triple antibiotic paste with the recommended concentrations may have detrimental effects as discoloration, cytotoxicity, sensitization, and development of resistant bacterial strains (7). Furthermore, prolonged use of calcium hydroxide as an intra canal medicament may weaken the physical and mechanical properties of dentin (8). This has led to the recent advocation of single visit regenerative endodontic procedures.

Therefore, this report documents the treatment of three cases of traumatized immature necrotic permanent maxillary central incisors with periapical lesions using single visit regenerative endodontic procedures.

MATERIALS AND METHODS

Four patients (one girl aged 11 years and two boys aged 12 and 13 years) were referred from the clinics of the Conservative Dentistry and Pediatric Dentistry Departments, Faculty of Dentistry, Alexandria University, Egypt for endodontic management of traumatized immature permanent maxillary central incisors.

All procedures performed were in consistency with the ethical standards of the Research Ethics Committee, Faculty of Dentistry, Alexandria University and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The clinical work was performed after the approval of the Research Ethics Committee, Faculty of Dentistry, Alexandria University (IRB 00010556) -(IORG 0008839) on 4/7/2018. The purpose, hazards, and benefits of the study procedures were explained to the parents/guardians. Written informed consent was signed by the parents/ guardian of the patient.

The patients were free from any systemic disease. All the patients had a history of trauma exceeding six months before their presentation to the office requiring restorations of their traumatized teeth. Teeth responded negatively to percussion, palpation and, thermal and electric pulp testing during clinical examination. Periodontal examination showed physiologic mobility and normal probing depth (2-3 mm) around the involved teeth. A sinus tract was observed on the labial aspect of one tooth (case#1). It was traced using a Guttapercha cone.

Cone-beam computed tomographic images were taken using Veraviewepocs 3D R 100 (J Morita Corp, Koyoto, Japan) operating at 90 kV and 8mA with an exposure time of 9.4 seconds, field of view of 40 x 40, and voxel size of 0.125mm. Lesion size was determined according to Estrela et al Cone-beam computed tomographic periapical index score (CBCT PAI) (9) and lesion volume was calculated with the medical image computing platform OsiriX MD software.

Cone-beam computed tomographic assessment was performed for all patients to compare between the lesion size (CBCT PAI) (9) and volume before and after 12 months of treatment.

The preoperative CBCT image for each case showed an immature tooth with open apex of at least stage nine according to Nolla's stages of tooth development (10) combined with a large radiolucent area scoring four and five according to CBCT PAI.

On the basis of clinical and radiographic findings, a diagnosis of pulp necrosis with asymptomatic apical periodontitis was made for case# 2 and 3 while case#1 was diagnosed with pulp necrosis and chronic apical abscess.

Treatment Procedures

Removal of plaque and calculus was performed. Mepivacaine hydrochloride 3% local anesthesia without vasoconstrictor was given as infiltration opposite to each treated tooth (Septodont, Cedex, France). The tooth was then isolated with a rubber dam (Sanctuary, Selangor, Malaysia). The access cavity was prepared with a sterile high-speed diamond round bur.

Slight dentinal wall filing was performed using a sterile stainless-steel hand file of 2% taper of minimum size #50 (Dentsply Maillefer, Ballaigues, Switzerland) to the estimated

working length at the radiographic terminus confirmed by a periapical radiograph.

The canals were irrigated in the following sequence with 2.5% sodium hypochlorite (NaOCl, 20 mL, 5 min), sterile saline (10 mL), and 17% Ethylenediaminetetraacetic acid (EDTA,20 mL/canal, 5 min) (Meta biomed Inc., Cheongju, Korea), and sterile saline (10 mL) using a 27- gauge side-vented irrigation needle (Kerr, Switzerland) positioned within two mm of the root apex.

A pre-curved K-file # 20 (Dentsply Maillefer, Ballaigues, Switzerland) was inserted 2mm beyond the apical foramen to produce bleeding filling the canal through laceration of the periapical tissue. The bleeding was stopped 3-4 mm apical to the to the level of the cemento–enamel junction to allow the placement of three millimeters of Biodentine (Septodont, Cedex, France) over the blood clot. A 3-4 mm layer of glass ionomer (Riva LC, SDI, Australia) was placed over the Biodentine. Then, a composite resin restoration (Ivoclar Vivadent, Schaan, Liechtenstein) was accomplished to seal the access cavity (5).

After 12 months follow up, if no pain on percussion or palpation, no attachment loss, no mobility and no sinus tract existed, the clinical success was reported with absence of signs and symptoms.

The healing of periapical lesions was assessed according to reduction in Estrela et al (2008) (9) CBCT periapical index score (CBCT PAI) and volume measured on CBCT.

Measuring of the root length and thickness was performed on the preoperative and follow up images. For the length, measuring the line from the cementoenamel junction (CEJ) to the radiographic apex of the tooth was performed. While for the thickness, measurements of the pulp space and root width were carried at the level of the apical third. The subtraction of the pulp space from the root width brought the value of dentin thickness (11).

RESULTS

(Figures 1-3, Tables 1,2)

Cases #2 and 3 were asymptomatic and responded negatively to percussion, palpation and to both electric pulp testing and cold testing with Ethyl chloride spray (Walter Ritter, Germany) during one year of follow up period. The sinus tract of case#1 had resolved during the intermediate follow up visits. However, after twelve months, it was re-apparent.

Comparing the pre-treatment CBCT images with those obtained after one year follow up period, there was evidence of root elongation, narrowing of apical diameter; increased root thickness and increased bone density. Persistent periapical lesions were examined in all cases. Healing of the destruction of the buccal plate of bone was examined in case# 2 and 3. Case#2 showed an increase in both CBCT PAI and volume.

Table (1): Assessment of the lesions size, volume and density before and after single visit regeneration.

Patient	Age	Gender	Diagnosis	CBCT PAI		E/D		Largest lesion diameter		Volume (mm^3)		Lesion density	
				Before	After	Before	After	Before	After	Before	After	Before	After
1	12	M	Pulp necrosis & chronic apical abscess	5	5		-	14.15	8.32	3.430	1.140	67.6	119.7
2	11	F	Pulp necrosis & asymptomatic apical periodontitis	4	4	D	-	7.7	7.84	1.486	3.497	-129.9	-92.1
3	13	M	Pulp necrosis & asymptomatic apical periodontitis	5	4	D	-	12.5	6.1 3	3.345	1.308	-85	124.8

Table (2): Assessment of the root thickness, length and apical diameter before and after single visit regeneration.

Patient	Age	Gender	Diagnosis	Thickness		Apical diameter		Length	
				Before	After	Before	After	Before	After
1	12	Male	Pulp necrosis & chronic apical abscess	1.57	1.88	0.47	0.31	14.79	14.92
2	11	Female	Pulp necrosis & asymptomatic apical periodontitis	2.82	3.14	1.41	1.41	14.2	14.8
3	13	Male	Pulp necrosis & asymptomatic apical periodontitis	1.88	2.35	0.78	0.63	14.92	15.71

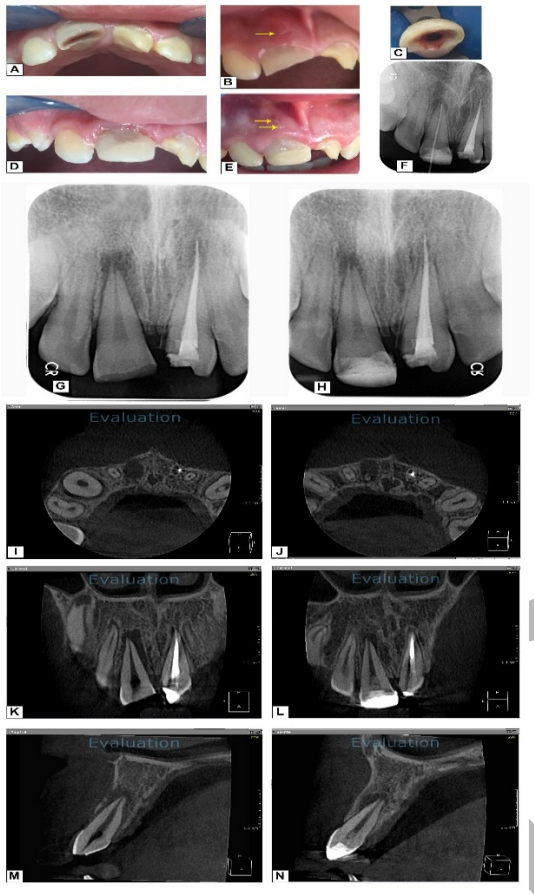

Figure (1): Case #1 tooth #8. A: Preoperative photograph, **B:** Preoperative photograph showing a sinus tract, **C:** Intraoperative photograph showing induction of bleeding, **D:** Postoperative photograph, **E:** After twelve months follow up photograph showing the healing of the previous sinus tract and the appearance of another one more apically, **F:** A periapical x-ray showing tracing of the second sinus tract, **G:** Preoperative periapical radiograph, **H:** After twelve months follow up periapical radiograph showing incomplete resolution of the lesion, **I,K,M:** Preoperative CBCT showing the lesion in axial, coronal and sagittal planes respectively, **J,L,N:** After twelve months follow up CBCT in axial, coronal and sagittal planes showing incomplete resolution of the lesion, **L,N:** Coronal and sagittal planes showing narrowing of the apical diameter.

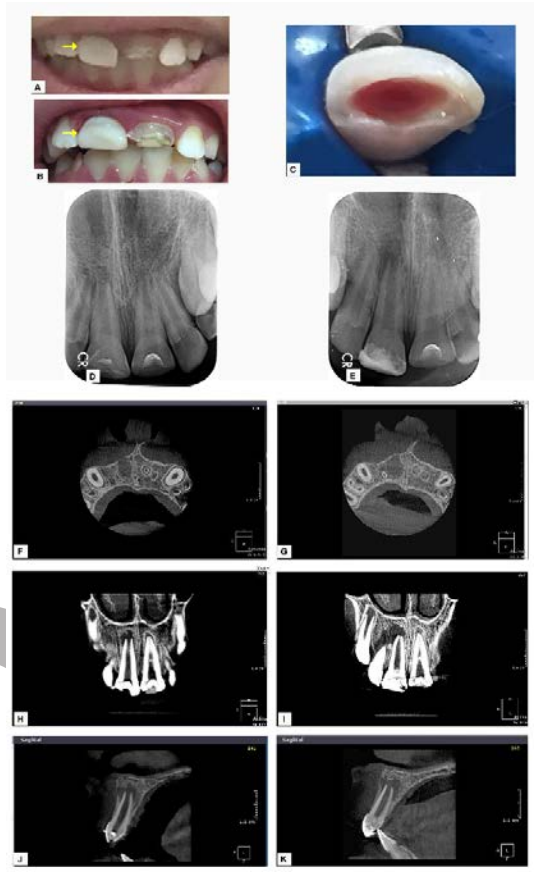

Figure (2): Case #2 tooth #8. A: Preoperative photograph showing an acrylic crown, **B:** Postoperative photograph, **C:** Intraoperative photograph showing induction of bleeding, **D:** Preoperative periapical radiograph, **E:** After twelve months follow up periapical radiograph showing an increase in the size of the lesion, **F,H,J:** Preoperative CBCT showing the lesion in axial, coronal and sagittal planes respectively, **G,I,K:** After twelve months follow up CBCT in axial, coronal and sagittal planes showing an increase in the size of the lesion, **I:** Coronal plane showing narrowing of the apical diameter.

Figure (3): Case #3 tooth #9. A: Preoperative photograph, B: Intraoperative photograph showing induction of bleeding**, C: Preoperative periapical radiograph, D:** After twelve months follow up periapical radiograph showing incomplete resolution of the lesion, **E,G,I:** Preoperative CBCT showing the lesion in axial, coronal and sagittal planes respectively, **F,H,J:** After twelve months follow up CBCT in axial, coronal and sagittal planes showing incomplete resolution of the lesion, **H,J:** Coronal and sagittal planes showing narrowing of the apical diameter.

DISCUSSION

This study shows questionable results of using single visit regenerative endodontic therapy for the treatment of three cases of traumatized necrotic immature permanent teeth with promising results in only one case despite the presence of periapical lesion larger than 8 mm in diameter.

All cases had an etiology of trauma with CBCT PAI which scored four or five indicating long standing challenging infections (12). For accurate assessment of lesion size changes, both volume and CBCT PAI index were calculated before and 12 months after treatment as previously shown (13). In addition to CBCT PAI volume was also measured since CBCTPAI is a linear measurement of the largest diameter of the lesion in one plane either bucco-lingual or corono-apical or mesio-distal. Volumetric assessment allows accurate assessment of endodontic lesion volume in three dimensions and better assessment of periapical healing during follow up (14,15).

The single visit endodontic regeneration procedure without any intracanal medicaments has been introduced by few case reports and two clinical trials (16-22). The irrigation solution is the unique disinfectant. This single visit procedure offers many advantages as the elimination of: subsequent appointments, the harmful sequels of bad patient compliance, risks of defective temporary restoration and microbial re-infection of the root canal (16).

Sodium hypochlorite irrigation was selected due to its powerful antimicrobial activity, and organic tissue dissolution ability (23). The high concentration of 2.5% used in the current study single visit regeneration was the least concentration used in previous studies to achieve acceptable antimicrobial efficacy with minimal harmful effects to stem cells (16) and is within the most recent recommended guidelines of the American Association of Endodontics (5).

The use of EDTA 17% before the induction of bleeding has many functions that support stem cell survival as: release of growth factors from the dentine matrix, inducing cellular attachment & differentiation at the dentine interface (24), as well as the other well-known functions of EDTA as: decalcification of the dentine surface, removal of the smear layer, and exposure of dentinal tubules and collagen fibrils (25).

Biodentine was the bioceramic used as the cervical plug because it is highly advantageous over Mineral trioxide aggregate (MTA) including: faster setting time, higher push-out bond strength at 24 hours, tooth colored, prevention of coronal discoloration due to presence of zirconium oxide as radiopacifier, greater ability to produce apatite crystals and release of dental elements, superior antimicrobial action (26-28). Moreover, Biodentine was found to activate the formation of reparative dentin and transforming growth factor beta 1 (TGF-b1). Furthermore, Biodentine stimulate the dental pulp stem cells (DPSCs) proliferation, migration and adhesion (29,30).

Although two cases showed increased bone density; healing after twelve months remained incomplete. This may be due to delayed healing response as a result of some residual infection

(31). In the current study, case #1 displayed exacerbation of chronic periapical abscess and reappearance of a sinus tract, after one year although clinical signs and symptoms had resolved during follow-up visits. During this year the patient had had orthopedic surgery in his lower limb. This surgery might have lowered his immunity causing a secondary infection appearing after 12 months.

Although root development has been shown to occur despite the residual microorganisms; bacteria can be the cause of weakened hard tissue deposition and delayed bone healing (32,33). The lack of calcium hydroxide or triple antibiotic paste may have eliminated the additional beneficial antimicrobial effects needed to stimulate complete bony healing (32,34). Additionally, calcium hydroxide has been shown to enhance hard tissue deposition and osteoblast differentiation (35,36).

The root thickening and lengthening might be explicated by the viability of Hertwig's epithelial sheath performing its function in root development and stimulating the odontoblast differentiation from stem cells from the apical papilla (SCAP) (37).

The current study results agreed with Estefan et al 2016 (38) who found that immature teeth of younger patients (9–13 years) showed significant root lengthening and thickening with decrease in their apical diameter than older participants (14–18 years old). Furthermore, hard tissue deposition and root maturation were examined in failed case reports without any regenerated tissue in the canal space or with persistent apical periodontitis attributing this to the viability of the Hertwig's epithelial sheath (39-41).

Longer follow up periods for the patients showing a persistent radiolucency periapically following the single visit protocol are recommended. The effects of single-visit regenerative endodontic management of immature necrotic teeth with periapical lesions need further investigations via clinical trials. Studies of other disinfection protocols as photodynamic, nanobubble water (NBW) or cold atmospheric plasma irrigation may be suggested for the single visit procedure. Additional research must also be carried out to detect the nature of the tissue causing the persistence of radiolucency periapically following the single visit protocol using a noninvasive technique as magnetic resonance imaging (MRI) rather than invasive ones as tissue biopsy.

CONCLUSION

Single-visit regenerative endodontic therapy may present questionable clinical and radiographic outcomes for treatment of traumatized necrotic immature permanent teeth with periapical lesions. More clinical trials are needed to validate the efficiency of this treatment.

REFERENCES

1. Diogenes A, Ruparel NB, Shiloah Y, Hargreaves KM. Regenerative endodontics: a way forward. J Am Dent Assoc. 2016;147:372–80.
2. Yang J, Yuan G, Chen Z. Pulp regeneration: Current approaches and future challenges. Front Physiol. 2016;7:58.
3. Almutairi W, Yassen GH, Aminoshariae A, Williams KA, Mickel A. Regenerative endodontics: A systematic analysis of the failed cases. J Endod. 2019;45:567-77.
4. Lee BN, Moon JW, Chang HS, Hwang IN, Oh WM, Hwang YC. A review of the regenerative endodontic treatment procedure. Restor Dent Endod. 2015;40:179–87.
5. AAE. AAE Clinical Considerations for a Regenerative Procedure Revised 5/18/2021. Available online: https://f3f142zs0k2w1kg84k5p9i1o-wpengine.netdna-ssl.com/specialty/wp-content/uploads/sites/2/2021/08/ClinicalConsiderationsApprovedByREC062921.pdf.
6. Galler KM, Krastl G, Simon S, Van Gorp G, Meschi N, Vahedi B, et al. European society of endodontology position statement: Revitalisation procedures. Int Endod J. 2016;49:717-23.
7. Khoshkhounejad M, Shokouhinejad N, Pirmoazen S. Regenerative endodontic treatment: Report of two cases with different clinical management and outcomes. J Dent (Tehran). 2015;12:460–8.
8. Amonkar AD, Dhaded NS, Doddwad PK, Patil AC, Hugar SM, Bhandi S, et al. Evaluation of the effect of long-term use of three intracanal medicaments on the radicular dentin microhardness and fracture resistance: An in vitro study. Acta Stomatol Croat. 2021;55:291-301.
9. Estrela C, Bueno MR, Azevedo BC, Azevedo JR, Pécora JD. A new periapical index based on cone beam computed tomography. J Endod. 2008;34:1325-31.
10. Nolla CM. The development of the permanent teeth journal of dentistry for children. J Dent Child. 1960;27:254-66.

11. Bose R, Nummikoski P, Hargreaves K. A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. J Endod. 2009;35:1343-9.
12. Ng YL, Mann V, Gulabivala K. A prospective study of the factors affecting outcomes of nonsurgical root canal treatment: part 1: periapical health. Int Endod J. 2011;44:583-609.
13. Campello AF, Gonçalves LS, Guedes FR, Marques FV. Cone-beam computed tomography versus digital periapical radiography in the detection of artificially created periapical lesions: A pilot study of the diagnostic accuracy of endodontists using both techniques. Imaging Sci Dent. 2017;47:25-31.
14. Ahlowalia MS, Patel S, Anwar HMS. Accuracy of CBCT for volumetric measurement of simulated periapical lesions. Int Endod J. 2013;46:536-46.
15. Schloss T, Sonntag D, Kohli MR, Setzer FC. A Comparison of 2- and 3-dimensional Healing Assessment after Endodontic Surgery Using Cone-beam Computed Tomographic Volumes or Periapical Radiographs. J Endod. 2017;43:1072-9.
16. Topçuoğlu G, Topçuoğlu HS. Regenerative endodontic therapy in a single visit using platelet-rich plasma and biodentine in necrotic and asymptomatic immature molar teeth: A report of 3 cases. J Endod. 2016;42:1344-6.
17. Chaniotis A. The use of a single-step regenerative approach for the treatment of a replanted mandibular central incisor with severe resorption. Int Endod J. 2016;49:802-12.
18. Shin SY, Albert JS, Mortman RE. One step pulp revascularization treatment of an immature permanent tooth with chronic apical abscess: a case report. Int Endod J. 2009;42:1118-26.
19. McCabe P. Revascularization of an immature tooth with apical periodontitis using a single visit protocol: a case report. Int Endod J. 2015;48:484-97.
20. Aldakak MM, Capar ID, Rekab MS, Abboud S. Single-visit pulp revascularization of a nonvital immature permanent tooth using biodentine. Iran Endod J. 2016;11:246-9.
21. Botero TM, Tang X, Gardner R, Hu JCC, Boynton JR, Holland GR. Clinical Evidence for Regenerative Endodontic Procedures: Immediate versus Delayed Induction? J Endod. 2017;43:S75-S81.
22. Cerqueira-Neto ACCL, Prado MC, Pereira AC, Oliveira ML, Vargas-Neto J, Gomes BPFA, et al. Clinical and radiographic outcomes of regenerative endodontic procedures in traumatized immature permanent teeth: Interappointment dressing or single-visit? J Endod. 2021;47:1598-608.
23. Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M. Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. J Endod. 2010;36:1558-62.
24. Pang NS, Lee SJ, Kim E, Shin DM, Cho SW, Park W, et al. Effect of EDTA on attachment and differentiation of dental pulp stem cells. J Endod. 2014;40:811-7.
25. Sen BH, Wesselink PR, Türkün M. The smear layer: a phenomenon in root canal therapy. Int Endod J. 1995;28:141-8.
26. Guneser MB, Akbulut MB, Eldeniz AU. Effect of various endodntic irrigants on the push-out bond strength of biodentine and conventional root perforation repair materials. J Endod. 2013;39:380-4.
27. Bhavna V, Chaitanya KP, Gandi P, Patel J, Dola B, Reddy RB. Evaluation of antibacterial and antifungal activity of new calcium-based cement (Biodentine) compared to MTA and glass ionomer cement. J Conser Dent. 2015;18:44-6.
28. Kaur M, Singh H, Dhillon JS, Batra M, Saini M. MTA versus Biodentine: Review of Literature with a Comparative Analysis. J Clin Diagn Res. 2017;11:ZG01-ZG05.
29. Laurent P, Camps J, About I. Biodentine (TM) induces TGF-b1 release from human pulp cells and early dental pulp mineralization. Int Endod J. 2012;45:439-48.
30. Luo Z, Li D, Kohli MR, Yu Q, Kim S, He WX. Effect of Biodentine on the proliferation, migration and adhesion of human dental pulp stem cells. J Dent. 2014;42:490-7.

31. Nair PNR, Sjögren U, Kahnberg KE, Krey G, Sundqvist G. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. J Endod. 1990a;16:580-8.
32. de-Jesus-Soares A, Prado MC, Nardello LCL, Pereira AC, Cerqueira-Neto ACCL, Nagata JY, et al. Clinical and molecular microbiological evaluation of regenerative endodontic procedures in immature permanent teeth. J Endod. 2020;46:1448-54.
33. Arruda MEF, Neves MAS, Diogenes A, Mdala I, Guilherme BPS, et al. Infection control in teeth with apical periodontitis using a triple antibiotic solution or calcium hydroxide with chlorhexidine: A randomized clinical trial. J Endod. 2018;44:1474-9.
34. Nagata JY, Soares AJ, Souza-Filho FJ, Zaia AA, Ferraz CC, Almeida JF, et al. Microbial evaluation of traumatized teeth treated with triple antibiotic paste or calcium hydroxide with 2% chlorhexidine gel in pulp revascularization. J Endod. 2014;40:778-83.
35. Paula-Silva FW, Ghosh A, Arzate H, Kapila S, da Silva LA, Kapila YL. Calcium hydroxide promotes cementogenesis and induces cementoblastic differentiation of mesenchymal periodontal ligament cells in a CEMP1- and ERK-dependent manner. Calcif Tissue Int. 2010;87:144-57.
36. Narita H, Itoh S, Imazato S, Yoshitake F, Ebisu S. An explanation of the mineralization mechanism in osteoblasts induced by calcium hydroxide. Acta Biomater. 2010;6:586-90.
37. Palma PJ, Martins J, Diogo P, Sequeira D, Ramos JC, Diogenes A, et al. Does apical papilla survive and develop in apical periodontitis presence after regenerative endodontic procedures? Appl Sci. 2019;*9*:3942.
38. Estefan BS, El Batouty KM, Nagy MM, Diogenes A. Influence of age and apical diameter on the success of endodontic regeneration procedures. J Endod. 2016;42:1620-5.
39. Lin LM, Kim SG, Martin G, Kahler B. Continued root maturation despite persistent apical periodontitis of immature permanent teeth after failed regenerative endodontic therapy. Aust Endod J. 2018;44:292-9.
40. Žižka R, Buchta T, Voborná I, Harvan L, Šedý J. Root Maturation in Teeth Treated by Unsuccessful Revitalization: 2 Case Reports. J Endod. 2016;42:724-9.