

An In Vitro Comparison of Microleakage in Root Canals Obturated with MTA, Cold Ceramic, Gutta-Percha, and AH26 Sealer Using the Dye Penetration Method

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Article Type ABSTRACT

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Background and Objective: Root canal treatment involves the removal of microorganisms and diseased tissues from within the root canal and filling the space with materials to prevent or treat the periapical region. If microorganisms survive and proliferate within the canal and extend to the periapical tissues of the tooth, they can lead to root canal treatment failure. An impermeable seal of the root canal is the main factor in the success of root canal treatment. The aim of the present study is to compare the microleakage of canals obturated with MTA, cold ceramic, gutta-percha, and AH26 sealer using the dye penetration method.

Methods: In this in vitro study, 64 freshly extracted human maxillary central teeth were prepared and shaped, and the canals were randomly divided into three experimental groups (18 samples each) and two control groups (5 samples each). The canals were filled using the materials according to the manufacturer's instructions, and the last three millimeters were filled using a temporary dressing. After one week of incubation at 100% humidity and 37°C, the samples were immersed in methylene blue for 4 days, and the amount of dye penetration was measured.

Findings: The mean microleakage among the three study groups demonstrated a statistically significant difference, with the highest microleakage (6 mm) observed in the gutta-percha and AH26 sealer group and the lowest in the cold ceramic group (0 mm). The results showed that the mean microleakage was 1.21 ± 0.94 mm in the cold ceramic group, 1.55 ± 1.19 mm in the MTA group, and 2.33 ± 1.71 mm in the gutta-percha and AH26 sealer group. There was a statistically significant difference between the cold ceramic group and the gutta-percha and AH26 sealer group ($p < 0.05$); however, there was no statistically significant difference between the MTA and cold ceramic groups, nor between the MTA and gutta-percha and AH26 sealer groups.

Conclusion: The results of the study showed that cold ceramic and MTA are better filling materials compared with gutta-percha and AH26 sealer.

Keywords: Dental Microleakage, Root Canal Filling Materials, In Vitro.

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Introduction

In root canal treatment, the primary aim is the elimination or reduction of microorganisms within the root canal. This aim is achieved by instrumenting the canals, using antimicrobial agents, and completely filling the empty spaces of the canal (1). Complete elimination of all microorganisms is not possible due to the anatomical complexity of the root canal, even with the most advanced cleaning instruments and techniques. If microorganisms survive and proliferate within the canal and extend to the periapical tissues of the tooth, they can lead to root canal treatment failure (2, 3). Therefore, the ultimate goal of root canal treatment is to create a complete seal throughout the entire length of the canal, from the coronal orifice to the apical end of the root, and to achieve the best possible adaptation of the filling material to the canal wall in order to prevent recontamination of the root canal. After preparation of the root canal, a material is placed in the canal as an obturator (4). The ideal filling material should seal all communication pathways between the root canal system and the periapical tissues. It should also be non-toxic, non-carcinogenic, biocompatible, insoluble in tissue fluid, and dimensionally stable (5).

In the past, various materials were used for this purpose, each with its own advantages and disadvantages. The most common materials were gutta-percha and sealers, which demonstrated good properties; however, they also had disadvantages such as the inability to bond to the tooth structure. Therefore, efforts to find better root canal filling materials continue. MTA (Mineral Trioxide Aggregate) was introduced in 1993 at Loma Linda University in the United States. This material is used for root-end filling, apical plug, direct pulp capping, sealing perforations, and also for complete canal filling. This cement consists of dicalcium silicate, tricalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite (6). The cement is composed of a hydrophilic powder that sets in the presence of moisture (6). When this cement is mixed with water, the resulting mixture transforms into a firm and hard mass within 4 hours (7). The initial pH of the mixture is 10.2, which increases to 12.5 three hours after mixing (6). Calcium hydroxide is the most important compound that MTA releases in water (6). The marginal adaptation of MTA is better than that of amalgam, IRM, and EBA-Super (7). It also has lower cytotoxicity compared with other materials (7). However, despite its many advantages, MTA has been criticized for its difficult handling and slow setting reaction, which can lead to leakage, surface degradation, and loss of marginal adaptation and material cohesion (8).

Recently, another material called cold ceramic has been introduced. This material is also used for root-end filling, apical plug, direct pulp capping, sealing perforations, and complete canal filling. This root filling material is calcium hydroxide-based and comes in powder and liquid form, which is used after mixing the two components. Based on the results obtained from X-ray fluorescence analysis, it is evident that four compounds—calcium oxide, silicon oxide, barium oxide, and sulfur oxide—together constitute approximately 93% of the chemical composition of this material (9). The initial setting time is approximately 15 minutes, and it completely sets after 24 hours (10). Various studies have shown favorable properties such as the ability for marginal adaptation with a high seal, appropriate biocompatibility, and low toxicity. A study comparing tissue reaction to MTA and cold ceramic has shown that both MTA and cold ceramic are well tolerated by tissue (11-17). The present study was conducted with the aim of an in vitro comparison of the microleakage of canals obturated with MTA, cold ceramic, gutta-percha, and AH26 sealer using the dye penetration method.

Methods

After approval by the Research Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, under code IR.SSU.DENTISTRY.REC.1401.035, this in vitro study was conducted on 64 freshly extracted human maxillary central teeth. These teeth had mainly been extracted due to periodontal problems. The inclusion criteria consisted of single-root and single-canal anatomy, complete root development, straight root, and absence of cracks, fractures, root caries, calcification, or root resorption. The exclusion criteria consisted of teeth that developed fracture or cracks during canal cleaning or filling. In order to control infection and remove surface debris, the teeth were immersed in 5.25% sodium hypochlorite (Chloran Chemical Production Co., Iran) for 30 minutes and then stored in normal saline until the time of the study.

In order to standardize the root length, the crowns of the teeth were cut using a diamond disc (Tizkavan, 010, Tehran, Iran) with a high-speed handpiece, such that a standard length of 13 mm was maintained in all samples. In the next step, to determine the working length, a size 15 K-file (Dentsply Maillefer, Tulsa) was inserted into the canal until the tip of the file was visible at the apical foramen region. By subtracting 1 mm from this length, the working length was determined. Canal preparation was performed using the step-back technique with Gates-Glidden drills numbers 1 to 4 (Dentsply, Milford, DE, USA). The canals were cleaned to the working length using K-files up to number 40 and enlarged up to number 80.

Between each instrument, the canal was irrigated with 1 mL of 5.25% sodium hypochlorite using a sterile irrigation syringe with a 27-gauge needle. After preparation, the root canals of all teeth were filled with 1 mL of 17% EDTA (Ethylenediaminetetraacetic Acid) (Ariadent, Tehran, Iran) for 3 minutes, with the aim of removing the smear layer and as part of the standard cleaning protocol, and then rinsed with 5 mL of normal saline solution and dried with paper points. In the next step, the prepared roots were randomly divided into three experimental groups: obturation with MTA Angelus, cold ceramic, and gutta-percha with AH26 sealer (18 samples each), and two positive and negative control groups (5 samples each).

In the two experimental groups 1 and 2, the canal filling materials were prepared according to the manufacturer's instructions and placed inside the canal using an MTA carrier (Dentsply Maillefer), and were condensed using a hand plugger (Dentsply Maillefer) such that 10 mm of the canal space was completely filled; the remaining 3 mm was covered with a temporary restoration (Cavit, 3M ESPE, Germany). In experimental group 3, the canals were filled with gutta-percha and AH26 sealer using the lateral compaction technique, and the remaining 3 mm was covered with a temporary restoration (Cavit, 3M ESPE, Germany). To ensure the density of the filling materials, radiographs were taken of all samples (Figure 1). Then, the entire root surface, except for the apical 2 mm, was covered with two layers of nail varnish.

In the positive control group, the canals were filled with gutta-percha (META, Korea) without sealer, and then the entire root surface, except for the apical 2 mm, was covered with two layers of nail varnish. In the negative control group, the canals were filled with gutta-percha and AH26 sealer (Dentsply, Tulsa), and finally the entire root surface was covered with two layers of nail varnish.

All samples were stored inside an incubator at 100% humidity and 37°C for one week. In the next step, all samples were immersed in 2.5% methylene blue dye solution for 96 hours. After 96 hours, the samples were removed from the dye and rinsed under running water for one hour and then dried. To facilitate sectioning of the roots for measuring dye penetration, the roots were mounted in acrylic (Figure 2).

The samples were then divided from the center of the longitudinal section of the root into two buccal and lingual halves using a diamond disc (Tizkavan, Iran). Methylene blue microleakage was measured in millimeters on two opposing surfaces using a stereomicroscope and recorded as the mean for each sample. Finally, the data were entered into a computer in the SPSS 25 software environment and analyzed using ANOVA and Scheffe statistical tests, and $p < 0.05$ was considered significant.

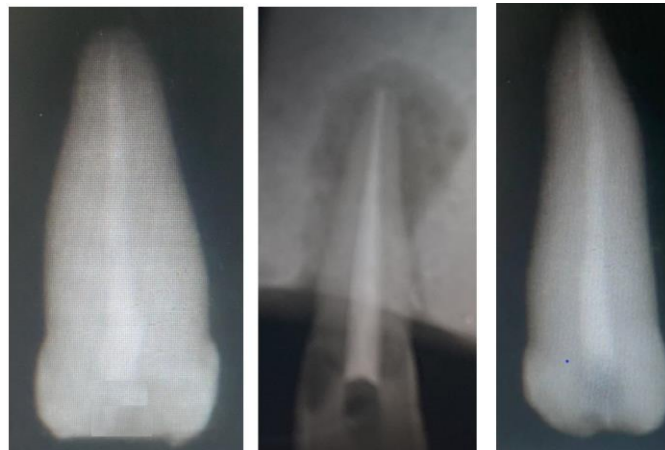


Figure 1. Radiographs obtained from the examined samples, in order from the right: cold ceramic, gutta-percha with AH26 sealer, and MTA



Figure 2. Tooth mounted in acrylic, in order from the right: gutta-percha, cold ceramic, and MTA

Results

The mean apical microleakage observed in the cold ceramic group was 1.21 ± 0.94 mm, in the MTA group 1.55 ± 1.19 mm, and in the gutta-percha-AH26 sealer group 2.33 ± 1.71 mm. Based on the obtained results, the mean microleakage among the three groups—MTA, cold ceramic, and gutta-percha-AH26 sealer—demonstrated a statistically significant difference ($p < 0.05$) (Table 1), such that the highest microleakage among these three groups was observed in the gutta-percha-AH26 sealer group and the lowest in the cold ceramic group.

Table 1. Mean apical microleakage in the study groups (mm)

Group	Sample size	Mean $\pm$ SD	p-value*
Cold ceramic ^a	18	1.21 ± 0.94	0.018
MTA ^b	18	1.55 ± 1.19	0.018
Gutta-percha-AH26 sealer ^c	18	2.33 ± 1.71	0.018
Negative control ^d	5	0.44 ± 0.2	0.018
Positive control ^e	5	6.5 ± 3.11	0.018

*ANOVA test

In the pairwise comparison of the study groups using the Scheffe statistical test, it was observed that the difference in mean microleakage between the cold ceramic group and the gutta-percha-AH26 sealer group was statistically significant ($p < 0.05$); however, this difference between the MTA group and the cold ceramic group, and between MTA and the gutta-percha-AH26 sealer group, was not statistically significant.

Discussion

In this study, which compared the sealing ability of gutta-percha with AH26 sealer, MTA, and cold ceramic, the results showed that cold ceramic and MTA are better filling materials compared with gutta-percha and AH26 sealer.

In studies, various methods are used to evaluate apical microleakage, such as dye penetration, radioisotope, bacterial penetration, impedance or electrical resistance, distance measurement with scanning electron microscopy, or fluid filtration. There is no evidence indicating that the use of any particular method is superior to another. Each technique has significant limitations that can lead to error (10). The dye penetration method has several disadvantages. These include examining microleakage only in two dimensions, the semi-quantitative nature of the data, loss of the sample during the test procedure, the impossibility of re-testing it, and the impossibility of examining microleakage at different times. Despite these limitations, it remains one of the most popular and common techniques for examining microleakage. This is due to its simplicity, low cost, and ease of performance. Consequently, this method was used in the present study to examine the amount of microleakage (18).

2% methylene blue dye was used in this study. Methylene blue is composed of fine particles smaller than a micron, which increases the accuracy of the test. If a material can prevent the microleakage of methylene blue dye, which has dimensions much smaller than bacteria, it will probably also prevent the penetration of bacteria and their endotoxins into the root canal, which have larger dimensions (19). The results of this study showed that there was a statistically significant difference between the mean microleakage in the three groups: MTA, cold ceramic, and gutta-percha-AH26 sealer. The highest microleakage was observed in the gutta-percha-AH26 sealer group and the lowest in the cold ceramic group. The difference in microleakage between the gutta-percha-AH26 sealer group and the cold ceramic group was statistically significant; however, the microleakage in the two groups of MTA and cold ceramic, as well as in the two groups of MTA and gutta-percha-AH26 sealer, did not show a statistically significant difference.

The results of the study by Hasheminia et al. showed that the sealing ability of cold ceramic in a blood-contaminated environment is significantly greater than that of MTA. However, in dry and moist environments, there was no statistically significant difference between their sealing abilities. Additionally, the results of their study showed that although the marginal adaptation of cold ceramic is greater than that of MTA over the long term, this difference was not statistically significant (10). Mokhtari et al. also showed that although the sealing ability of cold ceramic is greater than that of MTA, this difference was not statistically significant (1). In another study, Mokhtari et al. reported that based on the electrical conductivity test, the mean microleakage in cold ceramic was significantly less than that of gutta-percha (20).

Based on the results of the study by Zahedinejad, the mean linear distance between the root filling materials and the canal wall in the cold ceramic group was significantly less than in the gutta-percha and MTA sealer (Endoseal) group (21). Bakhtiyarinia also showed that the mean marginal adaptation in the cold ceramic group was significantly less than in the gutta-percha-AH26 sealer group (22). The results of all the mentioned studies confirm the results of the present study.

Previous studies have shown that in cases of using bioceramic materials such as MTA, a layer of apatite crystals precipitates between the MTA and the dentinal wall, which can increase the sealing ability (23, 24). Farahnak noted that the reason for the greater adaptation of cold ceramic to the dentinal wall appears to be the release of calcium from cold ceramic and its reaction with phosphorus present in the tooth structure, creating hydroxyapatite crystals that can partially fill the microscopic space between the wall and the material (25). The points mentioned can to some extent justify the better sealing property of cold ceramic and MTA compared with gutta-percha and AH26 sealer; however, further studies are needed to confirm the obtained results and to investigate the possible reasons for these results.

Based on the results of the present study and considering the limitations of an in vitro study, it appears that the bioceramics cold ceramic and MTA exhibit superior sealing ability as root canal filling materials when compared with gutta-percha-AH26 sealer. Consequently, provided that their remaining properties are satisfactory, they may be successfully utilized as root canal fillers. However, further studies are needed to confirm the obtained results and to investigate the possible reasons for these results. It is suggested that, in order to confirm the use of cold ceramic as a root canal filling material, its sealing ability be examined using other criteria for evaluating microleakage.

Conflicts of Interest: None of the authors of this study, individuals, or organizations have any conflicts of interest regarding the publication of this article.

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References

1. Mokhtari F, Koopaei K, Modaresi J, Hemati HR, Zandi H. Experimental evaluation of the sealing ability of MTA and cold ceramic by using bacterial leakage method. *J Dent Med* 2017;30(3):150-5. [In Persian]
2. Baer J, Maki JS. In vitro evaluation of the antimicrobial effect of three endodontic sealers mixed with amoxicillin. *J Endod*. 2010;36(7):1170-3.
3. Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2005;99(2):231-52.
4. Torabinejad M, Pitt Ford TR. Root end filling materials: a review. *Endod Dent Traumatol*. 1996;12(4):161-78.
5. Torabinejad M, Fouad AF, Shabahang S. *Endodontics: Principles and practice*, 6th ed. Elsevier; 2020. p. 120-64.
6. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review--Part III: Clinical applications, drawbacks, and mechanism of action. *J Endod*. 2010;36(3):400-13.
7. Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review--part II: leakage and biocompatibility investigations. *J Endod*. 2010;36(2):190-202.
8. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review--Part I: chemical, physical, and antibacterial properties. *J Endod*. 2010;36(1):16-27.
9. Modaresi J, Talakoob MH. Comparison of two root-end filling materials. *J Isfahan Med Sch*. 2015;11(5):379-86. [In Persian]
10. Hasheminia SM, Nejad SL, Dianat O, Modaresi J, Mahjour F. Comparing the sealing properties of mineral trioxide aggregate and an experimental ceramic based root end filling material in different environments. *Indian J Dent Res*. 2013;24(4):474-7.
11. Modaresi J, Yavari SA, Dianat SO, Shahrabi S. A comparison of tissue reaction to MTA and an experimental root-end restorative material in rats. *Aust Endod J*. 2005;31(2):69-72.
12. Kolahdüzan AR, Abbasi M, Rezai N, Araghi S, Sadeghi D, Esmaeili SH. Comparison of Coronal Microleakage of Canals Filled with Gutta Percha and Resilon with a Glass Ionomer Coronal Barrier. *J Islam Dent Assoc Iran*. 2014;26(1):18-23.
13. Razmi H, Bolhari B, Karamzadeh Dashti N, Fazlyab M. The Effect of Canal Dryness on Bond Strength of Bioceramic and Epoxy-resin Sealers after Irrigation with Sodium Hypochlorite or Chlorhexidine. *Iran Endod J*. 2016;11(2):129-33.
14. Setia P, Sikri VK, Sroa RB, Sidhu B. Apical sealing ability of two novel root canal sealers: An: ex-vivo: study. *J Int Clin Dent Res Org*. 2013;5(1):9-13.
15. Shahraki HA, Saberi EA, Maserrat V, Dalaei M, Nejad SM. In Vitro Comparison of Apical Micro Leakage in Root Canal Prepared Wave One and Reciproc Files. *J Biochem Technol*. 2020;11(2):65-70.
16. de Almeida-Gomes F, Maniglia-Ferreira C, de Moraes Vitoriano M, Carvalho-Sousa B, Guimaraes NL, dos Santos RA, et al. Ex vivo evaluation of coronal and apical microbial leakage of root canal--filled with gutta-percha or Resilon/Epiphany root canal filling material. *Indian J Dent Res*. 2010;21(1):98-103.
17. Prithviraj KJ; Sreegowri; Manjunatha RK, Horatti P, Rao N, Gokul S. In Vitro comparison of the microbial leakage of obturation systems: Epiphany with resilon, guttaflow, and ah plus with gutta percha. *Indian J Dent Res*. 2020;31(1):37-41.
18. Camps J, Pashley D. Reliability of the dye penetration studies. *J Endod*. 2003;29(9):592-4.
19. Wu MK, Wessellink PR. Endodontic leakage studies reconsidered. Part I. Methodology, application and relevance. *Int Endod J*. 1993;26(1):37-43.

- 20.Mokhtari F, Modaresi J, Zandi H, Ramezani Nik I. In-vitro comparison of sealing ability of gutta-percha and AH26 sealer with cold ceramic through bacterial leakage and electrochemical method. J Dent Med. 2021;34:25. [In Persian]
- 21.Zahedinejad AA. Investigation of marginal adaptation of channels filled with cold ceramic and gutta-percha-endoseal MTA bioceramic sealer by electron microscopy [dissertation]. Yazd: Shahid Sadoughi University of Medical Science; 2022.
- 22.Bakhtiyarinia Z. Experimental evaluation and comparison of marginal adaptation of obturated root canals with gutta percha and cold ceramic [dissertation]. Yazd: Shahid Sadoughi University of Medical Science; 2021.
- 23.Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I. Physicochemical basis of the biologic properties of mineral trioxide aggregate. J Endod. 2005;31(2):97-100.
- 24.Bird DC, Komabayashi T, Guo L, Opperman LA, Spears R. In vitro evaluation of dentinal tubule penetration and biomineralization ability of a new root-end filling material. J Endod. 2012;38(8):1093-6.
- 25.Farahnak S. Comparison of marginal adaptation of two retrofilling materials following root end cavity preparation with Er:YAG laser and ultrasonic using SEM [dissertation]. Yazd: Shahid Sadoughi University of Medical Science; 2016.