

CALCIUM ENRICHED MIXTURE USED FOR PERMANENT MOLAR PULPOTOMY IN TEETH DIAGNOSED AS IRREVERSIBLE PULPITIS: A CASE SERIES

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ABSTRACT

Aim: To evaluate the clinical and radiographic pulpotomy outcomes in permanent molars diagnosed as irreversible pulpitis using Calcium Enriched Mixture (CEM) as the pulpotomy material.

Materials and Methods: Eight patients with 8 permanent molars demonstrating signs and symptoms of symptomatic / asymptomatic irreversible pulpitis were included. For all patients, after rubber dam application, standardized pulpotomy was performed. Sodium hypochlorite 5 % was used to obtain haemostasis. A mixture of CEM was placed in the pulp chamber. Molars were restored with a glass ionomer restoration. After seven days, the glass ionomer restoration was covered by stainless steel crowns. Recall assessment was appointed at 3, 6, 12 months.

Results: Patients' age at the time of treatment was at range from 9.10 to 15 years. Most of the molars (75%) were having symptomatic irreversible pulpitis, with 88% demonstrating symptomatic apical periodontitis. To stop pulpal hemorrhage time range was between 1-12 minutes (mean 4.8 ± 4.5 minutes). The follow-up examination period ranged from 15- 17 months with a mean of 16.6 ± 0.5 months. At the beginning of the study 3 molars that had open apices (38%) all of them showed continued root maturation. Four molars that showed radiographic apical radiolucencies (50%), all lesions healed completely by the end of the follow-up period. One case (13%) showed partial with no complete root canal obliteration. There were neither periradicular bone nor root resorption. Additionally, no evidence of internal root resorption was noticed.

Conclusion: Successful outcomes of this case series recommend the use of CEM as a simple, useful and applicable pulpotomy agent in the treatment of permanent molars having irreversible pulpitis. However, more studies are needed to assess this procedure including larger number of patients over longer period of recalls.

KEY WORDS: CEM , Irreversible pulpitis , Molar, Pulpotomy.

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INTRODUCTION

Whenever there is a carious vital exposed pulp, the operator may decide to seal the pulp by direct pulp capping, pulpotomy or to begin a conventional root canal treatment (RCT) ⁽¹⁾. Treatment modality depends upon several factors such as age, degree of pulp inflammation, remaining tooth structure and patient's medical history. The successful prognosis of RCT is well accepted ^(2,3). On the other hand, it is complicated and time consuming. RCT has been accused by increasing the probability to fracture because of the loss of tooth structure coronally and apically during cleaning and shaping ⁽⁴⁾. Moreover, it is recommended that after RCT, most of the teeth should be protected by an extra coronal restoration. In some areas of the world, extraction might be the treatment of choice because of the cost. ⁽⁴⁾

Pulpotomy is defined as the "surgical removal of the coronal portion of the pulp and the placement of a therapeutic agent to preserve the health of the remaining vital tissues" ^(1,2). Pulpotomy is a worldwide documented procedure in immature permanent teeth having exposed pulps ^(1,2). Additionally, pulpotomy is one of the most commonly adopted emergency treatment protocols for pain relief in case of irreversible pulpitis ⁽²⁾. If pulpotomy fails, this will not affect the chances of starting RCT ⁽⁵⁾.

The use of pulpotomy technique by applying a well-accepted biomaterial gained a great popularity as an alternative treatment to RCT. In 1998, Nosrat and Nosrat, recommended partial pulpotomy as a treatment option in case of pulpal exposure in deep carious lesions. They used calcium hydroxide (CH) to seal pulp then zinc oxide eugenol was added, and finally a semi-permanent filling. They found hard tissue bridge formation by the end of three months and all treated teeth were asymptomatic ⁽⁶⁾.

Recently, MTA has been introduced for pulpotomy in permanent molars ⁽⁷⁻¹¹⁾. MTA has many merits such as its great biocompatibility, excellent sealing ability, and the efficiency to initiate healing of the pulpal tissue ^(7,8). In spite of its great characteristics, it has been reported that MTA displays demerits as an unreliable antibacterial ability, difficult management, expanded setting time, and the most important is the high cost ⁽⁹⁻¹¹⁾.

CEM is a new favorable efficient hydrophilic tooth colored cement ⁽¹⁰⁻¹²⁾. It is composed of different calcium compounds such as calcium oxide, sulfur tricalcium, calcium phosphate, calcium carbonate, calcium silicate, calcium hydroxide and calcium chloride ⁽¹¹⁾. It has several excellent biological and physical properties ⁽¹⁰⁻¹²⁾. Compared to MTA, it is characterized by its shorter setting time, good handling, better flowability and less film thickness ⁽¹⁰⁻¹²⁾. It is also characterized by its sealing ability, biocompatibility, ability to form hydroxyapatite and ability to induce cementum ^(11,12). It is widely used as a pulp capping agent, retrograde filling and in repairing root perforation ⁽¹²⁾. In addition, its cost-wise property is advantageous in comparison to many other biomaterials and it demonstrated stimulation of dentinal bridge formation, besides, its setting affinity in aqueous media. Likewise, the antibacterial effect of CEM is exceeding MTA but equivalent to CH ⁽¹³⁾.

Several studies displayed high successful outcome clinically and radiographically when MTA was used as a pulpotomy agent in cariously exposed permanent teeth diagnosed as irreversible pulpitis ^(3,14-22). Few studies investigated the clinical and radiographic success when CEM was used as a pulpotomy agent in cariously exposed permanent molars diagnosed by irreversible pulpitis ⁽²²⁻²⁵⁾. So, this case series expressed clinical and radiographic

pulpotomy outcomes in carious vital pulp exposure of eight permanent molars diagnosed as irreversible pulpitis using CEM .

MATERIALS AND METHODS

The current case series was approved by the ethical committee, College of Dentistry, Suez Canal University. Teeth were examined and after discussing the benefits and risks of the procedure, a written consent was signed by the guardians. The study was designed to include children and adolescents attending Suez Canal University, dental clinics (Endodontic and Pedodontic departments) having the following inclusion criteria: (1) Signs and symptoms clinically ascertained irreversible pulpitis diagnosis of deeply carious permanent mature/immature molars. The tentative diagnosis was obtained if they were presented with one of the following chief complaints: a. spontaneous or intermittent, dull or sharp, localized, diffuse, or referred pain; b. lingering pain after exposure to thermal changes even after stopping the insult, or c. no signs or symptoms but pulpal hemorrhage as a result of caries removal; (2) Restorable molars or (3) Uncooperative children to conventional RCT. Exclusion criteria were : (1) Irrestorable; (2) Mobile molars; (3) Existence of sinus tract; (4) No pulpal bleeding after exposure; (5) Swelling of soft tissues; (6) Radiographic appearance of internal or external resorption or furcation radiolucency.

One Endodontist finished medical and dental history, clinical examination and the pulpotomy procedure for all patients. Thermal test with refrigerant spray (Endo ICE- Coltene/Whaledent Inc., OH, USA) and electrical pulp test (Pulp tester- Analytic Technology, WA, USA) were used to prove the irreversible pulpitis diagnosis. Rinn® XCP holder (Dentsply, IL, USA) was used to standardize the

preoperative and postoperative periapical radiographs.

The teeth were anesthetized with 4% Articaine HCL and 1:200,000 epinephrine (Septocaine, Septodont, Novocol pharmaceutical of Canada, Canada). Under rubber dam isolation, the tooth and the clamp were wiped by 2% chlorhexidine (Consepsis V, Ultradent Products, Inc., South Jordan, UT, USA) . Caries was removed by a large round bur in a low speed handpiece. Under water coolant, pulpotomy was completed using a sterile round and/or flame shape diamond burs mounted in a high speed hand piece. Sodium hypochlorite 5% was used to achieve hemostasis. An assistant checked the time for bleeding to control for all teeth. CEM (Bionique Dent, Tehran, Iran) was manipulated according to manufacturer's instructions. Then it was applied in the pulp chamber (2 mm) using a plastic filling instrument and excess CEM was removed. All teeth were temporized with Equia Forte fil glass ionomer filling (GC America inc., USA).

Later, patients were recalled after 24 hr for evaluation of the postoperative pain. Subsequently, the patients came back after seven days for evaluation of postoperative pain and to place the stainless steel crown (3M/ ESPE, St. Paul, MN, USA) by the same pedodontist. Spofa Dental Kavitan Pro glass ionomer cement (SpofaDental, Zhengzhou Shengxin Medical Instrument Co., Ltd., Henan, China) was used to lute all crowns. Guardians were instructed to replace the stainless steel crown by a permanent one once adult teeth had fully erupted and the dento-alveolar position was constant. On the next recall sessions, in addition to radiographic examination, testing for thermal, percussion, and palpation were carried out, to reveal any signs of inflammation or infection. After 3, 6 and 12 months, patients were recalled for clinical examination. Radiographs were

assessed at 6 and 12 months. The radiographs were assessed independently by two investigators for root development if they were immature, hard tissue bridge formation, canal obliteration, and any periradicular changes.

By the end of the follow-up period, failure was recorded if there was one or more of the following: continuous and persistent pain, mobility, severe response to percussion and/or palpation, swelling, or sinus tract. Signs of radiographic failure were: increase of a preoperative periradicular radiolucency, existence of either a new periradicular or furcal pathology, root resorption or incomplete development of root in immature teeth. Statistical analysis was performed using Statistical Package for the Social Sciences software version 22 (Armonk, NY: IBM Corp, USA).

RESULTS

Ten patients (4 males and 6 females) having 10 permanent molars were intended to be in the study. Two cases were dropped out of the study because they failed to show up in the recall periods. Telephone communication was made; when they were asked, they answered that teeth were painless but they won't be able to come. So, eight participants (3 males and 5 females) were included in the study. The age of patients at the time of treatment ranged between 9.10 to 15 years (mean=11.6 ± 2.5 yrs.). Table 1 showed the features of patients and the condition of the teeth that were included in the study. Two maxillary first molars and six mandibular first

molars were treated. Three restored (carious under the filling) and five carious were included in the case series. Most of the molars (75%) were having symptomatic irreversible pulpitis, with 78.5% of the cases demonstrating symptomatic apical periodontitis. To stop pulpal hemorrhage time range was between 1-12 minutes (mean 4.8 ± 4.5 minutes). The patients were asked to come after 3, 6, 12 months. In spite of the continuous contact to confirm the appointments, the follow-up examination period ranged from 15-17 months with a mean of 16.6 ± 0.5 months.

Clinically, all pulpotomy procedures represented successful outcome by the end of the study. There were no signs or symptoms of pain either continuous or persistent pain. Periapical examination revealed no tenderness to percussion. Palpation of alveolar areas demonstrated no signs of inflammation. Using refrigerant spray showed positive response. No signs of swelling or sinus tract were noticed in any of the treated teeth. Radiographically, a hard tissue barrier was noticed in 3 (37.5%) teeth (**Figure. 1,2**). At the beginning of the study, 3 molars that had open apices (37.5%), all of them showed continued root maturation (**Figure 2**). Four molars that showed radiographic apical radiolucencies (50%) (**Figure. 1,2**), all lesions healed completely by the end of the follow-up period. One case (12.5%) showed partial with no complete obliteration of the root canal (**Figure. 2**). There were neither periradicular bone nor root resorption. Additionally, no evidence of internal root resorption was noticed.

TABLE (1) The features of patients and the condition of the included molars treated by CEM pulpotomy.

case	Gender	Age Yr.	Tooth #	Condition of the tooth	Pulpal diagnosis	Apical Diagnosis	Periapical radiographic changes	Control of bleeding	Root maturation	Canal obliteration	Dentin bridge formation	Follow up (mth)
1	F	8.11	36	carious	SIP	SAP	Yes	1 min	mature	No	yes	17
2	F	10.1	46	carious	SIP	None	Yes	1 min	immature	Partly	Yes	17
3	F	14.5	46	restored	SIP	SAP	Yes	2 min	mature	No	No	17
4	M	11.5	16	carious	SIP	SAP	No	3 min	mature	No	No	16
5	M	15	36	carious	AIP	SAP	No	1 min	mature	No	No	16
6	M	12.9	36	restored	AIP	SAP	yes	8 min	immature	No	No	17
7	F	11.9	26	restored	SIP	SAP	No	10 min	mature	No	Yes	16
8	F	13.2	36	carious	SIP	SAP	No	12 min	immature	No	No	17

SIP: symptomatic irreversible pulpitis, AIP: Asymptomatic irreversible pulpitis.

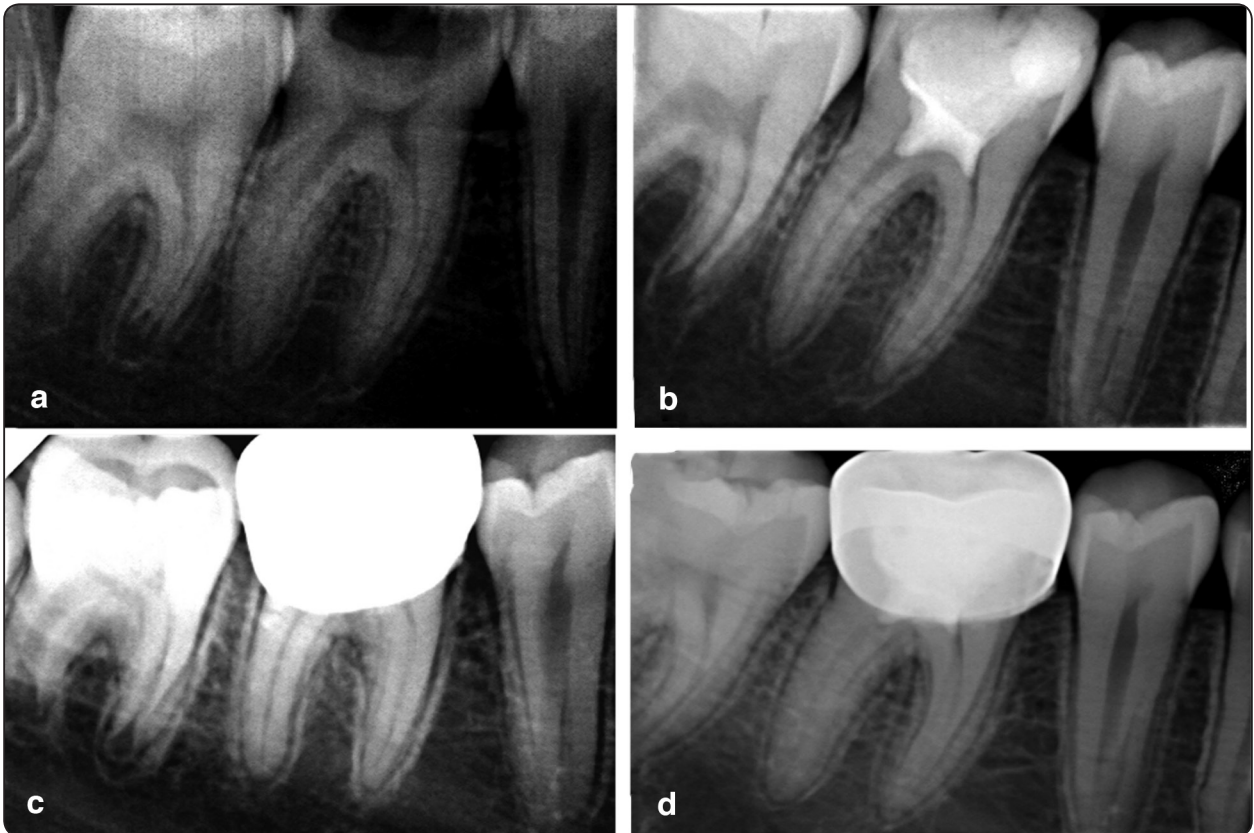


Fig. (1): Case #1 radiographic evaluation (A) Preoperative radiograph of an immature root of #36 showing a periapical radiolucency(B) Immediately after CEM pulpotomy (C) Postoperative radiograph after 6 months (D) Postoperative radiograph after 17 months showing dentinal bridge formation and healing of the periapical radiolucency.

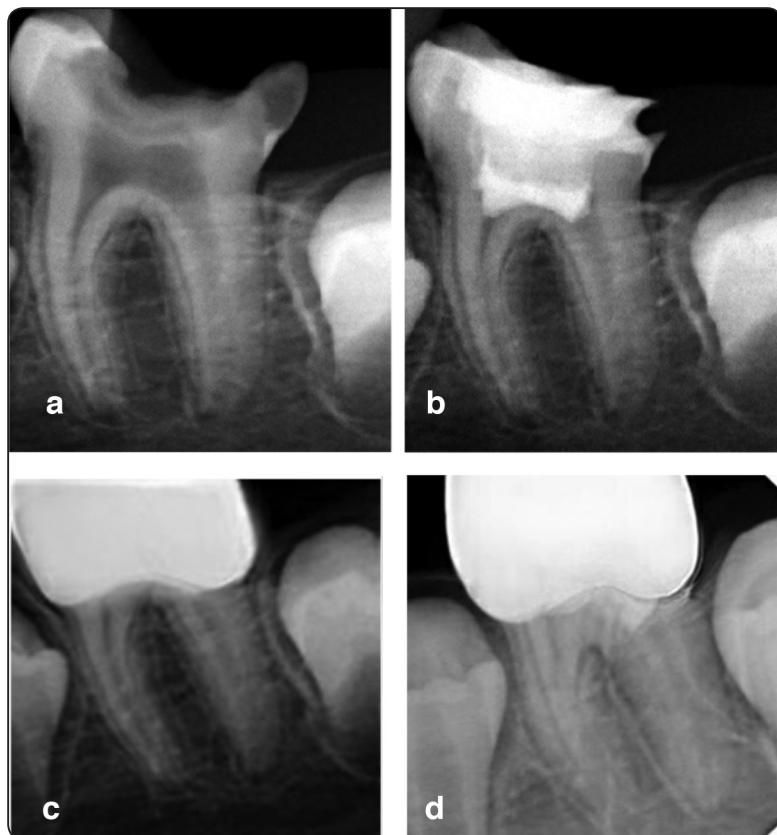


Fig. (2): Case #2 radiographic evaluation (A) Preoperative radiograph of an immature root of #36 showing a periapical radiolucency (B) Immediately after CEM pulpotomy (C) Postoperative radiograph after 6 months (D) Postoperative radiograph after 17 months showing dentinal bridge formation, partial root canal obliteration, mature roots and healing of the periapical radiolucency.

DISCUSSION

Successful vital pulpotomy depends on many factors, such as proper diagnosis, case selection, the operator's ability, materials feasibility and patient's cooperation^(1-3,19). The recent development of biomaterials in addition to the understanding of the pulp biology and reparative processes foster the clinical application of adult pulpotomy over RCT⁽³⁾. In a systematic review, it was demonstrated that permanent teeth with cariously exposed pulp can be treated successfully with vital pulp therapy⁽²⁶⁾. Sometimes it is hard to diagnose precisely the pulpal status of symptomatic teeth. That is why researchers found no clear association between clinical signs or symptoms of pulpitis and pulp histologic conditions⁽²⁷⁾. All teeth enrolled in the present study, were diagnosed by irreversible pulpitis, however, it is hard to determine the accurate histologic diagnosis. It was reported that, in some conditions the coronal

tissues of pulp were irreversibly inflamed whereas the radicular pulp were reversibly inflamed⁽²⁷⁾. That is the fact that favors the concept of vital pulp generally and pulpotomy in specific. This might explain the high successful outcome clinically and radiographically in our study.

This study is a case series designed to investigate the outcome (clinically and radiographically) of complete pulpotomy in cariously exposed mature and immature permanent teeth with inflamed symptomatic/asymptomatic pulps diagnosed as irreversible pulpitis using CEM. The overall success outcome after 18 months was 100 %, which is partly in agreement with others who demonstrated 78%-98% success rate⁽²⁰⁻²⁵⁾. Differences between results might be due to variation of follow-up periods.

It is well known that the primary function of the dental pulp is the formation of dentin. The capability of young pulps in immature teeth is faster than that

in mature ones⁽²⁷⁾. Moreover, caries and restorative procedures may hinder the ability of odontoblasts to lay down dentine⁽²⁸⁾. During and after CEM setting, it releases CH. As a result, CEM initiates hydroxyapatite crystals formation. Because the distribution configuration of calcium, phosphorus, and oxygen ions is the same as in human dentin, the surface features of CEM are similar to dentin^(10,12). The previously mentioned CEM characteristics might clarify the existence of dentinal bridge by the adjacent pulp cells. Recently, it was shown by Ricucci et al. that fibroblast-like cells (pulpal fibroblasts) produced a calcific material that looks like pulp stones rather than tubular dentine under CH as a direct pulp capping procedure which is presented as a repair other than regeneration⁽²⁹⁾. We found dentin bridge in 25% of the treated molars. Partial canal obliteration was displayed in 13% of the treated molars. This might be due to the action of the odontoblast which leads to root canal narrowing⁽³⁾. But still, this result is not a failure because the radicular pulp might be still vital and pulpotomy might be considered a success whenever there is no clinical symptoms. Fortunately, pulp necrosis frequency following canal obliteration is generally considered low⁽³⁰⁾.

Interestingly, it seems that preoperative periapical diagnosis, bleeding stopping duration and root maturation did not affect the formation of dentin bridge. The reason why the hard tissue barrier is not formed in all cases is not well understood⁽³⁰⁾. It was suggested that the success outcome of the pulpotomy is related to the duration to stop pulpal bleeding after removal of pulp in the pulp chamber. According to recommendation the appropriate time to stop pulp bleeding ranged from 1 to 12 minutes⁽⁵⁾. This ascertained that inflammation in the cases we treated was confined to the pulp chamber with no progression into the radicular pulp. Many researchers documented the repair of periapical radiolucencies after indirect pulp capping and pulpotomy^(5,31-34). Some studies noticed radiolucency resolution in addition to complete hard tissue bridge after starting the vital

pulp therapy by one year.^(31, 34) By the end of this case series, all teeth that had preoperative periapical radiolucencies (3/8) demonstrated healing of the radiolucencies. One of the 3 teeth presented by preoperative periapical radiolucency displayed a hard tissue calcific bridge formation after 1 year.

The coronal seal of the permanent restoration is critical to the long term success of vital and functional pulpotomized tooth⁽³⁵⁾. Therefore, it is crucial to have a well sealed coronal restoration to avoid any bacterial penetration. Some researchers affirmed that the coronal seal is more significant than the agent placed in pulpotomy⁽³⁵⁾. We could postulate that, because of the good seal offered by the biomaterial CEM and the final stainless steel crown restoration, a high success outcome was the result .

CONCLUSION

According to the findings from this case series we concluded that:

1. After a year follow-up, CEM pulpotomy offers highly successful outcome clinically and radiographically in symptomatic / asymptomatic carious permanent molars diagnosed as irreversible pulpitis.
2. CEM pulpotomy could be a successful alternative treatment modality for RCT.
3. In order to ensure the effectiveness of CEM pulpotomy, increasing the number of patients over a longer-term observation should be considered.

REFERENCES

1. Hasselgren G, Reit C. Emergency pulpotomy: pain relieving effect with and without the use of sedative dressings. *J Endod.* 1989;15:254–256.
2. Bender IB. Reversible and irreversible painful pulpitis: diagnosis and treatment. *Aust Endod J.* 2000;26:10–14.
3. Alqaderi HE, Al-Mutawa SA, Qudeimat MA . MTA pulpotomy as an alternative to root canal treatment in children's permanent teeth in a dental public health setting. *J Dent.* 2014; 42: 1390–1395.

4. Kishen A, Kumar G.V, Chen NN. Stress-strain response in human dentine: Rethinking fracture predilection in post-core restored teeth. *Dent Traumatol.* 2004;20:90-100.
5. Waterhouse PJ and Whitworth JM, Camp JH. Pediatric endodontics: endodontic treatment for the primary and young permanent dentition. In: Hargreaves KM, Cohen S, eds. *Cohen's Pathways of the pulp*, 11th ed. St Louis: Mosby Elsevier, 2016 pp. 941-946.
6. Nosrat IV, Nosrat CA. Reparative hard tissue formation following calcium hydroxide application after partial pulpotomy in cariously exposed pulps of permanent teeth. *Int Endod J.* 1998;31:221-226.
7. Asgary S, Eghbal MJ, Parirokh M, Ghanavati F, Rahimi H. A comparative study of histologic response to different pulp capping materials and a novel endodontic cement. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2008;106:609-614.
8. Aqrawabi J. Sealing ability of amalgam, super EBA cement, and MTA when used as retrograde filling materials. *Br Dent J.* 2000;188:266-268.
9. Asgary S, Kamrani FA. Antibacterial effects of five different root canal sealing materials. *J Oral Sci.* 2008;50:469-474.
10. Asgary S, Shahabi S, Jafarzadeh T, Amini S, Kheirieh S. The Properties of a new endodontic material. *J Endod.* 2008;34:990-993.
11. Chng HK, Islam I, Yap AU, Tong YW, Koh ET. Properties of a new root-end filling material. *J Endod.* 2005;31:665-666.
12. Asgary S, Eghbal MJ, Parirokh M. Sealing ability of a novel endodontic cement as a root-end filling material. *J Biomed Mater Res.* 2008;87:706-709.
13. Asgary S, Kamrani FA. Antibacterial effects of five different root canal sealing materials. *J Oral Sci.* 2008; 50: 469-474.
14. Witherspoon DE, Small JC, Harris GZ. Mineral trioxide aggregate pulpotomies: a case series outcomes assessment. *J Am Dent Assoc.* 2006;137:610-618.
15. Eghbal MJ, Asgary S, Baglue RA, Parirokh M, Ghoddusi J. MTA pulpotomy of human permanent molars with irreversible pulpitis. *Aust Endod J.* 2009;35:4-8.
16. Barrieshi-Nusair KM, Qudeimat MA .A prospective clinical study of mineral trioxide aggregate for partial pulpotomy in cariously exposed permanent teeth. *J Endod.* 2006; 32: 731-735.
17. Chueh LH, Chiang CP. Histology of irreversible pulpitis premolars treated with mineral trioxide aggregate pulpotomy. *Oper Dent.* 2010; 35, 370-374.
18. Qudeimat MA, Barrieshi-Nusair KM, Owais AI . Calcium hydroxide vs mineral trioxide aggregates for partial pulpotomy of permanent molars with deep caries. *Europ Arch Paed Dent.* 2007; 8: 99-104.
19. Taha NA, Ahmad MB, Ghanim A. Assessment of Mineral Trioxide Aggregate pulpotomy in mature permanent teeth with carious exposures. *Int End J.* 2017; 50: 117-125.
20. Nosrat A, Seifi A, Asgary S. Pulpotomy in caries-exposed immature permanent molars using calcium-enriched mixture cement or mineral trioxide aggregate: a randomized clinical trial. *Int J Paediatr Dent.* 2013; 23: 56-63.
21. Asgary S and Eghbal M . Treatment outcomes of pulpotomy in permanent molars with irreversible pulpitis using biomaterials: A multi-center randomized controlled trial .*Acta Odont Scand.* 2013; 71: 130-136.
22. Asgary S, Eghbal MJ, Ghoddusi J, Yazdani S. One-year results of vital pulp therapy in permanent molars with irreversible pulpitis: an ongoing multicenter, randomized, non-inferiority clinical trial. *Clin Oral Investig.* 2013; 17: 431-439.
23. Nosrat A, Asgary S. Apexogenesis of a symptomatic molar with calcium enriched mixture. *Int Endod J.* 2010; 43: 940-944.
24. Asgary S, Eghbal MJ. The effect of pulpotomy using a calcium-enriched mixture cement versus one-visit root canal therapy on postoperative pain relief in irreversible pulpitis: a randomized clinical trial. *Odontology.* 2010; 98: 126-133.
25. Asgary S, Eghbal MJ, Fazlyab M, Baghban AA, Ghoddusi J . Five-year results of vital pulp therapy in permanent molars with irreversible pulpitis: a non-inferiority multicenter randomized clinical trial. *Clin Oral Invest.* 2015; 19: 335-341.
26. Aguilar P, Linsuwanont P .Vital pulp therapy in vital permanent teeth with cariously exposed pulp: a systematic review. *J Endod.* 2011 ;37: 581-587.
27. Ricucci D, Loghin S, Lin LM, Spangberg LS, Tay FR . Is hard tissue formation in the dental pulp after the death of primary odontoblasts a regenerative process or a reparative process? *J Dent.* 2014; 42: 1156-1170.

28. Matsuzaka K, Muramatsu T, Katakura A et al. Changes in the homeostatic mechanism of dental pulp with age: expression of the core-binding factor alpha-1, dentin sialoprotein, vascular endothelial growth factor, and heat shock protein 27 messenger RNAs. *J Endod.* 2008 ; 34 :818–821.
29. Schmalz G, Smith AJ . Pulp development, repair, and regeneration: challenges of the transition from traditional dentistry to biologically based therapies. *J Endod.* 2014; 40: S2–5.
30. McCabe PS, Dummer PM .Pulp canal obliteration: an endodontic diagnosis and treatment challenge. *Int End J.* 2012; 45: 177-197.
31. Foreman PC. Resolution of a periapical radiolucency following renewal of the pulpotomy dressing. *Int End J.* 1980; 13, 41-43.
32. Russo MC, Holland R, de Souza V .Radiographic and histological evaluation of the treatment of inflamed dental pulps. *Int End J.* 1982; 15:137-142.
33. Moule AJ, Oswald RJ . Resolution of periapical radiolucency following pulpotomy. *J Endod.*1983; 9: 388-389.
34. Trope M . Regenerative potential of dental pulp. *J Endod.* 2008; 34: (Suppl), S13-17.
35. Saunders WP, Saunders EM . Assessment of leakage in the restored pulp chamber of endodontically treated multirooted teeth. *Int End J.*1990 ;23: 28-33.