

In-vitro Evaluation of Microleakage in Root Canal Obturation with Mineral Trioxide Aggregate and Calcium-enriched Mixture Cement Using Fluid Filtration

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Abstract

Introduction: Endodontic treatment failure is caused by the leakage of microorganisms and endotoxins, which leads to pathological lesions. Adequate seal of the root canal is essential to preventing recontamination and ensuring the long-term clinical success rate. Mineral trioxide aggregate (MTA) and calcium-enriched mixture cement (CEM) are common types of cement with adequate sealing capability in endodontics. The present study aimed to compare the microleakage in the root canals filled with ProRoot MTA and CEM cement using fluid filtration. **Methods:** This experimental, *in-vitro* study was conducted on 46 root canals of extracted mandibular premolars. After preparation and disinfection with 3% sodium hypochlorite, the crowns were resected from the cervical region. The teeth were randomly divided into two experimental groups of 20 based on the tested materials (ProRoot MTA and CEM cement), as well as two negative and positive control groups of three. All the samples were instrumented and obturated using the step-back technique, and fluid filtration was used to evaluate sealing ability and leakage inhibition. Data analysis was performed using the Mann-Whitney U test ($\alpha=0.05$). **Results:** Mean microleakage in the ProRoot MTA and CEM cement was 2 ± 0.79 and 3.02 ± 1.38 $\mu\text{L}/8$ min, respectively. In addition, a significant difference was observed between the two groups in this regard ($P<0.011$). **Conclusion:** According to the results,

ProRoot MTA provided significantly less microleakage compared to the CEM cement. Therefore, the sealing ability of ProRoot MTA was higher than the CEM cement, which makes MTA a better material than CEM cement for canal obturation.

Keywords: Calcium-enriched Mixture, Fluid Filtration, Mineral Trioxide Aggregate.

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Introduction

One of the major issues in endodontic treatment is the elimination of microorganisms from the complex three-dimensional root canal system (1-3). Microorganisms are the main cause of failure in endodontic treatment, which leads to apical periodontitis (4, 5). Endodontic treatment is aimed at disinfection using mechanical and chemical techniques, as well as replacing the inflamed pulp with a neutral substance in order to prevent reinfection in the blood flow, saliva leakage, coronal area, and microorganism invasion in the periodontal zone (6-8).

Endodontic treatment failure is caused by the leakage of microorganisms and endotoxins, which leads to pathological lesions (9). Therefore, selecting the materials that can seal the root canal could remarkably affect the treatment prognosis. Recently, several materials have been introduced for filling the canal, while the most commonly used substance is gutta-percha (10, 11).

Within the past decade, the mineral trioxide aggregate (MTA) has been utilized as an efficient substitution in dentistry, demonstrating satisfactory clinical outcomes (12). MTA is composed of various alkaline mineral oxides and has antimicrobial properties, tissue compatibility, and the ability to seal the canal in the presence of blood and moisture (13). Furthermore, MTA could be used as an alternative to gutta-percha as a filling material (14).

Although MTA is considered to be an effective filling substance, some of its limitations include the long duration of hardening, difficulty of use, and high costs. Considering the advantages and disadvantages of MTA, other substances such as calcium-enriched mixture (CEM) cement and novel endodontic cement (NEC) have recently been introduced for such purposes (15). CEM cement mainly contains CaO, SO₃, P₂O₅, and SiO₂. It is alkaline cement with several advantages, including tissue biocompatibility, hard tissue induction, high sealing ability, ability to be set in an aqueous environment, antibacterial properties, and resistance to washing out (16). Therefore, CEM has been reported to have comparable results with MTA and has been recommended as a proper material for the filling of the root canal. In addition, CEM cement can be used in vital pulp therapy in the impact, mature teeth (17).

Using MTA and CEM cement as filling substances has been associated with various limitations. For instance, after the complete setting of these substances, their removal for nonsurgical retreatment and post preparation is extremely difficult.

Due to the limited number of the studies focusing on root canal filling using ProRoot MTA and CEM cement, the present study aimed to compare the microleakage of the root canals filled with ProRoot MTA and CEM cement. The null hypothesis was the lack of a significant difference between the microleakage of these canal fillers.

Materials and Methods

This experimental, in-vitro study was conducted on 46 root canals of extracted mandibular premolars. In order to disinfect the teeth, all the samples were placed in 3% sodium hypochlorite for two hours. To facilitate the cleaning and shaping process, the crowns of all the teeth were cut using a high-speed hand piece at the cemento-enamel junction. Afterwards, a K-file #15 (Mani, INC, Japan) was inserted into the root canal to the length that the tip could be seen in the apex. The succeeding file was subtracted by an increment of one millimeter from the file and was considered as the working length. The step-back technique with the manual K-files was initiated with a K-file #25, which was proceeded by a master apical file #40. Shaping continued to K-file #80.

After canal preparation, the roots were randomly divided into four groups. Group one included 20 teeth filled with ProRoot MTA (Maillfer, Dentsply, Switzerland), group two included 20 teeth filled with the CEM cement (BioniqueDent, Tehran, Iran), group three was the negative control consisting of three teeth without root filling and the root surface and apical foramen lined with two layers of nail polish, and group four was the positive control consisting of three teeth, which were filled by a single gutta-percha point #40 (Meta Biomed Co. Chung-Ju, South Korea), and the root surface was covered with two layers of nail polish, with the exception of the apical foramen.

ProRoot MTA and CEM cement were combined in accordance with the instructions of the manufacturers in order to reach the suitable consistency and were applied using a cotton-tipped K-file #30 and hand plugger. Following the obturation, all the teeth were wrapped in sterile gauze moistened with sterile normal saline and placed in a plastic bag for seven days. The gauze was moistened with normal saline every day in order to provide 100% humidity.

After seven days, two layers of nail polish were applied to the root surfaces of all the teeth so as to seal all the superficial cracks in the tooth structure and prevent fluid extravasation. In the experimental groups and positive control group, the root surface was covered with nail polish, with the exception of the apical

foramen. In the negative control group, nail polish was applied to the entire access cavity, as well as the root surface and apical foramen. Following that, the teeth were mounted and exposed to the fluid filtration system.

Microleakage Testing

The roots of all the teeth were coated with a double-layer, waterproof nail polish in order to seal the superficial cracks in the tooth structure and prevent fluid extravasation. Afterwards, plastic tubes (internal diameter: 5 mm, length: 30 mm) were prepared and attached to the tooth apex as the apex was placed in the tube. The outer surface of the tube in the attachment area was sealed by cyanoacrylate in order to prevent potential penetration from this area. After sample preparation, the fluid level of the pipette (TPC, Thebarton, Australia) was adjusted at zero using a tube attached to a syringe containing a colored liquid in one end and a barometer and nitrogen gas capsule system in the other end.

The pipette had an accuracy of 0.1 μL , and the pressure was set at 50 KPa. The duration of each experiment for the samples was 10 minutes. Within the initial two minutes, the tube attached to the system was expanded, and a sustainable condition was maintained in the system. After two minutes, the fluid level in the pipette was recorded, and after eight minutes, the final fluid level in the pipette was recorded. In addition, the

reduction of the fluid level was measured and considered as microleakage (microliter/minute).

The duration of infiltration was recorded in each group. The induction of the fluid pressure behind the experimental surface was assessed, and the volume of the fluid passing through the surface was determined based on a specific time.

Data analysis was performed in SPSS version 22 using the Mann-Whitney U test at the significance level of $P < 0.05$.

Results

Considering the failure to establish normal distribution conditions in one of the groups in the present study, the comparison of the two groups was performed using the Mann-Whitney U test. Mean microleakage for the ProRoot MTA and CEM cement was estimated at 2.01 ± 0.79 and 3.02 ± 1.38 $\mu\text{L}/8$ min, respectively (Fig. 1) (Table I). Mean microleakage in the positive control group was calculated to be 20 $\mu\text{L}/8$ min, while it was 00 $\mu\text{L}/8$ min in the negative control group. However, no microleakage was observed in the negative control group, while the positive control group showed maximum microleakage. Furthermore, a statistically significant difference was denoted between the two groups in this regard ($P < 0.05$).

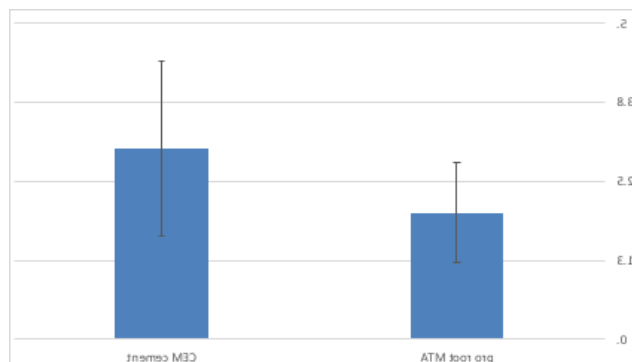


Figure 1. Mean Microleakage (per 8 min; substance descriptive error bar: ± 1 SD)

Table I. Mean Values of Microleakage ($\mu\text{L}/8$ min) in Experimental Groups

Groups	Mean	Minimum and Maximum	Kolmogorov-Smirnov Test P-value
MTA	2.01	1.22 2.80	0.005
CEM	3.02	1.64 4.40	0.2

Discussion

According to the results of the present study, the mean microleakage in the ProRoot MTA group was significantly lower compared to the CEM cement group, which indicated that the sealing ability of ProRoot MTA was higher than the CEM cement. As such, the null hypothesis was ruled out.

Evaluation of microleakage is performed using various methods, including bacterial penetration, fluid filtration, dye penetration tests, penetration of radioisotopes, gas chromatography, and electrochemical tests (18). In the present study, the fluid filtration technique was used for the measurement of microleakage; this technique has been reported to be a reliable approach in the study by Javidi et al. (19) with its advantages over the common dye penetration method. In fluid filtration, the samples are not destroyed, allowing the evaluation of sealing efficiency over time. Moreover, no tracer is required for the problems associated with the molecular size, which are among the major setbacks in dye penetration. It is notable that no intricate materials are required in bacterial penetration and radioactive tracer studies.

The purpose of inserting a substance into the canal is to induce proper sealing in order to prevent the recontamination of the periapical tissues. Various materials are used for obturation, each of which has advantages and disadvantages. However, no substance has yet been produced with all the essential laboratory conditions for sealing (20, 21).

As a common substance in endodontic treatment, MTA has been reported to have favorable tissue compatibility and the ability to stimulate osteogenesis (22). Furthermore, MTA has exhibited a high success rate in the sealing of perforated areas, as well as the restoration of the root-end cavities in periapical surgeries (14, 23). Some of the main limitation of MTA include the high costs, long duration of hardening, and possibility of color change in the crown. On the other hand, the antibacterial properties of this substance are unpredictable, and its application is not clinically ideal and simple (15, 24). To overcome the mentioned shortcomings, the CEM cement has recently been introduced with various calcium components (17). The CEM cement has favorable sealing ability, and unlike MTA, it contains the chemical elements that are required for the formation of hydroxyapatite crystals (25).

Since the purpose of root canal filling is to induce appropriate sealing with the minimum level of microleakage, we compared the microtubular leakage of root canal obturation using the ProRoot MTA and CEM

cement. Similarly, Razmi et al. (25) compared the sealing ability of two root-end filling materials (MTA and CEM cement) following the preparation of ultrasonic cavities or laser. According to the obtained results, the minimum leakage rate was significantly lower in the laser group with the CEM cement compared to the MTA group. In another study by Moghadam et al. (26), no statistically significant difference was reported between the CEM cement and MTA in terms of sealing strength.

In this regard, Kazem et al (27). Compared the microleakage in amalgam, root MTA, white ProRoot MTA, and CEM cement using color infiltration and microbial leakage. In both experiments, the leakage was observed to be higher in the CEM cement group compared to the root MTA group, which indicated the reduced ability for sealing. This finding is consistent with the results of the present study. In contrast, the findings of Shahriary demonstrated no significant difference between the two materials in terms of the sealing ability although MTA was subtly more sealable compared to the CEM cement. Unlike the present study, microbial leakage was used instead of infiltration in the mentioned research (28).

In the study by Asgary et al. (29), the microleakage of the CEM cement was assessed and compared with intermediate restorative material (IRM) and three types of MTA (American, Brazilian, and Iranian) for apical filling in various environments. The obtained results indicated that the seal formed by the CEM cement was stronger compared to that of MTA, and both materials were more sealable compared to IRM (29). On the other hand, Hasheminia et al (30). Reported that the sealing ability of CEM was higher compared to MTA, which is inconsistent with our findings.

In another research by Khademi et al (31), two types of MTA (ProRoot MTA and Bio MTA) were compared after root canal filling. In the mentioned study, the fluid infiltration technique was used to determine the microleakage level. After the test, no significant difference was observed in the sealing ability of ProRoot MTA and Bio MTA (31). In the previous studies in this regard, various methods were applied for leakage measurement (e.g., leakage duration), which could explain the discrepancies in the obtained results. For instance, in the study by Ghorbani et al. (32), the maximum sealing ability of the CEM cement was observed after 12 hours, while the rate of sealing ability in the MTA was observed after 24 hours. In addition, Mousavi et al (33). compared the sealing ability of ProRoot MTA, Biodentine, and Ortho MTA in canal obturation using fluid infiltration, reporting that the

microleakage values were similar to ProRoot MTA, Biodentine, and Ortho MTA.

The literature is replete with data on MTA for the management of various endodontic conditions, such as root-end filling, repair of root perforations and resorptions, vital pulp therapy, closure of an open immature apex using an apical plug, and other root-end inductive procedures. This could be attributed to endodontically favorable physicochemical and biological properties, such as superior sealing, proper marginal adaptation, minimal microleakage, high biocompatibility, and bioinductive and antimicrobial properties. These properties could be associated with the slow leaching of calcium hydroxide and calcium ion in MTA (34). As a result, MTA has been strongly recommended and successfully utilized by eminent clinicians and researchers in order to obturate the entire canal (35). However, orthograde MTA compaction remains challenging and technique-sensitive, and porosities and inadequacies are commonly reported (36).

Conclusion

According to the results, ProRoot MTA has a lower microleakage rate as a canal obturation substance compared to the CEM cement. Therefore, MTA is considered to be a better material than the CEM cement for canal obturation. Considering the contradictory results of the CEM cement and ProRoot MTA microleakage, further investigations are required in this regard.

Conflicts of interest

None declared.

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References

1. Berutti E, Marini R, Angeretti A. Penetration ability of different irrigants into dentinal tubules. *J Endod.* 1997;23(12): 725-727.
2. Siqueira JF Jr, Machado AG, Silveira RM, Lopes HP, De Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of *Enterococcus faecalis* from the root canal, in vitro. *Int Endod J.* 1997;30(4): 279-282.
3. Safavi KE, Spangberg LS. Root canal dentinal tubule disinfection. *J Endod.* 1990;16(5): 207-210.
4. Heling I, Chandler NP. Antimicrobial effect of irrigant combination within dentinal tubules. *Int Endod J.* 1998;31(1): 8-14.
5. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surg Oral Med Oral Pathol.* 1965;20(3): 340-349.
6. Mamootil K, Messer HH. Penetration of dentinal tubules by endodontic sealer cements in extracted teeth and in vivo. *Int Endod J.* 2007;40(11): 873-881.
7. Von Fraunhofer JA, Fagundes DK, McDonald NJ, Dumsha TC. The effect of root canal preparation on microleakage within endodontically treated teeth: an in vitro study. *Int Endod J.* 2000;33(4): 355-360.
8. Gençoglu N, Oruçoglu H, Helvacioğlu D. Apical leakage of different gutta-percha techniques: Thermafil, Js Quick-Fill, Soft Core, Microseal, System B and lateral condensation with a computerized fluid filtration meter. *Eur J Dent.* 2007;1(2): 97-103.
9. Pappen AF, Bravo M, Gonzalez-Lopez S, Gonzalez-Rodriguez MP. An in vitro study of coronal leakage after intraradicular preparation of cast-dowel space. *J Prosthet Dent.* 2005;94(3): 214-218.
10. Shahi S, Zand V, Oskoe SS, Abdolrahimi M, Rahnema AH. An in vitro study of the effect of spreader penetration depth on apical microleakage. *J Oral Sci.* 2007;49(4): 283-286.
11. Baumgardner KR, Taylor J, Walton R. Canal adaptation and coronal leakage: Lateral condensation compared to Thermafil. *J Am Dent Assoc.* 1995;126(3): 351-356.
12. Witherspoon DE. Vital pulp therapy with new materials: new directions and treatment perspectives-permanent teeth. *J Endod.* 2008;34(7 Suppl): S25-28.
13. Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Cytotoxicity of four root end filling materials. *J Endod.* 1995;21(10): 489-492.
14. Torabinejad M, Fouad A, Walton RE. *Endodontics: Principles and practice.* 5th ed. St. Louis: Elsevier Health Sciences; 2015.
15. Eghbal MJ, Torabzadeh H. Review of high level endodontic research in PubMed index journals from Iran. *Iran Endod J.* 2012;7(3): 109-114
16. Sadeghi S, Khoshdel M. In-vitro sealing ability of calcium enriched mixture cement versus amalgam as retrograde filling materials. *J Dent Mater Tech.* 2018;7(4): 152-155.
17. Tavassoli-Hojjati S, Kameli S, Rahimian-Emam S, Ahmadyar M, Asgary S. Calcium enriched mixture cement for primary molars exhibiting root perforations and extensive root resorption: report of three cases. *Pediatr Dent.* 2014;36(1): 23E-27E.

18. Jacobson SM, Fraunhofer JA von. The investigation of microleakage in root canal therapy. An electrochemical technique. *Oral Surg Oral Med Oral Pathol.* 1976;42(6): 817-823.
19. Javidi M, Naghavi N, Roohani E. Assembling of fluid filtration system for quantitative evaluation of microleakage in dental materials. *Iran Endod J.* 2008;3(3): 68-72.
20. Veríssimo DM, do Vale MS. Methodologies for assessment of apical and coronal leakage of endodontic filling materials: A critical review. *J Oral Sci.* 2006;48(3): 93-98.
21. Suprabha BS, Sudha P, Vidya M. A comparative evaluation of sealing ability of root canal-sealers. *Indian J Dent Res.* 2002;13(1): 31-36.
22. Tani-Ishii N, Hamada N, Watanabe K, Tujimoto Y, Teranaka T, Umemoto T. Expression of bone extracellular matrix proteins on osteoblast cells in the presence of mineral trioxide. *J Endod.* 2007;33(7): 836-839
23. Tabarsi B, Parirokh M, Eghbal M, Haghdoost A, Torabzadeh H, Asgary S. A comparative study of dental pulp response to several pulpotomy agents. *Int Endod J.* 2010;43(7): 565-571.
24. Vasudev SK, Goel BR, Tyagi S: Root end filling materials-A review. *Endodontology.* 2003;15(2): 12-18.
25. Razmi H, Shokouhinejad N, Fekrazad R, Motahhary P, Alidoust M. Comparison of the sealing ability of two root-end filling materials (MTA and CEM cement) following retropreparation with ultrasonic or Er, Cr: YSGG laser. *J Dent Med.* 2009;22(4): 144-151.
26. Moghadam N, Abdollahi A, Aghabalayi Fakhim H, Borna Z. In-vitro sealing properties of calcium-enriched mixture and mineral trioxide aggregate orifice barriers during Intra-coronal bleaching. *Iran Endod J.* 2017;12(2): 231-235.
27. Kazem M, Eghbal MJ, Asgary S. Comparison of bacterial and dye microleakage of different root-end filling materials. *Iran Endod J.* 2010;5(1): 17-22.
28. Shahriari S, Faramarzi F, Alikhani MY, Farhadian M, Hendi SS. Apical sealing ability of mineral trioxide aggregate, intermediate restorative material and calcium enriched mixture cement: a bacterial leakage study. *Iran Endod J.* 2016;11(4): 336-340
29. Asgary S, Eghbal MJ, Parirokh M, Torabzadeh H. Sealing ability of three commercial mineral trioxide aggregates and an experimental root-end filling material. *Iran Endod J.* 2006;1(3): 101-105.
30. Hasheminia M, Loriaeinejad S, Asgary S. Sealing ability of MTA and a new endodontic material as root-end fillings in dry, saliva or blood-contaminated conditions. In: 2nd IADR Iranian Section General Meeting; 2007 Dec 6-7; Tehran, Iran.
31. Khademi A, Mousavi SA, Moraditalab A, Shahnasari S, Khazaei S, Tajali R. Comparative evaluation of sealing of ProRoot MTA and Bio MTA in canal obturation by fluid infiltration. *J Dent Med.* 2018; 31(1): 11-17.
32. Ghorbani Z, Kheirieh S, Shadman B, Eghbal MJ, Asgary S. Microleakage of CEM cement in two different media. *Iran Endod J.* 2009;4(3): 87-90.
33. Mousavi SA, Khademi A, Soltani P, Shahnasari S, Poorghorban M. Comparison of sealing ability of ProRoot mineral trioxide aggregate, biodentine, and ortho mineral trioxide aggregate for canal obturation by the fluid infiltration technique. *Den Res J.* 2018;15(5): 307-312.
34. O'Sullivan SM, Hartwell GR. Obturation of a retained primary mandibular second molar using MTA: A case report. *J Endod.* 2001;27(11): 703-705.
35. Adiga S, Ataide I, Fernandes M, Adiga S. Nonsurgical approach for strip perforation repair using mineral trioxide aggregate. *J Conserv Dent.* 2010;13(2): 97-101.
36. Torabinejad M, Smith PW, Kettering JD, Pitt Ford TR. Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root end filling materials. *J Endod* 1995;21(6): 295-299.

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