Original

In vitro evaluation of the antimicrobial activity of a new resin-based endodontic sealer against endodontic pathogens

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Abstract: The purpose of this study was to compare the antimicrobial activities of a new resin-based SuperBond (SB) Sealer and five other sealers/cements against endodontic pathogens. The antimicrobial activities of SB Sealer, Sealapex, AH plus, Roeko Seal Automix, Canals N, and ProRoot mineral trioxide aggregate (MTA) were examined using a double-layered method. The microorganisms *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida albicans*, *Streptococcus mutans*, and *Streptococcus sanguinis* were used. Live microorganisms were stained using triphenyltetrazolium chloride, and the zones of inhibition of microorganism growth were measured. The antimicrobial activity of SB Sealer was significantly lower than that of the other sealers, except for Pro Root MTA, against *S. aureus*, *C. albicans*, *S. mutans*, and *S. sanguinis*, but no activity against *E. faecalis* was detected. On the other hand, AH plus exhibited the highest antimicrobial activity. Pro Root MTA showed no antimicrobial activity against any of the microorganisms tested. SB Sealer offered no antimicrobial advantage over the other sealers tested except for Pro Root MTA. (J. Oral Sci. 50, 309-313, 2008)

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Introduction

One important goal of endodontic therapy is to remove bacteria from root canals and thus prevent further infection (1,2). However, it has been reported that bacteria remain in root canals even after root canal preparation using a reamer or a file and final irrigation with NaOCl and EDTA (3). This is probably because some bacteria survive in the dental tubules and apical rami due to their complicated anatomical structures (4). Formaldehyde and phenol agents are used for sterilization, but it is difficult to completely sterilize the root canal (5). Root canal filling is performed using gutta-percha and sealers. Filling the spaces between the gutta-percha and the root canal wall is important for preventing reinfection (6). To suppress the growth of residual bacteria, antimicrobial agents have therefore been added to the sealers (7).

Root canal-filling sealers with calcium hydroxide-, epoxy resin-, or zinc oxide eugenol-based components are clinically applied. Grossman et al. proposed that the best sealers should have good biocompatibility, good sealing ability, safety, and antimicrobial activity (8). However, no sealers satisfying these characteristics have yet been developed. Many studies have examined the antimicrobial activity of sealers, and it has been reported that para-formaldehyde, eugenol, and thymol have such antimicrobial actions (9-11). Sealapex becomes strongly alkaline due to discharged hydroxide ions, thus exhibiting antimicrobial activity (12,13).

SuperBond (SB), a 4-META/MMA-TBB resin cement, is widely used for adhesion of orthodontic brackets and the fixation of mobile teeth (14). Recently, SB Sealer with high adhesiveness has been developed. SB has been reported to have almost no cytotoxicity to dental pulp cells (15), although its antimicrobial activity remains unclear. Facultative anaerobic microorganisms such as *Enterococcus faecalis*, *Staphylococcus aureus* and *Candida albicans* are considered to have the highest resistance in the oral cavity, with the potential to cause failure of root canal treatment (16). Therefore the present study was performed to compare the antimicrobial activities of a new resin-based SB Sealer and five other sealers/cements against various endodontic pathogens.

Materials and Methods

The sealers tested in this study were SuperBond (SB) Sealer (Sun Medical, Shiga, Japan), Sealapex (Sybron Kerr, Romulus, MI, USA), AH plus (Dentsply DeTrey, Konstanz, Germany), Roeko Seal Automix (Coltene/Whaledent, Germany), Canals N (Showa Yakuhin Kako, Tokyo, Japan), and ProRoot mineral trioxide aggregate (MTA) (Dentsply, Johnson City, TN, USA). The compositions of the sealers are shown in Table 1. The pH of Pro Root MTA in 3 h was measured with a pH meter (Horiba Instruments Ltd., Kyoto, Japan) using a temperature-compensated electrode.

These materials were evaluated in triplicate for antimicrobial activity using the double-layered method as described previously (12,13). The base layer comprised 10.0 ml of sterilized Brain Heart Infusion (BHI) agar (Difco, Detroit, MI, USA) poured into 20 mm × 100 mm sterilized Petri dishes. Six wells (diameter 7 mm, depth 2 mm) were made by removal of agar at equidistant points, and these were filled immediately with sealers after being mixed according to the manufacturer's instructions. The strains used for analysis were *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 10541, *Candida albicans* ATCC 10231, *Streptococcus mutans* Ingbritt, and *Streptococcus sanguinis* ATCC 10556.

The microorganisms were maintained on BHI agar at 4°C. A few colonies were picked from the BHI agar, and then inoculated into BHI broth. All microorganisms were grown at 37°C for 24 h and then seeded into 15.0 ml of the BHI agar to produce a turbidity of 0.5 on the McFarland scale, which corresponds to a concentration of 10^8 colony-forming units per ml. The seeded agar was added over the plate immediately after insertion of the sealers. The plates were maintained at room temperature for 2 h for prediffusion of the materials, and then incubated at 37°C for 24 h. Aliquots of 5.0 ml of triphenyltetrazolium chloride (TTC, Sigma) gel prepared with 1.0% BHI agar and 0.05% of TTC were added for optimization (12,13). After solidification, they were incubated at 37°C for 30 min. The same procedure was conducted using a plate without the sealers (negative control). The diameter of the zone was measured as the inhibition zone (mm) with a millimeter ruler to an accuracy of 0.5 mm. The data are expressed as the mean ± SD. The data were compared using one-way analysis of variance (ANOVA) followed by Tukey's test at a level of significance of 5% ($P < 0.05$). The different letters indicate significant differences between the sealers.

Results

To examine the antimicrobial activities of the six sealers/cement (SB Sealer, Sealapex, AH plus, Roeko seal Automix, Canals N, and ProRoot MTA) against endodontic pathogens, the diameters of the inhibition zones were measured by the double-layered method. 0.05% TCC was added to the media so that live microorganisms were dyed

Table 1 Composition of the materials tested for antimicrobial activity

Materials	Manufacturer	Composition
SB Sealer	Sun Medical	MMA, 4-META, pulverized PMMA, tri-*n*-butylborane derivative
Sealapex	Sybron Kerr	Calcium oxide, Barium sulphate, Silica, Titanium dioxide, Zinc stearate
AH plus	Dentsply Detrey	Diepoxide, Calcium tungstate, Zirconium oxide, Aerosil, Pigment, 1-adamantane amine, N,N'-dibenzyl-5-oxa-nonandiamine-1,9, TCD-diamine
Roeko Seal	Coltene/Whaledent	Polydimethylsiloxane, Silicone oil, Paraffin-based oil, Hexachloroplatinic acid, Zirconium dioxide
Canals N	Showa Yakuhin	Zinc oxide, Barium sulfate, Rosin, Bismuth, Fatty acid, Propylene glycol
ProRoot MTA	Tulsa Dentsply	Tricalcium silicate, Tricalcium aluminate, Tricalcium oxidate, Silicate oxide, Bismuth

MMA: methyl methacrylate
4-META: 4-methacryloxyethyl trimellitate anhydride
PMMA: poly(methyl methacrylate)

red, and the unstained area was defined as the inhibition zone (Fig. 1). The antimicrobial activity of SB Sealer was significantly lower than that of the other sealers except for Pro Root MTA against *S. aureus*, *C. albicans*, *S. mutans*, and *S. sanguinis*, but no activity against *E. faecalis* was detected (Table 2). Among the sealers, AH plus showed the highest antimicrobial activity against *C. albicans* and *S. mutans*. Canals N showed high antimicrobial activity against *S. aureus* and *S. sanguinis*, comparable to that of AH plus. Sealapex and Roeko Seal Automix exhibited mild antimicrobial activities against all of the bacteria. No antimicrobial activity of Pro Root MTA against any of the bacteria was detected. The pH of Pro Root MTA was 11.5 in 3 h. Among the bacteria, *E. faecalis* showed the highest resistance against the sealers.

Discussion

The antimicrobial activity of the sealers we tested, except for Pro Root MTA, was detected by the double-layered method. Since live microorganisms alone were stained red by addition of 0.05% TTC to the agar media, areas with component diffusion alone and dead bacteria could be distinguished. Previous reports have suggested that there is a relationship between antimicrobial activity and size of the inhibition zone. However, expression of the inhibition zone is influenced by several factors (12). Furthermore, there is a possibility that antimicrobial substances are produced when components of sealers come into contact with plate media.

Any microorganism remaining in a root canal has the potential to cause treatment failure. The presence of *E. faecalis*, *S. aureus*, and *C. albicans* in root canals has been reported in therapy-resistant periapical periodontitis (16). *E. faecalis* is the most drug-resistant of these bacteria, and can survive in root canals for 12 months even under nutrient-deprived conditions (6,17). Therefore, sealers are required to have antimicrobial activity in addition to sealing

ability and biocompatibility. In this study, the antimicrobial activity of sealers against endodontic pathogens which are almost universally present in the human oral cavity was examined. We found that *E. faecalis* was the bacterium most resistant to all of the sealers, in agreement with the previous study (12).

Currently, root canal sealers consisting of various components are used clinically. Many studies have reported the antimicrobial activities of such sealers, but the results have not always been concordant (9-11). This is because
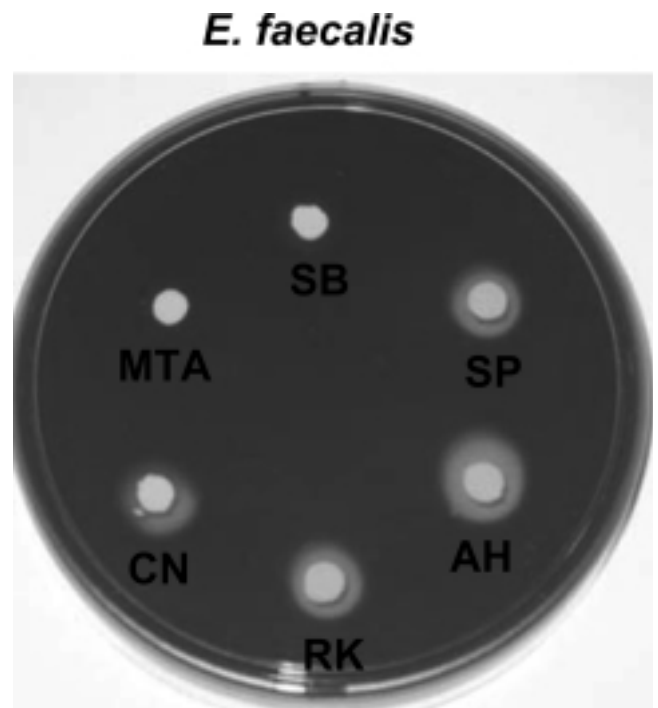

Fig. 1 Representative inhibition zones of *Enterococcus faecalis* determined by the double-layered method. SB: Superbond Sealer, SP: Sealapex, AH: AH plus, RK: Roeko Seal Automix, CN: Canals N, MTA: ProRoot MTA.

Table 2 Comparison of *in vitro* antimicrobial activity of six endodontic sealers/cements

Sealer	Pathogens				
	S. aureus	*E. faecalis*	*C. albicans*	*S. mutans*	*S. sanguinis*
SB Sealer	8.17 ± 0.76 [c]	0	7.50 ± 0.50 [c]	7.67 ± 0.29 [c]	7.50 ± 0.50 [c]
Sealapex	11.33 ± 0.76 [b]	10.67 ± 0.76 [a]	12.17 ± 1.76 [b]	11.00 ± 0.56 [b]	11.83 ± 1.04 [b]
AH plus	16.00 ± 1.50 [a]	12.33 ± 1.53 [a]	16.67 ± 1.26 [a]	13.67 ± 1.04 [a]	16.83 ± 0.29 [a]
Roeko Seal	10.67 ± 1.26 [b]	10.00 ± 0.50 [a]	11.00 ± 0.50 [b]	10.67 ± 0.29 [b]	12.17 ± 0.29 [b]
Canals N	14.50 ± 1.00 [a]	11.33 ± 1.04 [a]	11.50 ± 0.50 [b]	11.50 ± 0.87 [b]	16.33 ± 0.58 [a]
ProRoot MTA	0	0	0	0	0

The data are expressed as means ± SD of the inhibition zones (mm) determined by the double-layered method. The data were compared using one-way analysis of variance (ANOVA) followed by Tukey's test at a level of significance of 5% (*P* < 0.05). The different letters indicate significant differences between sealers.

variations in the concentrations of both agar and the tested bacteria, the amount and hardening time of the sealers, the incubation time, and evaluation methods employed affect the outcome of the agar diffusion test. It has been reported that eugenol, which is continuously eluted from zinc oxide eugenol-based sealers such as Canals, induces a strong inflammatory reaction (18,19). Therefore, Canals N with the same components as Canals, excluding eugenol, was used in the present study for examination of antimicrobial activity. This sealer showed the second highest antimicrobial activity, suggesting that zinc oxide may be involved in this effect. Some studies of AH26, which is an epoxy resin-based sealer, have reported that formaldehyde eluted from it has strong antimicrobial activity and cytotoxicity, and that another component, bisphenol A diglycidylether, is mutagenic (20,21). AH plus, which has recently been improved by its manufacturer, showed the strongest antimicrobial activity among the sealers tested. Since AH26 has been reported to discharge almost no formaldehyde (22), other components such as epoxy resin and amines must therefore be responsible for the strong antimicrobial activity of AH plus. Sealapex, which is a calcium hydroxide-based sealer, has been well studied, because it accelerates the closure of apical foramina (23). A high pH due to hydroxide ions discharged from Sealapex is unfavorable for bacteria, and their growth is therefore suppressed (24). This sealer showed mild antimicrobial activity, but its strong alkalinity could damage the surrounding tissues. MTA contains calcium oxide, which forms calcium hydroxide on contact with water (25-27). It has been reported that this cement has antimicrobial activity similar to that of calcium hydroxide-based sealers (25), and that its activity against *C. albicans* is particularly strong (28,29). However, MTA showed no antimicrobial activity against any of the microorganisms tested in this study. Although Torabinejad et al. observed that the pH of MTA was 12.5 in 3 h (25), that of Pro Root MTA was 11.5 in the present study. Considering that microorganisms cannot grow above pH 12, pH is considered to be one of the reasons for the conflicting results. However, previous reports have also suggested that there is little difference in antimicrobial activity between MTA and Pro Root MTA (29). Therefore, further investigations are needed to clarify the mechanism responsible for the antimicrobial activity of these cements.

Conventional sealers are used only for filling the spaces between the gutta-percha and the root canal wall. On the other hand, SB Sealer has strong adhesiveness and high sealing ability due to the formation of resin tags and hybrid layers in the dental tubules (14). Although it is ideal for sealers to exert antimicrobial activity while maintaining their adhesiveness, the present study revealed that SB Sealer has only low antimicrobial activity. Since a previous study demonstrated that both bacterial penetration into dentin and caries spread were significantly reduced by sealing with a resin composite (30), SB Sealer with a high degree of adhesiveness is considered to be useful as a sealer. In conclusion, SB Sealer showed the second lowest antimicrobial activity among the sealers/cements tested in this study.

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