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Koichiro Inokuchi [†], [Naoya Higuchi](#) [†], [Masanori Fujita](#), [Takahiko Morotomi](#) ^{*}

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Article

Evaluation of the Sealing Ability of a Dual-Cure Self-Adhesive Methacrylate Resin Sealer Using a Novel Bacterial Leakage Test Model

Koichiro Inokuchi [†], Naoya Higuchi [†], Masanori Fujita and Takahiko Morotomi ^{*}

Department of Endodontics, School of Dentistry, Aichi Gakuin University, Nagoya, Japan

^{*} Correspondence: morotomi@dpc.agu.ac.jp; Tel.: +81-52-759-2111

[†] These authors contributed equally to this work.

Abstract

Background/Objectives: This study aimed to evaluate the bacterial invasion inhibitory ability of MetaSEAL Soft Paste, which is a root canal sealer that has good sealing properties in dye penetration tests, compared with conventional sealers, using a novel bacterial leakage test model. **Methods:** We compared MetaSEAL Soft Paste (methacrylate-based resin sealer) with Canals (zinc oxide-eugenol-based sealer), AH Plus Jet (epoxy-based resin sealer), and Dentalis NX (calcium hydroxide-based sealer). In the bacterial leakage tests, the center of the root of a bovine incisor was sliced horizontally. The area of the root canal prepared using a diamond point was filled with a sealer, and the specimen was placed on a brain heart infusion agar medium. Specimens with a filter paper disc containing *Enterococcus faecalis* were maintained until colonization was observed. The antibacterial properties of the sealers were evaluated using the agar diffusion test and the direct contact test. **Results:** AH Plus Jet tended to take the longest time to show colonies in the bacterial leakage test; however, this result was not significant compared with MetaSEAL Soft Paste. In the direct contact test, significantly fewer bacteria were detected in MetaSEAL Soft Paste and Canals than in AH Plus Jet or positive controls ($p < 0.01$). **Conclusions:** This study suggests that the novel bacterial leakage test model is effective for accurately evaluating the ability of sealers to prevent bacterial leakage, and further suggests that the sealing ability of MetaSEAL Soft Paste may not be superior to that of AH Plus Jet.

Keywords: root canal sealer; bacterial leakage test; MetaSEAL Soft Paste; AH Plus Jet

1. Introduction

The purpose of root canal filling is to prevent bacterial invasion into the aseptic root canal, which is achieved by root canal cleaning and disinfection (i.e., to prevent reinfection) and to entomb trace amounts of remaining bacteria in the root canal, including the dentinal tubules [1–3]. Coronal and apical leakage are the main causes of periapical lesions after root canal filling [4–9]. Root canal sealers improve sealing between the root canal wall and root canal filling materials and among filling materials themselves. Therefore, these sealers play an important role in maintaining an aseptic root canal and are considered essential materials for root canal filling [10–12]. Dye penetration and bacterial leakage tests have been mainly used to evaluate the sealing ability of root canal sealers [13–15]. The dye penetration test is relatively simple, but it does not use bacteria and cannot always provide an accurate assessment of bacterial invasion. In recent years, the fluid transport test has become widely used, allowing for quantitative evaluation, and is considered to be more accurate than the dye penetration test [16–18]. However, the equipment for the fluid transport test is complicated, with strict control of the conditions required, and similar to the dye penetration test, it does not actually use bacteria. The bacterial leakage test model currently used also has its weaknesses. Even greater care is required to prevent contamination in the bacterial leakage test, and the steps are even more complicated than those of the dye penetration test and the fluid transport test. Therefore,

evaluation of the sealing ability of each root canal sealer is affected by the test conditions and varies depending on each test [19,20]. The bacterial leakage test is often performed using the entire tooth root. This method can simulate an environment closer to that of clinical practice. However, differences between each evaluation are suggested to be due to anatomical variations, such as side branches and apical bifurcations, the experimental environment (e.g., variations in the prepared root canal shape and equipment attached to specimens), and the complexity of aseptic procedures including sterilization.

Currently, there is a wide variety of root canal sealer products in use. A zinc oxide-eugenol sealer, which was developed in the 1930s, has been widely used worldwide with some improvements since its invention [20,21]. However, several types of resin-based sealers have become more popular in recent years, especially in Europe and the United States. Eleazer *et al.* [22] reported that the most commonly used sealers by general dentists registered with the National Dental Practice-Based Research Network were zinc oxide-eugenol-based (43%), resin-based (40%), and calcium hydroxide-based (26%) sealers. Resin sealers are broadly divided into epoxy resin and methacrylate resin types. Among the epoxy resin sealers, AH Plus® (Dentsply Sirona Inc., Charlotte, NC, USA) is often used as the gold standard comparison when evaluating physical properties of other sealers, such as methacrylate resin sealers [16,20]. When methacrylate resin sealers come into contact with the root canal wall, a resin tag and resin-impregnated layer are formed at the interface, resulting in good adhesion [23–25]. MetaSEAL®/Hybrid Root Seal® (Sun Medical Company, Ltd., Shiga, Japan), which is methacrylate resin-based root canal sealer, is a dual-cure self-adhesive resin sealer containing 4-META. The sealing ability of this sealer when used in combination with gutta-percha or Resilon was evaluated in a fluid transport test, and no significant difference compared with AH Plus was found [16]. However, similar studies have also reported that the sealing ability of AH Plus is significantly higher than that of MetaSEAL ($p < 0.001$ for both studies) [17,18]. Previously, MetaSEAL was too hard to remove during retreatment. Therefore, MetaSEAL Soft was developed for easier removal during retreatment. Furthermore, MetaSEAL Soft Paste was developed as a paste instead of a powder–liquid product to prevent changes in physical properties due to mixing. A previous study by Ito *et al.* [26] reported that MetaSEAL Soft Paste had better sealing properties than other sealers, including AH Plus Jet, in dye penetration tests. However, the bacterial leakage properties of these sealers have not been compared using bacterial leakage tests.

Enterococcus faecalis is a gram-positive, facultative anaerobic coccus frequently detected in infected root canals with asymptomatic persistent periapical lesions and in infected root canals that require retreatment [27–29]. This bacterium is believed to be difficult to remove or disinfect through standard root canal treatment because: 1) it can penetrate deep into the dentinal tubules; 2) it is resistant to alkalinity; 3) it can survive in nutrient-poor, starved environments; and 4) it has the ability to form biofilms, making various antibacterial agents less effective against it [28,29].

This study aimed to establish a novel bacterial leakage test model using *E. faecalis*. We also aimed to compare the ability of various commonly used sealers, including zinc oxide eugenol-based, epoxy resin-based, calcium hydroxide-based, and methacrylate resin-based sealers, to prevent bacterial leakage.

2. Materials and Methods

We performed a series of experiments. In these experiments, the equipment that came into direct contact with the specimens was sterile and disposable or sterilized by autoclave or gas sterilization using ethylene oxide gas. The sealers evaluated in this study are shown in Table 1.

Table 1. List of sealers used in the tests.

Type	Product name	Composition	
	(manufacture, country)		
Zinc oxide-eugenol	Canals® (GC SHOWAYAKUHIN, Japan)	Powder	Zinc oxide, Barium sulfate, Bismuth subcarbonate, Rosin
		Liquid	Clove oil, Olive oil
Epoxy resin	AH Plus Jet® (Dentsply Sirona, Germany)	Paste A	Bisphenol A epoxy resin, Zirconium oxide, Bisphenol F epoxy resin, Calcium tungstate, Iron oxide, Silica
		Paste B	N,N-dibenzyl-5-oxanonadiamin-1.9, Amantameamine, Tricyclodecane-diamine, Calcium tungstate, Zirconium oxide
Calcium Hydroxide	Dentalis® NX (Neo Dental Chemical Products, Japan)	Powder	Zinc oxide, Calcium hydroxide, Other
		Liquid	Eugenol, Other
Methacrylate resin	MetaSEAL® Soft Paste (Sun Medical, Japan)	Base Paste	Methacrylate esters (HEMA, 4-META, others), water, Organic fillers, others
		Catalyst Paste	Methacrylate esters (HEMA, others), X-ray contrast agents, polymerization initiator, other

2.1. Bacterial Leakage Test

A schematic diagram of the bacterial leakage test model used in this study is shown in Figure 1. Thirty-six specimens were prepared, 12 of which were used for each test, and each test was carried out 3 times. Three of each of the four sealer types were randomly assigned to each test. The root of a bovine incisor was cut horizontally in the center of the root to a thickness of 3 mm, and the inner diameter was prepared to a diameter of 3 mm using a diamond point (Horiko® Sinter diamond point SHM111031; Hopf, Ringleb & Co. GmbH, Berlin, Germany). Ultrasonic cleaning was performed for 1 minute each using a 3%–6% sodium hypochlorite solution (DENTAL ANTIFORMIN; Nippon Dental Pharmaceuticals Co., Ltd., Yamaguchi, Japan), a 3% EDTA solution (Smearclean; Nippon Dental Pharmaceuticals Co., Ltd.), and sterile saline solution (Otsuka Saline Injection; Otsuka Pharmaceutical Factory, Tokushima, Japan). Thereafter, the specimens were sterilized in an autoclave (121°C, 15 minutes) and then immersed in sterile physiological saline for 24 hours. Thereafter, excess moisture was removed from the surface of the specimen with sterile KimWipes® (Kimberly-Clark Corp., Irving, TX, USA). Each sealer was mixed in the proportions specified by the manufacturer, and then it was inserted into the prepared root canal using a syringe (EndNozzle; Sun Medical Co., Ltd., Shiga, Japan). The specimens were stored at 37°C and 100% humidity. Eight hours after filling, the excess sealer was cut off with a surgical blade (FEATHER® No. 11; Feather Safety Razor Co., Ltd., Osaka, Japan) to flatten the sealer surface of the specimen. The sealer-filled specimens were hardened again under the same conditions for up to 24 hours after filling, and they were placed on brain heart infusion (BHI) agar medium (Difco™ Brain Heart Infusion Agar; Becton Dickinson and Company, Franklin Lakes, NJ, USA). A filter paper disc (4 mm in diameter, thickness of 1.5 mm, PAPER DISC ADVANTEC®; Toyo Roshi Kaisha, Ltd., Tokyo, Japan) containing 20 µl of *E. faecalis* (ATCC19433) bacterial suspension (1.0 × 10⁷ CFU/ml) was placed on the sealer (3 mm in diameter) on top of the specimen (Figure 1). The presence or absence of colony formation around the specimen was checked every 24 hours, and the filter paper was replaced every 48 hours.

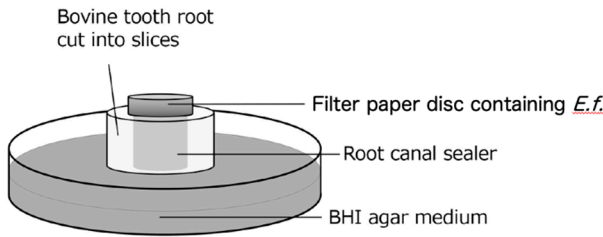


Figure 1. Schematic diagram of the bacterial leakage test model.

2.2. Antibacterial Test

2.2.1. Agar Diffusion Test

Thirty-six cylindrical polypropylene molds (diameter: 7 mm, height: 3 mm) were prepared to be filled with the sealers, 12 of which were used in each test, and the test was carried out three times. Three polypropylene molds were used for each of the four types of sealers in each test. Each sealer was injected into the polypropylene mold using the syringe, and it was stored at 37°C and 100% humidity. In the bacterial leakage test, the excess sealer was cut off and removed, and the specimen was allowed to harden for 24 hours under the same conditions. The turbidity of the *E. faecalis* solution (1.0×10^7 CFU/ml, 100 μ l) was adjusted using a spectrophotometer and applied to the surface of the BHI agar medium, after which the specimens were left to stand. After culturing for 24 hours under the same conditions aerobically, the diameter of the inhibition zone formed around the sealer was measured at three random locations, and the average of the three measurements was taken.

2.2.2. Direct Contact Test

A fixed amount (80 μ l) of each sealer was injected into a 96-well microplate using a syringe and allowed to harden for 24 hours under 37°C and 100% humidity. The prepared *E. faecalis* bacterial solution (1.0×10^7 CFU/ml, 200 μ l) was poured into each well and cultured aerobically for 24 hours, after which the bacterial solution was collected. Serial dilutions were made and plated on BHI agar medium. After 24 hours of incubation, the colonies were counted, and the number of viable bacteria was calculated. A positive control group containing only the bacterial solution without any sealer filling was added. Three wells were used for each sealer and control group per test, and the test was carried out three times.

2.3. Statistical Analysis

SPSS software (version 26; IBM SPSS Inc., Armonk, NY, USA) was used to analyze the data. The Kruskal–Wallis test was performed to examine whether there were significant differences in the bacterial leakage test and the antibacterial test. The level of significance was set at $\alpha = 0.05$.

3. Results

3.1. Bacterial Leakage Test

As shown in Kaplan–Meier survival curves (Figure 2a), colony formation was observed in 10 to 15 days with Dentalis NX. Additionally, colony formation was observed in all specimens of Canals and MetaSEAL Soft Paste at approximately 30 days. However, colony formation was not observed until 54 days in all specimens of AH Plus Jet. The mean number of days required for colony formation in each sealer is shown in Figure 2b. The mean duration required for colony formation was significantly longer for AH Plus Jet than for Canals and Dentalis NX ($p < 0.01$), and longer for MetaSeal Soft Paste than for Dentalis NX ($p < 0.01$) (Figure 2b). No significant difference in the mean duration required for colony formation was observed between MetaSEAL Soft Paste and AH Plus Jet or between MetaSEAL Soft Paste and Canals, but AH Plus Jet tended to take the longest time to observe colony formation (Figure 2b).

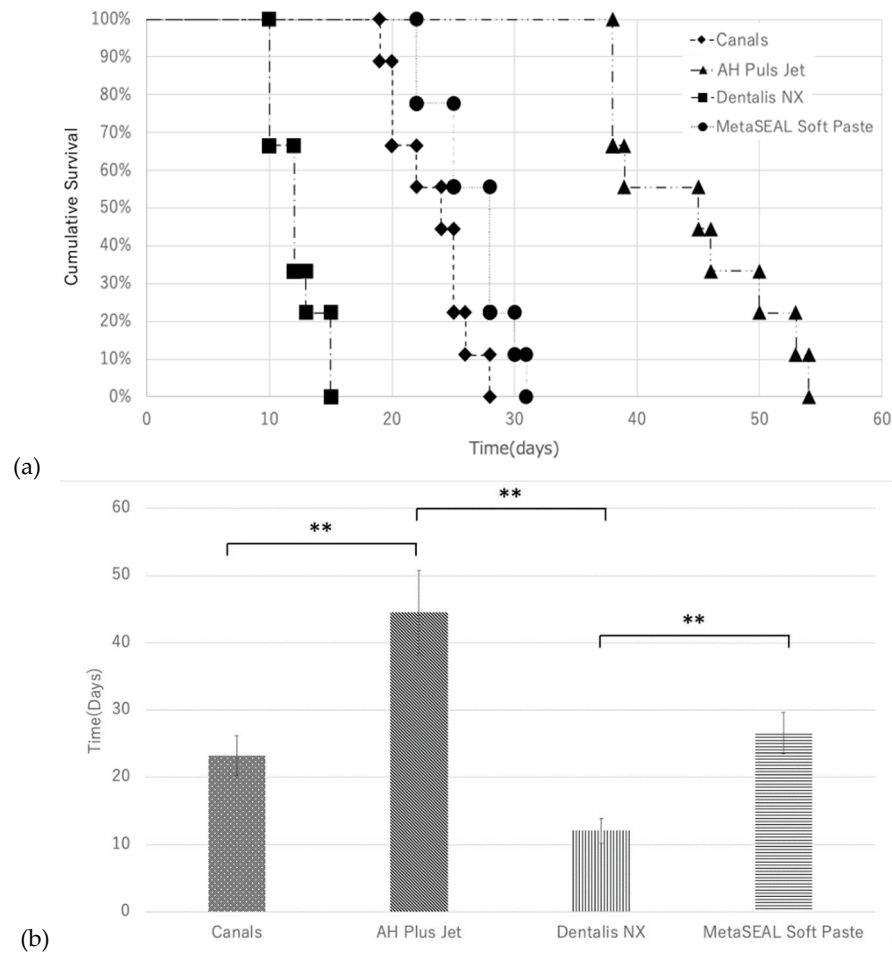
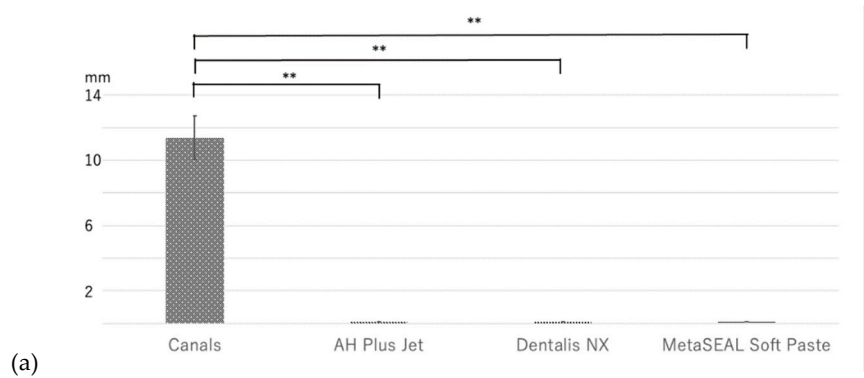


Figure 2. Comparison of preventing bacterial leakage between the various sealers. (a) Kaplan–Meier survival curves of bacterial leakage between various sealers; (b) comparison of the mean number of days required for colony formation between various sealers. ** $p < 0.01$.

3.2. Antibacterial Test

In the agar diffusion test (ADT), an inhibition zone was observed with Canals, but this was not observed with AH Plus Jet, Dentalis NX, or MetaSEAL Soft Paste (Figure 3a). In the direct contact test (DCT), significantly fewer bacteria were detected in MetaSEAL Soft Paste and Canals than in positive controls ($p < 0.01$) (Figure 3b). However, no significant difference in the number of bacteria was observed between MetaSEAL Soft Paste and Canals (Figure 3b). There was also no significant difference in the number of bacteria between AH Plus Jet or Dentalis NX and positive controls (Figure 3b). Additionally, no significant difference in the number of bacteria was observed between these two sealers (Figure 3b). MetaSEAL Soft Paste and Canals showed significantly fewer bacteria than AH Plus Jet ($p < 0.01$) (Figure 3b).



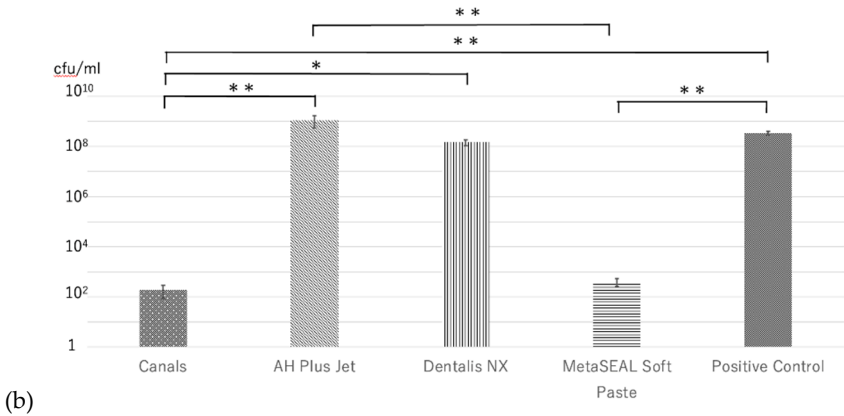


Figure 3. Comparison of antibacterial properties of the sealers. (a) Agar diffusion test; (b) direct contact test. **p < 0.01, *p < 0.05.

4. Discussion

This study showed that in bacterial leakage tests, AH Plus Jet took a significantly longer time for colony formation than Canals and Dentalis NX. Furthermore, although no significant difference in the time for colony formation was observed between AH Plus Jet and MetaSEAL Soft Paste, a strong tendency was observed. In the DCT, significantly fewer bacteria were detected in MetaSEAL Soft Paste and Canals than in AH Plus Jet or positive controls.

AH Plus Jet took a significantly longer time for colony formation than Canals and Dentalis NX in bacterial leakage tests in this study. Many studies have suggested that AH Plus has a higher sealing ability than zinc oxide-eugenol sealers [15,30–32]. Previous studies on the comparison between AH Plus and calcium hydroxide-based sealers have been conflicting. In two studies that evaluated AH Plus and the hydroxide-based sealer Apexit, using similar bacterial leakage tests, one reported that AH Plus was superior in its sealing ability [14] and the other was reported as inferior [33]. Similarly, the comparison between AH Plus and Sealapex using the bacterial leakage test has shown conflicting results [30,32]. The bacterial leakage test model used in this study can provide a simple and standardized method to evaluate the ability of sealers to prevent bacterial leakage in a more reliable and accurate manner than other tests.

In this study, AH Plus Jet showed the highest ability to prevent bacterial leakage compared with the other sealers. However, this result may only represent the sealer’s antimicrobial properties and may not indicate its sealing properties. Therefore, we also evaluated the antibacterial properties of each sealer. We found that Canals, a zinc oxide-eugenol-based sealer, showed antibacterial activity in the ADT and the DCT. Eugenol contained in zinc oxide eugenol-based sealers exerts antibacterial effects by inhibiting bacterial cell membrane function, suppressing the production of toxins and enzymes and inhibiting biofilm formation [34]. Additionally, eugenol exhibits antibacterial properties against *E. faecalis* [35,36]. Therefore, the antibacterial properties of Canals in this study may be related to free eugenol. Furthermore, eugenol is widely known to possess cytotoxic and tissue-toxic properties and to act as an inflammatory agent [20,37]. MetaSEAL Soft Paste showed no antibacterial effect in the ADT, whereas it showed an antibacterial effect in the DCT. This finding may have been due to the excessive number of bacteria used in the ADT, or alternatively, the antibacterial components in MetaSEAL Soft Paste may have had difficulty diffusing into the agar medium. On the other hand, no antibacterial effect was observed in AH Plus Jet in the ADT and the DCT.

Although AH Plus Jet did not show antibacterial properties, it had the longest duration for confirmation of colony formation in the bacterial leakage test, which suggested that it had the highest sealing ability. Furthermore, MetaSEAL Soft Paste, which has been attracting attention in recent years, had higher antibacterial properties than AH Plus Jet, but showed a tendency for earlier colony formation in the bacterial leakage test, which suggested that its sealing ability was inferior to that of

AH Plus Jet. This result is different from a report [26] in which MetaSEAL Soft Paste showed a better sealing ability than AH Plus Jet and Canals in a dye penetration test. Therefore, dye penetration testing methods may not be able to properly evaluate the sealing ability of root canal sealers. The bacterial leakage test performed in this study may not fully reflect actual clinical conditions because the entire root or gutta-percha was not used. However, we believe that this model is reliable for evaluating the ability of the sealer to prevent bacterial leakage.

Nevertheless, long-term sealing depends on many properties, such as adhesion, antibacterial properties, dimensional stability, solubility, and flow. There have been few reports of sealing experiments using MetaSEAL Soft Paste, and further accumulation and examination of experimental data are necessary. Additionally, MTA/bioceramic sealers, which have been attracting attention in recent years, have been reported to have better sealing properties than AH Plus [33,38], but also have inferior sealing properties [4,30,31]. Therefore, we plan to conduct a comparative study using this test model in future studies.

5. Conclusions

This study suggests that the novel bacterial leakage test model is effective for accurately evaluating the ability of sealers to prevent bacterial leakage, and further suggests that the sealing ability of MetaSEAL Soft Paste may not be superior to that of AH Plus Jet.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

- BHI: brain heart infusion
- ADT: agar diffusion test
- DCT: direct contact test
- MTA: mineral trioxide aggregate

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