Antibacterial Activity of Endodontic Sealers against Planktonic Bacteria and Bacteria in Biofilms

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Abstract

Introduction: The aim of this study was to investigate the antibacterial activity of 4 endodontic sealers against bacteria planktonic grown or in biofilms commonly detected from persistent and secondary endodontic infections. **Methods:** The antibacterial activity of the sealers AH Plus, TotalFill BC sealer, RoekoSeal, and Guttaflow 2 was investigated for planktonic grown and 24-hour-old biofilms of *Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus*, and *Streptococcus mutans*. **Results:** AH Plus had high antibacterial activity toward all species investigated, both planktonic and in biofilms. However, the antibacterial activity was lost after 24 hours. TotalFill BC sealer showed marked antibacterial effect on planktonic bacteria up to 7 days after setting. TotalFill BC sealer had lower antibacterial activity against biofilms of *S. aureus* and *E. faecalis* compared with AH Plus when direct contact between the sealer and biofilm was investigated and for all species investigated when a membrane was used to separate the biofilm and sealer. Guttaflow 2 and RoekoSeal had no antibacterial activity against planktonic bacteria or bacteria in biofilms. **Conclusions:** Bacteria in biofilms showed higher susceptibility for AH Plus compared with TotalFill BC sealer during the first 24 hours after setting. Investigating the antibacterial activity of endodontic sealers and materials against bacteria in biofilms is highly important to evaluate the materials' ability to eradicate bacteria from the infected root canal. *(J Endod 2017;■ :1–6)*

Key Words

Antibacterial activity, bacteria, biofilm, endodontic sealer, planktonic

Significance

Endodontic sealers should ideally eliminate residual bacteria and prevent reinfection after chemomechanical treatment and obturation of the root canal. The present study investigated the antimicrobial effect of 4 endodontic sealers against gram-positive planktonic and biofilm bacteria.

The main objective of endodontic treatment is to eradicate microorganisms from the infected root canal system and prevent recontamination. However, complete elimination of all microorganisms imposes a great challenge. It has been reported that about 35% of the root canal area is left untouched when conventional rotary and hand instruments are used (1). Therefore, bacteria may remain in the root canal system even after mechanical and chemical treatment, which may affect the periapical healing (2–5).

Endodontic sealers have an important function in endodontic infection control by entombing residual bacteria and preventing leakage of nutrients and reinfection of the root canal. In addition, some sealers have antimicrobial activity, which is considered beneficial for reducing and preventing growth of residual bacteria (6).

Microorganisms are established in biofilms in the infected root canal system. Bacteria living in biofilms are intrinsically more resistant to antimicrobials than their planktonic counterparts (7). In addition to its technical and biological requirements, an ideal root canal filling material should have antimicrobial and anti-biofilm activity to eradicate residual biofilm and bacteria after instrumentation and irrigation (8).

Enterococcus faecalis is often detected in persistent and secondary endodontic infections in addition to *Streptococcus* and *Staphylococcus* spp. (9–12). Some freshly prepared sealers have been reported to effectively kill *E. faecalis* (13–15). However, the antibacterial activity of sealers has been reported to decrease over time (15).

The antibacterial effect of endodontic sealers has most often been studied by using the agar diffusion test (ADT) or the direct contact test (DCT) (15–21). Neither of these tests measures the antibacterial activity of the materials on established biofilms.

The biofilm in post-treatment apical periodontitis may be formed by bacteria that survive the endodontic treatment or by bacteria that gain access through leakage of the coronal restoration (22). However, few studies have investigated the potential of endodontic sealers' ability to disrupt and kill bacterial biofilms, whereas the efficacy of disinfectants against biofilms has often been investigated (23–26).

The aim of the present study was to investigate the antibacterial activity of the endodontic sealers AH Plus, TotalFill BC sealer, RoekoSeal, and Guttaflow 2 against established biofilms. The susceptibility of the gram-positive bacteria *E. faecalis, S. mutans*,

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S. epidermidis, and *S. aureus* was investigated after planktonic growth and formation of biofilm.

Materials and Methods

Endodontic Sealers

An epoxy resin–based sealer, AH Plus (Dentsply International Inc, York, PA), 2 silicon-based sealers, RoekoSeal and Guttaflow 2 (Coltène/Whaledent, Altstätten, Switzerland), and a calcium-silicate-phosphate–based bioceramic sealer, TotalFill BC sealer (Brasseler USA, Savannah, GA) were tested. All materials were handled in accordance with the manufacturers' recommendations. RoekoSeal served as a positive control in our study because the manufacturer states it does not possess antibacterial activity.

Bacteria and Media

E. faecalis American Type Cell Culture Collection (ATCC) 19434, *S. mutans* ATCC 700610, *S. epidermidis* ATCC 35984, and *S. aureus Newman* were grown overnight for 18 hours in tryptone soya broth (TSB) at 37°C, 5% CO_2 supplemented atmosphere. The bacteria were suspended in phosphate-buffered saline (PBS) to an optical density at 600 nm (OD_{600}) of 1.0, corresponding to approximately 2×10^8 colony-forming units (CFU)/mL for the modified direct contact test (MDCT) assay. For the antibacterial assays on biofilms, an OD_{600} of 0.1 was used.

Antibacterial Assay on Planktonic Bacteria: MDCT

The MDCT was used to investigate the antimicrobial activity of sealers according to Zhang et al (15). Briefly, a 96-well microtiter plate (Costar, flat bottom, ultra-low attachment; Corning Incorporated, Corning, NY) was held vertically. A fixed area on a side wall of the wells was carefully coated with the material of each sealer by using a small-size round-ended dental instrument. Materials were used freshly mixed or after 24 hours and 7 days stored in humidified atmosphere at 37°C. The setting times for the freshly mixed samples for AH Plus, RoekoSeal, and Guttaflow 2 were 20, 50, and 30 minutes, respectively. TotalFill BC sealer was covered with 30 μL sterile distilled water (SDW) and left to set for 1 hour at 37°C because moisture is needed to initiate its setting process (18). The set samples were stored for 24 hours and 7 days, either in SDW or without SDW. The MDCT was individually conducted for every bacterial species. An amount of 10 μL from each bacterial suspension was carefully placed on the surface of the mixed material. Another 10 μL from the same bacterial suspension was transferred to uncoated wells, serving as positive control. Plates were incubated at 37°C for 1 hour, while complete evaporation of the suspension's liquid was inspected. Subsequently, 300 μL PBS was transferred to each well. Colonies of surviving bacteria were calculated after serial dilution in PBS and plating on TSB agar plates incubated overnight at 37°C, 5% CO_2 supplemented atmosphere. Experiments were conducted in triplicate and with 3 parallels for each material investigated.

Antibacterial Assay on Established Monospecies Biofilm: DCT and Membrane Restricted Test

A droplet of 20 μL of each bacterial inoculum OD_{600} 0.1 was applied onto the outer surface of cell culture inserts (culture plate inserts, polytetrafluoroethylene membrane, pore size 0.4 μm, 12 mm in diameter) (Milicell CM-Merck KGaA, Darmstadt, Germany). The inserts were then placed with the bottom up inside TSB agar plates. Plates were incubated at 37°C in a 5% CO_2 supplemented atmosphere for 24 hours. After 24 hours, the inserts were removed from the agar and washed gently with PBS to remove loosely attached bacteria.

For the DCT, freshly mixed sealers were placed directly onto the biofilm formed on the surface of the inserts inside a 10-mm Teflon ring. For the membrane restricted test (MRT), the sealers were applied on the inner surface of the inserts. To initiate setting of TotalFill BC sealer, 20 μL SDW was placed on top of the sample. Samples were placed in a humidified chamber. The contact time was 24 hours at 37°C. Inserts with biofilm growth were stored in a humidified chamber for 24 hours and served as positive control. For negative control, sealers were placed onto the surface of sterile inserts.

After the contact time, inserts were separated from sealers. Each sealer sample and insert were put in a vial containing 10 mL PBS and vigorously vortexed with glass beads. After 5-fold serial dilutions in PBS, 2 droplets of 25 μL were placed on TSB agar plates. Colony-forming units were counted after incubation at 37°C in a 5% CO_2 supplemented atmosphere for 24 hours for *S. epidermidis, S. aureus*, and *E. faecalis* and for 48 hours for *S. mutans*. Experiments were performed in triplicate and with 3 parallels for each material investigated.

Carryover Effect Test

Inserts with monospecies biofilm served as positive controls and were placed in a vial containing 10 mL PBS. A sealer specimen inside a 10-mm Teflon ring was allowed to set independently for 24 hours at 37°C in a humidified chamber and was then put in the same vial. These samples were vigorously vibrated with glass beads. Possible carryover effect was measured after 5-fold serial dilutions, and CFU/mL was counted and calculated as described previously. Experiments for potential carryover effect were performed in triplicate.

Data Analysis

The experiments were analyzed by using one-way analysis of variance, followed by Tukey multiple comparisons test with GraphPadPrism version 6.00 for Windows (GraphPad Software, La Jolla, CA). The *P* value was set at .05.

Results

Antibacterial Activity against Planktonic Bacteria: MDCT

For freshly prepared samples of AH Plus, no surviving bacteria were recovered for any of the 4 bacterial species investigated (Fig. 1). This antibacterial activity was lost after 24 hours, because there were no differences between bacterial survival from the AH Plus and control samples after 24 hours or 7 days of setting time ($P < .05$). For the silicone-based sealer Guttaflow 2 and RoekoSeal, there was no difference in the number of bacteria recovered from samples compared with control during or after setting (Fig. 1). The bioceramic sealer, TotalFill BC sealer, showed antibacterial activity when freshly mixed and after 24 hours and 7 days for all conditions investigated. *S. aureus* was more resistant to the antibacterial effect of TotalFill BC sealer compared with the other bacterial species when sealer samples were stored in water conditions ($P < .05$) (Fig. 1). The overall results of the MDCT assay are shown in Table 1.

Antibacterial Activity against Established Monospecies Biofilms: DCT and MRT

Freshly made AH Plus killed all bacteria in the biofilm of *E. faecalis* and *S. epidermidis*, for both the DCT and the MRT (Fig. 2). Guttaflow 2 and RoekoSeal had no antibacterial activity against biofilm formed by any of the bacterial species investigated (Fig. 2). TotalFill BC sealer reduced bacterial survival for all bacterial biofilms investigated ($P < .05$). However, the MRT showed that AH Plus had higher antibacterial activity against all monospecies biofilms investigated compared