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Apical Microleakage of Endoseal MTA, AH26, and Sure-Seal Root Canal Sealers Using a Bacterial Leakage Model: An In-Vitro Study

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A B S T R A C T

Objectives: Lack of a proper apical seal is one of the most common reasons for root canal treatment failure. Several sealers have been developed in an attempt to enhance the quality of apical seal. Nevertheless, it is essential to evaluate their effectiveness in preventing bacterial penetration. The present study employed a bacterial leakage model to assess and compare the apical sealing ability of three root canal sealers namely AH26, Endoseal MTA, and Sure-Seal Root.

Materials and Methods: This in vitro study evaluated 148 extracted single-rooted permanent teeth with almost identical root lengths. The teeth were prepared using the modified crown-down technique, and underwent cleaning and shaping using ProTaper rotary files. Five teeth were selected as positive controls, and five as negative controls. The remaining teeth were randomly assigned to three groups (n=46), and obturated using the abovementioned root canal sealers according to the manufacturers' instructions. A bacterial leakage model was used to evaluate microleakage. The samples were evaluated daily regarding turbidity resulting from microbial microleakage. The data were analyzed with the Chi-square test and the log-rank test using SPSS 24 (alpha=0.05).

Results: The highest bacterial microleakage was recorded in the Sure-Seal Root group (7.21%), while the lowest microleakage was noted in the AH26 group (2.16%). However, there was no significant difference in bacterial microleakage among the three sealer groups (P=0.718).

Conclusion: According to the results of this in vitro study, the three sealers evaluated in the present study exhibited a similar performance regarding apical microleakage.

Keywords: Dental Leakage; Epoxy Resin AH-26; Mineral trioxide Aggregate

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INTRODUCTION

Root canal sealers were designed to adapt to discrepancies between the canal wall and the obturating core material, thereby improving the likelihood of producing a fluid-tight seal [1]. The main roles of sealers include occluding voids and accessory canals, sealing multiple foramina, enhancing the adhesion between the filling material and dentinal walls, providing lubrication during obturation, and encapsulating residual microorganisms [2].

AH26 is an epoxy resin sealer with good properties, including antimicrobial activity, adhesion, long working time, easy mixing, and outstanding sealing ability. However, its disadvantages include discoloration, relative toxicity until its setting reactions are completed, and some degrees of solubility in oral fluids [3].

In contrast, bioceramic sealers formulated with calcium silicate—such as Endoseal MTA and Sure-Seal Root—demonstrate favorable biological characteristics. These include the ability to induce dentin remineralization, low cytotoxicity within acceptable limits, antibacterial potential, and efficient penetration into dentinal tubules [4-8].

Lack of a proper apical seal is the main reason for failure of root canal treatment. Therefore, different sealers have been introduced to improve apical seal. However, it is important to evaluate the sealing ability of other sealers, especially in terms of bacterial microleakage, to identify the best sealers with the least microleakage for clinical success of root canal treatment. AH26, Endoseal MTA, and Sure-Seal Root sealers are the most commonly used endodontic sealers. Although several studies have evaluated the microleakage of different sealers [9,13], no study has compared the apical microleakage of AH26, Endoseal MTA, and Sure-Seal Root sealers. Therefore, the present in vitro study aimed to compare the apical microleakage of these three sealers.

MATERIALS AND METHODS

The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1401.491). A total of 148 single-rooted permanent teeth with almost equal root lengths, extracted for periodontal or orthodontic reasons, were included in the present in vitro study. The teeth had no cracks or fractures (especially root cracks), calcified root canals, open apices, long roots, resorptive lesions, severe caries, or severe root curvatures. The sample size was determined based on the microleakage values reported by Milani et al [14] who evaluated the presence or absence of leakage using AH Plus (0.00275±0.00363) and iRoot SP (0.00115±0.00160). Using these values for effect size estimation, and considering a significance level of α=0.05 and a power of 80%, the required sample size was calculated to be 148 teeth. They were randomly assigned to three groups (n=46), with two positive and negative control groups (n=5). The soft tissues attached to the tooth surfaces were removed with a periodontal curette. Teeth were immersed in 0.5% NaOCl for 24 hours for surface disinfection and finally stored in saline solution to prevent dehydration.

Procedural steps:

The tooth crowns were removed to achieve a standard root length of 12 mm. A barbed broach was used to remove the pulp residues from the root canals as much as possible. Then, a #15 hand K-file (Mani, Tochigi, Japan) was placed in the root canal until its tip was visible at the apical foramen. Then, the working length was recorded at 0.5 mm shorter. Subsequently, the root canals underwent cleaning and shaping with rotary ProTaper files (Dentsply Maillefer, Ballaigues, Switzerland) to F3 using the crown-down technique according to the manufacturer's instructions. The root canals were irrigated between the steps with saline solution. Finally, the root canals were irrigated with 5.25% NaOCl solution for 1 minute, 17% EDTA for 1 minute, and then the saline solution.

Five teeth were selected as positive controls, and five teeth as negative controls. Similar to a study by Haïkel et al, [15] the root apices in the negative control group were covered with two layers of nail varnish. In the positive control group, the root canals were only obturated with gutta-percha (Gapadent, Tianjin, China), without a sealer. The remaining teeth were randomly assigned to three groups (n=46) of (I) Endoseal MTA sealer (Maruchi, Wonju, Korea), (II) AH26 sealer (Dentsply, Baden, Switzerland), and (III) Sure-Seal Root sealer (Suredent, Seol, Korea). The root canals were obturated according to the manufacturers' instructions.

A bacterial leakage model was used to evaluate microbial microleakage. To this end, the teeth were transferred to a system used in a study by Pisano et al [16]. First, the tooth root and microtubes were separately autoclaved for sterilization. Then, the roots were passed through 1.5-mm microtubes (Eppendorf tubes) whose ends had been cut. To prevent percolation of the bacterial suspension at the root–microtube interface, the microtube ends were sealed with cyanoacrylate glue (Evo-Bond, Kaohsiung, Taiwan).

The tooth root and microtube assemblies were

placed under a UV lamp to prevent contamination. Then, the assemblies were transferred into 10-mL glass vials containing brain heart infusion broth culture medium (QUELAB, Canada) that had been autoclave-sterilized. To prevent the evaporation of the culture medium in glass vials, the microtube–vial outlet junction was closed with a Teflon band and glue. To ensure no contamination of the samples up to this step, the assemblies were incubated for 3 days (Fig. 1).

Fig 1. Bacterial leakage model

Absence of turbidity indicated the sterility of the assembly. Next, microleakage was evaluated (Fig. 2).

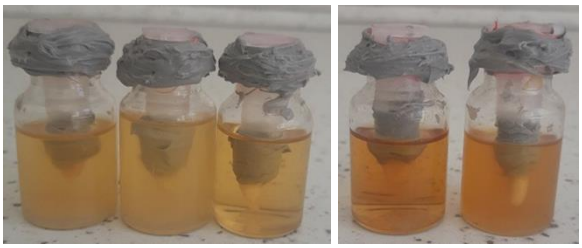

Fig 2. Samples placed into the nutrient solution during the follow-up; Right: turbidity of the broth as a sign of bacterial leakage; Left: no contamination.

In the next step, *Enterococcus faecalis* (ATCC29212) bacterial suspension was prepared at 0.5 McFarland standard concentration, containing 1.5×10^8 colony forming units per milliliter (CFU/mL). This suspension was injected into each microtube to cover the whole tooth surface. Then, the microtubes were incubated. The samples were evaluated daily for 60 days [3]. Upon observing a turbidity resulting from microbial leakage in a sample, the time was recorded for the respective sample, and it was excluded from the study.

Finally, the microleakage data for the three sealer groups were compared using the Chi-square test. The log-rank test was also used for the survival analysis. All statistical analyses were performed using SPSS version 24 (SPSS Inc., IL, USA) at 0.05 level of significance.

RESULTS

According to the results, 7 (15.2%) samples in the AH26 group, 9 (19.6%) in the Endoseal MTA group, and 10 (12.7%) in the Sure-Seal Root group exhibited microleakage (Table 1).

Table 1. Comparison of bacterial microleakage among the three sealer groups

Sealer	Bacterial microleakage		P-value
	Yes	No	
AH26	9 (19.6%)	37 (80.4%)	0.718
Endoseal MTA	7 (15.2%)	39 (84.8%)	
Sure-Seal Root	10 (21.7%)	36 (78.3%)	

The Chi-square test (Table 1) did not show any significant difference in bacterial microleakage among the three groups (P=0.718).

Survival time was defined as days free from turbidity (leakage). Samples that did not show turbidity by day 60 were treated as right-censored at 60 days. Mean leakage-free times and the number at risk at prespecified intervals are reported in Table 2.

Table 2. Survival status of the samples

Group	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
AH26	53.854	2.107	49.724	57.985
Endoseal	50.781	2.393	46.091	55.471
Sure seal	46.395	2.566	41.366	51.425

Std: standard

The result of the log-rank test showed the equality of survival distribution in the study groups. There was no significant difference in survival among the study groups (P=0.074).

DISCUSSION

Using a bacterial leakage model, the present study found that although the highest bacterial microleakage was recorded in the Sure-Seal Root sealer group, and the lowest in the AH26 sealer group, there were no significant difference in bacterial microleakage among the three sealer groups. We used the log-rank test because our primary outcome was time to bacterial leakage (days to turbidity) and several specimens remained leakage-free at the end of the 60-day observation period. The log-rank test compares the survival distributions across groups over the entire follow-up period. In our study the log-rank test showed no statistically significant difference in leakage-free survival among the three sealers (log-rank χ^2 = 5.211; df = 2; P = 0.074). Numerically, the AH26 group exhibited the longest mean leakage-free period (53.9 days) compared with Endoseal MTA (50.8 days) and Sure-Seal Root (46.4 days). These differences, while suggestive of a trend favoring AH26, did not reach conventional statistical significance at $\alpha = 0.05$. Clinically, a modest prolongation in leakage-free time in vitro may indicate slightly improved short-term resistance to bacterial ingress; however, the small absolute differences and the in vitro design limit direct extrapolation to patient outcomes. Therefore, although AH26 displayed the longest mean leakage-free interval, we recommend caution in translating this finding into clinical practice without corroboration from further studies. Additional investigations with larger sample sizes, longer follow-up, and complementary outcome measures would help determine whether the observed differences have meaningful effects on treatment durability. The present results were in line with those of Thakur et al, [17] who reported that MTA-based sealers exhibited clinical and radiologic efficacy similar to epoxy resin sealers in endodontic treatment, with no significant difference in the treatment outcomes. Forghani et al. [11] showed no significant difference in apical microleakage of root canals obturated with AH-Plus sealer and MTA Fillapex MTA-based sealer at any of the evaluated intervals (2 weeks and 3 months after sealing the root canals). Also Sonmez et al. [12] showed no significant difference in dye microleakage between AH-Plus and ProRoot MTA MTA-based sealer, reporting that both sealers exhibited optimal performance in creating an apical seal.

Inconsistent with the present findings, Asawaworarit et al. [18] reported that bioceramic sealers had a significantly better sealing ability than epoxy resin-based sealers. According to a study by Al-Ashou et al, [19] Sure-Seal Root sealer showed the best apical seal compared with AH Plus and GuttaFlow2. Although it is difficult to sort out these inconsistent results, the difference in results may be partly clarified by considering the differences in methodologies of the studies.

Different materials and methods are used to evaluate apical leakage, including endotoxin penetration [20], human saliva [21], fluid percolation [22,23], dye penetration [24], and bacterial penetration [25]. Although the dye penetration technique is the most commonly used method for evaluating the quality of root-end filling materials [26], in the present study, microleakage was evaluated using a bacterial model because it properly simulates the

clinical conditions of endodontic failure. The dye penetration technique has a high technical sensitivity and requires accurate standardization. In addition, in the dye penetration technique, the samples should be sectioned, there are many scoring systems for evaluation of microleakage, and evaluations are usually made by several observers, increasing the subjectivity of the results [27]. The findings of dye penetration studies evaluating bacterial microleakage can be different from reality because of the smaller molecular size of dyes than bacteria. On the other hand, the potential existence of voids and prevention of dye penetration could potentially compromise the findings of studies that use this method [28].

The present study evaluated AH26, Endoseal MTA, and Sure-Seal Root sealers. AH26 is the most commonly used epoxy resin sealer, and it is claimed that it has excellent sealing ability in terms of apical microleakage [29,30]. However, it has been reported that a lack of bonding between AH26 sealer and gutta-percha might increase microleakage [29]. In addition, apart from a proper working time, this sealer flows well and properly seals the dentinal walls. Generally, AH26 sealer has favorable properties, including adhesion to dental tissues, long working time, good seal, low solubility, low shrinkage, good biocompatibility, and antibacterial effects [31]. However, it has some disadvantages, including formaldehyde release, discoloration, and long setting time [32].

Endoseal MTA and Sure-Seal Root are bioceramic sealers. Bioceramic sealers do not shrink during setting, which increases their sealing ability [8]. In addition, bioceramic sealers can form chemical bonds with the root canal wall; this mechanical interlocking decreases microleakage [33,34]. Endoseal MTA is a MTA-based sealer. The main components of MTA are tricalcium aluminate, tricalcium oxide, and silicate oxide. The hydrophilicity of MTA leads to the formation of a colloidal gel, which sets in less than 4 hours, creating a strong, impermeable barrier. The impermeability of MTA is attributed to its hydrophilic nature and its minor expansion during the setting reaction [35]. One of the disadvantages of MTA is its long setting time [36]. Endoseal MTA and Sure-Seal Root, representing the bioceramic category, performed comparably in limiting bacterial ingress. Despite their beneficial biological characteristics, factors such as material solubility and potential dimensional alterations during the setting process may negatively influence their long-term sealing efficiency [37].

This study had some limitations that must be acknowledged. In vitro leakage models are unable to fully simulate the dynamic oral environment, and methodological variations across studies reduce the possibility of precise comparison. Additionally, treatment outcomes are influenced by a variety of factors, including the obturation technique applied, the clinician's expertise, and the complexity of root canal anatomy.

CONCLUSION

The results of this in vitro study showed that Endoseal MTA, AH26, and Sure-Seal Root sealers had a similar performance regarding apical microleakage. However, considering the importance of this clinical subject in success of root canal treatment, further studies are necessary for better decision-making.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Rai K, Mandhotra P, Sharma N, Patil L, Sharma A, Singh S. In vitro Assessment of Apical Microleakage of Teeth Sealed with Three Different Root Canal Sealers: A Comparative Study. J Pharm Bioallied Sci. 2021 Jun;13(Suppl 1):S375-S378.
2. Kaur A, Shah N, Logani A, Mishra N. Biotoxicity of commonly used root canal sealers: A meta-analysis. J Conserv Dent. 2015 Mar-Apr;18(2):83-8.
3. Kelmendi T, Koçani F, Kurti A, Kamberi B, Kamberi A. Comparison of Sealing Abilities Among

Zinc Oxide Eugenol Root-Canal Filling Cement, Antibacterial Bioceramic Paste, and Epoxy Resin, using Enterococcus faecalis as a Microbial Tracer. Med Sci Monit Basic Res. 2022 Jun 1;28:e936319.
4. Watson TF, Atmeh AR, Sajini S, Cook RJ, Festy F. Present and future of glass-ionomers and calcium-silicate cements as bioactive materials in dentistry: biophotonics-based interfacial analyses in health and disease. Dent Mater. 2014 Jan;30(1):50-61.
5. Li X, De Munck J, Van Landuyt K, Pedano M, Chen Z, Van Meerbeek B. How effectively do hydraulic calcium-silicate cements re-mineralize demineralized dentin. Dent Mater. 2017 Apr;33(4):434-445.
6. Chybowski EA, Glickman GN, Patel Y, Fleury A, Solomon E, He J. Clinical Outcome of Non-Surgical Root Canal Treatment Using a Single-cone Technique with Endosequence Bioceramic Sealer: A Retrospective Analysis. J Endod. 2018 Jun;44(6):941-945. doi: 10.1016/j.joen.2018.02.019. Epub 2018 Mar 29. Erratum in: J Endod. 2018 Jul;44(7):1199. doi: 10.1016/j.joen.2018.06.001.
7. Vouzara T, Dimosiari G, Koulaouzidou EA, Economides N. Cytotoxicity of a New Calcium Silicate Endodontic Sealer. J Endod. 2018 May;44(5):849-852.
8. Carvalho CN, Grazziotin-Soares R, de Miranda Candeiro GT, Gallego Martinez L, de Souza JP, Santos Oliveira P, et al. Micro Push-out Bond Strength and Bioactivity Analysis of a Bioceramic Root Canal Sealer. Iran Endod J. 2017 Summer;12(3):343-348.
9. Taylor JK, Jeansonne BG, Lemon RR. Coronal leakage: effects of smear layer, obturation technique, and sealer. J Endod. 1997 Aug;23(8):508-12.
10. Wu MK, De Gee AJ, Wesselink PR. Leakage of AH26 and Ketac-Endo used with injected warm gutta-percha. J Endod. 1997 May;23(5):331-6.
11. Forghani M, Bidar M, Sadeghalhoseini N, Naghavi N, Attaran Mashhadi N. In Vitro Evaluation of Apical Microleakage of MTA Fillapex, iRoot SP and AH-Plus. J Mashhad Dent Sch. 2014 Sep;38(3):1-2.
12. Sönmez IS, Oba AA, Sönmez D, Almaz ME. In vitro evaluation of apical microleakage of a new MTA-based sealer. Eur Arch Paediatr Dent. 2012 Oct;13(5):252-5.
13. Assmann E, Scarparo RK, Böttcher DE, Grecca FS. Dentin bond strength of two mineral trioxide aggregate-based and one epoxy resin-based sealers. J Endod. 2012 Feb;38(2):219-21.
14. Milani AS, Kuzegari S, Zand V, Mokhtari H, Rahbar M. Ability of Calcium Silicate and Epoxy Resin-based Sealers to Fill the Artificial Lateral Canals in the Presence or Absence of Smear Layer. Maedica (Bucur). 2021 Sep;16(3):458-462.
15. Haïkel Y, Wittenmeyer W, Bateman G, Bentaleb A, Allemann C. A new method for the quantitative analysis of endodontic microleakage. J Endod. 1999 Mar;25(3):172-7.
16. Pisano DM, DiFiore PM, McClanahan SB, Lautenschlager EP, Duncan JL. Intraorifice sealing of gutta-percha obturated root canals to prevent coronal microleakage. J Endod. 1998 Oct;24(10):659-62.
17. Thakur S, Emil J, Paulaian B. Evaluation of mineral trioxide aggregate as root canal sealer: A clinical study. J Conserv Dent. 2013 Nov;16(6):494-8.
18. Asawaworarit W, Pinyosopon T, Kijsamanmith K. Comparison of apical sealing ability of bioceramic sealer and epoxy resin-based sealer using the fluid filtration technique and scanning electron microscopy. J Dent Sci. 2020 Jun;15(2):186-192.
19. Al-Ashou WMO, Al-Shamaa RM, Hassan SS. Sealing Ability of Various Types of Root Canal Sealers at Different Levels of Remaining Gutta Percha After Post Space Preparation at Two Time Intervals. J Int Soc Prev Community Dent. 2021 Nov 30;11(6):721-728.
20. Stabholz A, Friedman S, Abed J. Marginal adaptation of retrograde fillings and its correlation with sealability. J Endod. 1985 May;11(5):218-23.
21. Al-Hezaimi K, Naghshbandi J, Oglesby S, Simon JH, Rotstein I. Human saliva penetration of root canals obturated with two types of mineral trioxide aggregate cements. J Endod. 2005 Jun;31(6):453-6.
22. Lamb EL, Loushine RJ, Weller RN, Kimbrough WF, Pashley DH. Effect of root resection on the apical sealing ability of mineral trioxide aggregate. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003 Jun;95(6):732-5.
23. Martin RL, Monticelli F, Brackett WW, Loushine RJ, Rockman RA, Ferrari M, et al. Sealing properties of mineral trioxide aggregate orthograde apical plugs and root fillings in an in vitro apexification model. J Endod. 2007 Mar;33(3):272-5.
24. Post LK, Lima FG, Xavier CB, Demarco FF, Gerhardt-Oliveira M. Sealing ability of MTA and amalgam in different root-end preparations and resection bevel angles: an in vitro evaluation using marginal dye leakage. Braz Dent J. 2010;21(5):416-9.
25. Yildirim T, Er K, Taşdemir T, Tahan E, Buruk K, Serper A. Effect of smear layer and root-end cavity thickness on apical sealing ability of MTA as a root-end filling material: a bacterial leakage study. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Jan;109(1):e67-72.
26. Valois CR, Costa ED Jr. Influence of the thickness of mineral trioxide aggregate on sealing ability of root-end fillings in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004 Jan;97(1):108-11.

27. Taylor MJ, Lynch E. Microleakage. J Dent. 1992 Feb;20(1):3-10.
28. Afkhami F, Nasri S, Valizadeh S. Bacterial leakage assessment in root canals sealed with AH Plus sealer modified with silver nanoparticles. BMC Oral Health. 2021 Nov 12;21(1):577.
29. 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 Sep;94(3):214-8.
30. Naulakha D, Hussain M, Alam M, Howlader M. An in vitro dye leakage study on apical microleakage of root canal sealers. J Nepal Dent Assoc. 2011;12(1):33-9.
31. Ørstavik D, Nordahl I, Tibballs JE. Dimensional change following setting of root canal sealer materials. Dent Mater. 2001 Nov;17(6):512-9.
32. YaghotiKhorasani M, Norozzadeh Z. Comparison of apical seal of three root canal sealers (AH26, AHPlus and Tubliseal) in root canal obturated by lateral condensation technique. J Mashhad Dent Sch. 2012 Oct;36(3):249-58.
33. Han L, Okiji T. Bioactivity evaluation of three calcium silicate-based endodontic materials. Int Endod J. 2013 Sep;46(9):808-14.
34. Jardine AP, Rosa RA, Santini MF, Wagner M, Só MV, Kuga MC, Pereira JR, Kopper PM. The effect of final irrigation on the penetrability of an epoxy resin-based sealer into dentinal tubules: a confocal microscopy study. Clin Oral Investig. 2016 Jan;20(1):117-23.
35. Torabinejad M, Rastegar AF, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root-end filling material. J Endod. 1995 Mar;21(3):109-12.
36. Rosa RA, Bier CAS, Só MVR, Tanomaru JMG, Tanomaru-Filho M. Push-out bond strength of Calcium hydroxide and Mineral Trioxide Aggregate based sealers to root canal dentin. Rev Odonto Cienc. 2012 Oct;27(4):320-4.
37. Dastorani M, Malekpour B, AminSobhani M, Alemrajabi M, Mahdian A, Malekpour B. Comparison of bacterial microleakage of three bioactive endodontic sealers in simulated underwater diving and aviation conditions. BMC Oral Health. 2021 Jul 15;21(1):345.