CJDR
Caspian Journal of Dental Research
Caspian Journal of Dental Research
e-ISSN: 2322-2395 p-ISSN: 2251-9890
Babol University Of Medical Sciences

Comparison of Bacterial Microleakage in Root Canals Filled with AH26 and Dia-Proseal Resin Sealers: An In Vitro Study

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Article type

Research Paper

Received: 2 Jun 2025
Revised: 29 Jun 2025
Accepted: 13 Jul 2026
Pub. online: 9 Aug 2026

ABSTRACT

Introduction: The primary objective of root canal treatment is to achieve three-dimensional obturation of the canal system and establish an effective seal to prevent bacterial contamination. Because resistance to microleakage is a critical property of endodontic sealers, this study aimed to compare bacterial microleakage in roots obturated with Dia-Proseal and AH26 sealers.

Materials & Methods: In this in vitro study, 60 sound, extracted human single-rooted teeth with a single canal were randomly assigned to two experimental groups (n = 25 each) and two control groups (positive and negative; n = 5 each). Following canal preparation, the experimental groups were obturated with gutta-percha and either Dia-Proseal or AH26 sealer. In the negative control group, the roots were obturated with gutta-percha and AH26 sealer, and the entire external root surface was completely coated with nail varnish. In the positive control group, the canals were filled with gutta-percha alone, without sealer. A custom-designed two-chamber apparatus was used to evaluate bacterial microleakage. The coronal portion of each root was positioned in the upper chamber containing an Enterococcus faecalis suspension. In contrast, the apical portion was immersed in Tryptic Soy Broth (TSB) in the lower chamber. The samples were incubated for 90 days and compared between the two experimental groups with respect to the time to onset and frequency of microleakage.

Results: No statistically significant difference was observed between the Dia-Proseal (56.2 ± 7.04 days) and AH26 (60.76 ± 7.02 days) groups in terms of the time to onset of bacterial microleakage. Similarly, the difference in the frequency of bacterial microleakage at the end of the 90-day period between the AH26 group (56%) and the Dia-Proseal group (48%) was not statistically significant.

Conclusion: Given the lack of difference in the sealing ability of the two materials studied, the sealer that is more readily available and suitable can be used.

Keywords: Endodontics, Epoxy Resins, Root Canal Filling Materials, Root Canal Therapy

Cite this article: Darjani S, Harandi A, Davoodabadi A, Khafri S, Mahmoudi E. Comparison of Bacterial Microleakage in Root Canals Filled with AH26 and Dia-Proseal Resin Sealers: An In Vitro Study. Caspian J Dent Res. 2025; 14: 67-76.

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Publisher: Babol University of Medical Sciences

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Introduction

Achieving three-dimensional obturation of the root canal system and an adequate seal to prevent bacterial contamination is a primary objective of root canal therapy. [1-3] Despite the variety of materials used for root canal filling, gutta-percha combined with a sealer remains the most widely applied option. [4, 5] Gutta-percha offers several advantages, including favorable physical and chemical properties, biocompatibility, and non-toxicity. It is also relatively easy to remove and flexible, allowing for various application techniques. [6, 7] However, gutta-percha's inability to adhere to dentin and its suboptimal sealing properties require its use with a sealer to achieve effective root canal obturation. [8, 9]

The primary role of a root canal sealer is to bond the root filling material to the dentin, sealing accessory canals and gaps between the gutta-percha points and the root canal walls. An ideal sealer should be biocompatible, non-toxic, radiopaque, easy to use, resistant to dissolution in tissue fluids, dimensionally stable, and capable of bonding strongly to the root canal walls. This ensures the formation of a tight seal that prevents leakage and reduces the risk of bacterial infiltration. [10, 11] The sealer's penetration into the dentinal tubules further creates a physical barrier that encapsulates residual bacteria and enhances mechanical retention by increasing the contact area between the root canal walls and the filling material. [12, 13]

Various types of endodontic sealers are available, including zinc oxide-eugenol, epoxy resin, glass ionomer, calcium hydroxide, silicone, and methacrylate-based sealers. [3] Recent reviews indicate that resin-based and bioceramic sealers have comparable physicochemical properties, sealing ability, dentinal tubule penetration, and antibacterial properties. [14-16] However, several studies have highlighted the superior performance of resin-based sealers in preventing apical microleakage. [17-21]

AH-26, a widely used epoxy resin-based sealer, is known for its favorable properties such as ease of mixing, extended working time, radiopacity, antimicrobial activity, and excellent sealing capability. However, it has certain drawbacks, including potential discoloration, limited solubility in solvents, relative toxicity before setting, and partial solubility in oral fluids. The working time of AH-26 is approximately 4–5 hours, with a final setting time of 24–48 hours. [22, 23]

Dia-Proseal, another resin-based sealer, has gained popularity due to its cost-effectiveness. It is characterized by low shrinkage, optimal flow, and radiopacity, although careful mixing is required. Dia-Proseal contains calcium hydroxide, which is believed to contribute to its antimicrobial properties by elevating pH levels. The working time for this sealer is 1 hour, and its setting time is approximately 7 hours. It is a two-paste system that is quick and easy to prepare, generating minimal waste. [20]

Among the various methods for evaluating the sealing ability of endodontic sealers, bacterial microleakage assessment is considered one of the most reliable and accurate techniques, as it more closely mirrors clinical conditions. [24] Therefore, the aim of the present study was to compare the bacterial microleakage of Enterococcus faecalis, the most commonly isolated bacterium from infected root canals, in teeth sealed with two resin-based materials: AH-26 and Dia-Proseal. [1, 25]

Materials & Methods

The protocol for this *in vitro* study was approved by the Ethics Committee of Babol University of Medical Sciences (Code: IR.MUBABOL.HRI.REC.1398.025).

Sample Selection and Preparation

Human single-rooted, single-canal extracted teeth with complete, straight roots and fully developed apices were selected. The teeth were evaluated clinically and radiographically, and teeth with vertical or horizontal fractures, additional canals, severe curvature, internal or external resorption, or calcification were excluded. [26]

The crowns were sectioned using a high-speed turbine and diamond bur, leaving a 12-mm root length. The working length was determined with a #15 K-file (Maillefer Dentsply, Ballaigues , Switzerland), set 1 mm shorter than the apical foramen. Root canal preparation was performed with Ni-Ti rotary files (Protaper Universal system, Dentsply Maillefer, Ballaigues, Switzerland). Instrumentation began with files SX, S1, and S2, followed by F1, F2, and F3 files for apical preparation. To maintain canal patency, the canals were irrigated with 5.25% sodium hypochlorite (Morvabon, Rey, Iran) and 0.9% sterile saline (Samen, Mashhad, Iran). A #10 K-file was used to remove debris from the apical region. After preparation, the samples were sterilized using ethylene oxide gas. [26]

Group Allocation

The samples were randomly assigned to four groups:

- Test Group 1 (n=25): Obturation with gutta-percha and AH26 sealer (De Trey, Dentsply, Konstanz, Germany).
- Test Group 2 (n=25): Obturation with gutta-percha and DIA-PROSEAL sealer (DiaDent, Seoul, Korea).
- Positive control group (n=5): Obturation with gutta-percha alone, without any sealer.
- Negative control group (n=5): Obturation with gutta-percha and AH26 sealer; additionally, the entire external root surface was completely coated with nail varnish.

In all groups, gutta-percha (Mani, Utsunomiya, Japan) was used, and the sealers were prepared according to the manufacturer's instructions. Root canal fillings were performed using a finger spreader (Maillefer Dentsply, Ballaigues, Switzerland) with the lateral condensation technique. The quality of obturation was verified by radiographic evaluation. The samples were then stored at 100% humidity and 37°C for one week to allow complete settings of the sealers.

The external surfaces of the roots, except for the apical 2 mm, were coated with two layers of nail varnish (Arcancil, Paris, France) in all experimental and positive control groups. In the negative control group, the entire external root surface, including the apical foramen and canal orifice, was coated. [26]

Preparation of Bacterial Microleakage Test Samples

To create a chamber in the coronal part of the roots, 0.5-mL microtubes with an internal diameter of 7 mm and a height of 30 mm were used. The ends of the microtubes were trimmed, and each tooth was inserted into the microtubes so that one-third of the apical root remained exposed. The interface between the root and the microtube was sealed with cyanoacrylate glue (Razi Co., Tehran, Iran).

For the apical chamber, 2-mL glass vials (diameter: 10 mm; height: 50 mm) were used. A 7-mm hole was drilled in the center of each vial cap using an electric drill. The microtubes containing the specimens were inserted through the holes in the caps, and the junction between the microtube and the vial cap was sealed with cyanoacrylate glue. The microtubes containing the specimens were inserted through the holes in the caps, and the junction between the microtube and the cap was sealed with cyanoacrylate adhesive. The cap, with the attached microtube and root specimen, was then secured onto the vial, forming a two-chamber system consisting of an upper and a lower compartment (Figure 1).

Figure 1: The custom-designed chamber system for evaluating bacterial microleakage

The entire assembly was sterilized with ethylene oxide gas and subsequently sealed in polyethylene packaging. [27]

Bacterial Microleakage Test

TSB was added to the lower chamber (vial) of each sample, ensuring the apical 2 mm of the root was submerged in the culture medium. To confirm sterility, the samples were incubated at 37°C for 3 days; the absence of turbidity in the culture medium indicated no contamination.

After confirming sterility, 0.3 mL of a bacterial suspension containing Enterococcus faecalis (ATCC 29212) at a concentration of 10^9 CFU/mL was inoculated into the upper chamber using a sterile syringe. The bacterial suspension was refreshed every 3 days to maintain viable microorganisms. The samples were incubated at 37°C for 90 days and monitored daily for turbidity in the culture medium, which would indicate bacterial penetration (Figure 2).

Figure 2: Bacterial microleakage caused turbidity in the culture medium in contact with the root apex (in the lower chamber).

If turbidity appeared, a sample was cultured on blood agar to confirm the presence of Enterococcus faecalis. [27]

Statistical Analysis

Data were analyzed using SPSS 26 (IBM, Armonk, USA). Kaplan-Meier survival analysis was used to compare the time to bacterial contamination between the experimental groups. The Chi-square test compared the frequency of bacterial contamination. A significance level of 0.05 was set for all statistical tests.

Results

The negative control group exhibited no evidence of bacterial microleakage throughout the 90-day study period. In contrast, all specimens in the positive control group demonstrated bacterial microleakage within the first week (Figure 2).

Frequency of Bacterial Microleakage

Table 1 summarizes the frequency of bacterial microleakage observed during the study period, categorized into 30-day intervals. At the end of the study, no statistically significant difference was observed in the frequency of bacterial microleakage between the AH26 group (56%) and the Dia-Proseal group (48%).

Table 1: Frequency of bacterial microleakage during the experimental period, categorized by 30-day intervals

Sealer \ Days	1-30 n (%)	31-60 n (%)	61-90 n (%)	1-90 n (%)	P-value*
AH26	8 (32%)	4 (16%)	2 (8%)	14 (56%)	0.57
Dia-Proseal	8 (32%)	2 (8%)	2 (8%)	12 (48%)	

*Chi-square test

Time to Bacterial Microleakage

Table 2 presents the mean and median time to bacterial microleakage in both the Dia-Proseal and AH26 groups. The mean time for the AH26 group was 56.2 days (SD = 7.04),

while the mean for the Dia-Proseal group was 60.76 days (SD = 7.02). The median time was 65 days for AH26 and 81 days for Dia-Proseal.

Table 2: Mean and median time to bacterial microleakage in the experimental groups

Sealer	Mean	Standard Deviation	95% CI for the Mean		Median
			Lower	Upper	
AH26	56.2	7.04	42.4	65	65
Dia-Proseal	60.76	7.02	47	81	81

Kaplan-Meier Survival Analysis

Kaplan-Meier survival analysis was used to compare the time to bacterial microleakage between the two groups. No statistically significant difference was found (Figure 3).

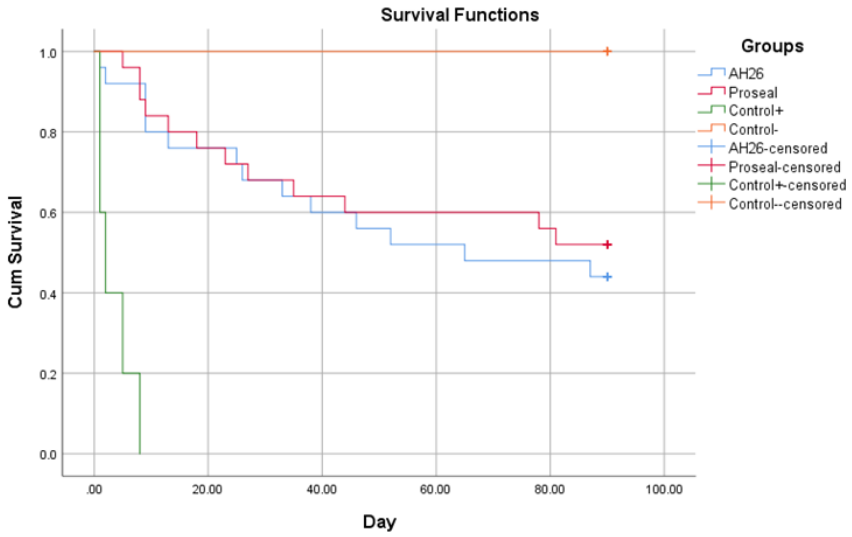

Figure 3: Kaplan-Meier survival curve comparing the time to bacterial microleakage between the AH26 and Dia-Proseal groups

Discussion

Statistical analysis revealed no significant difference in the frequency of bacterial microleakage between the two sealer groups at any of the three monthly intervals. In the first month, 32% of samples from both the AH26 and Dia-Proseal groups demonstrated microleakage. This rate decreased to 16% and 8% in the AH26 group during the second and third months, respectively. In contrast, the Dia-Proseal group maintained a consistent microleakage rate of 8% in both the second and third months. Over the entire 90-day period, 56% of AH26 samples and 48% of Dia-Proseal samples showed bacterial microleakage, while the remaining samples indicated no leakage.

Similar studies have reported variable rates of microleakage. Milani et al. reported leakage rates of 66% for AH26 and 93% for MTA Fillapex at 90 days. [18] Antunovic et al. observed rates of 14.2%, 78.6%, and 50% for Fill bioceramic, AH Plus, and MTA Plus, respectively, at

60 days. [28] Oliveira et al. found leakage in 33% of AH Plus samples and 61% of MTA Sealapex samples after one month. [29] Razavian et al. detected bacterial microleakage in 6% of AH26 samples and 28% of MTA Fillapex samples at 30 days. [19]

Furthermore, dye penetration studies have shown varied results. Vo et al. found the least microleakage with AH Plus, followed by Pulp Canal Sealer, NeoSEALER Flo, EndoSequence BC, and Super-Bond RC Sealer. [21] Soleymani et al. assessed apical leakage in root canals filled with AH-26 and MTA Fillapex under blood-contaminated conditions using a dye penetration method. Although AH26 showed the lowest apical leakage, the difference was not statistically significant. [30] Song et al. reported that AH Plus exhibited less leakage than Dia-Proseal and ADseal; however, the differences were not statistically significant. [20] Similarly, Yaghoti Khorasani and Norozzadeh concluded AH Plus performed better than AH26 without statistical significance. [31]

These variations highlight the complexities of evaluation, often due to methodological differences, duration, and materials. While some studies report less leakage with resin-based sealers, recent reviews find no significant difference between resin-based and bioceramic types, attributing discrepancies to sealer types, techniques, or methodologies. [15, 32]

Physicochemical properties such as polymerization shrinkage, solubility, and interfacial adaptation significantly impact sealing ability. [33] Resin-based sealers like AH26 have minimal solubility and strong adhesion, but shrinkage or poor flow can compromise the seal. Hydrophilicity and resistance to dissolution are also important for long-term sealing. [34]

Various techniques, including dye penetration, bacterial leakage, liquid filtration, and glucose infiltration, have been applied to assess the microleakage of sealers [35-41], but no standardized method has been universally adopted. Even within a specific technique, variations can lead to different results. [42] Among these methods, the bacterial model using Enterococcus faecalis is considered the most clinically relevant. [17, 43, 44]

The contamination observed in the positive control group samples during the first week underscores the critical importance of using effective sealers. The absence of contamination in the negative control group throughout the study demonstrates the reliability of the experimental model.

In the current study, both sealers performed similarly. The effectiveness of Dia-Proseal's performance may be attributed to its alkaline pH, antimicrobial properties, flowability, and adaptability. [20] Differences in filler particle size and resin viscosity between the two sealers may also account for the slight differences in leakage rates observed. [45] The 90-day duration corresponds with the typical leakage onset. [46] Future research should consider more precise models, such as evaluating saliva penetration or bacterial metabolites, and incorporate advanced analytical techniques, including electron microscopy, to further evaluate the sealing performance of endodontic sealers.

Conclusion

Given the lack of difference in the sealing ability of the two materials studied, the sealer that is more readily available and suitable can be used.

Acknowledgments

The authors express their sincere gratitude and appreciation to the staff of the Dental Materials Research Center and Microbiology Laboratory for their help during this study

Conflicts of Interest

The authors declare no potential conflict of interest with respect to the authorship and/or publication of this paper.

Author's Contribution

Shahab Darjani: Acquisition of data and drafting of the manuscript. Elham Mahmoudi: Study supervision, administrative, technical, and material support. Azadeh Harandi: Critical revision of the manuscript for important intellectual content. Abolfazl Davoodabadi: Analysis and interpretation of data. Soraya Khafri: Statistical analysis. All authors read and approved the final manuscript.

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