In Vitro Evaluation of the Antimicrobial Effect of Three Endodontic Sealers Mixed with Amoxicillin

Jeff Baer, DDS, and James S. Maki, PhD†*

Abstract

Introduction: The purpose of this *in vitro* study was to evaluate the antimicrobial effect of mixing amoxicillin with three different sealers when freshly mixed and set. **Methods:** Using a direct contact test, Pulp Canal Sealer EWT (SybronEndo Corporation, Orange, CA), AH Plus (Dentsply International Inc, York, PA), and RealSeal SE (Pentron Clinical Technologies LLC, Wallingford, CT) were freshly mixed with amoxicillin and placed on the side wall of the microtiter plate. A 10-μL bacterial suspension of *Enterococcus faecalis* was placed directly onto the fresh sealers, and sealers set 1 day, 3 days, and 7 days after mixing. The bacteria were allowed to dry in direct contact with the sealer sample. Fresh media was then added, and growth of the bacteria was measured by spectrophotometry over an 8-hour period. One-way analysis of variance (ANOVA), two-way ANOVA, and Tukey multiple comparison were used for statistical significance. **Results:** All sealers mixed with amoxicillin showed complete inhibition of the growth of *E. faecalis*. Sealers mixed with amoxicillin had no statistical difference in inhibiting growth between freshly mixed samples and samples set for 1 day, 3 days, or 7 days ($p > 0.05$). Sealers without amoxicillin did not inhibit growth of *E. faecalis*, and no statistical difference was found between freshly mixed and set samples ($p > 0.05$). Sealers with amoxicillin were statistically different than sealers without amoxicillin when freshly mixed and set ($p < 0.001$). **Conclusions:** Sealers mixed with amoxicillin inhibited the growth of *E. faecalis* significantly greater than sealers without amoxicillin ($p < 0.001$). *(J Endod 2010; 36:1170–1173)*

Key Words

Amoxicillin, antibiotics, direct contact test, root canal sealer

From the Departments of *Endodontics and †Biological Sciences, Marquette University School of Dentistry, Milwaukee, Wisconsin.

Address requests for reprints to Dr Jeff Baer, Marquette University School of Dentistry, Department of Endodontics, 1801 W Wisconsin Avenue, Milwaukee, WI 53233. E-mail address: jeff.baer@marquette.edu.

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An important goal of endodontic therapy is to eliminate or prevent the introduction of microorganisms into the root canal system and to prevent infection by a well-sealed obturation (1, 2). A complete chemomechanical preparation is essential to reducing the bacterial load within a canal system. It is well known, however, that regardless of instrumentation and technique, even well-treated canal systems often remain untouched during chemomechanical preparation (3). Also, studies have revealed that chemomechanical preparation with sodium hypochorite (NaOCl) does not eliminate all bacteria in root canals (4, 5). The persistence of microbes can result in periapical disease (6–10). The prognosis of endodontic therapy is significantly influenced by eliminating or reducing the bacterial concentration within a canal system (11–14). Although complete elimination of all microbes in a system is virtually impossible, aiming to lower the critical concentration of microbes and disrupting biofilms will give the best chance for a positive host response (15–17).

After chemomechanical preparation, endodontic obturation materials and sealers attempt to entomb residual bacteria (biofilms) that are untouched by irrigants and medicaments (18). However, if a microbial colony is able to adapt to a new environment and a source of nutrients is available, periapical disease results. To prevent new bacterial growth, obturation materials and sealers should have antimicrobial properties upon contact with microbes and biofilms and, ideally, over time be able to maintain this effect (19).

Adding antibiotics to a sealer can enhance their antimicrobial effect and could provide an important advantage in reducing the critical concentration of microbes necessary for a favorable host response. Historically, two different assays have been used to test the antimicrobial properties of sealers: the agar diffusion test (ADT) and the direct contact test (DCT). Hoelscher et al (20) used the ADT to determine the effect of different antibiotics mixed with a ZOE-based sealer on *Enterococcus faecalis*. Hoelscher et al found a significant difference in the zone of inhibition with fresh sealer mixed with amoxicillin compared with sealer without amoxicillin. Today, the ADT is no longer recommended because of its limitations (21–23).

The DCT, which was first introduced by Weiss et al (24), has been determined to be a reliable, quantitative, and reproducible assay that allows testing of insoluble materials and can be used in standardized settings (22, 24). The DCT has been shown in several studies to be a reproducible method for measuring the antimicrobial property of various sealers (21, 22, 25, 26). To date, no study has been performed with a DCT evaluating a sealer-antibiotic combination after being set for several days. The purpose of this study was to evaluate the antimicrobial effect of three different freshly mixed sealers with and without amoxicillin and to compare the effect after allowing the sealer to set 1 day, 3 days, and 7 days.

Materials and Methods

Sealers

Three commonly used sealers were tested. Pulp Canal Sealer EWT (SybronEndo Corporation, Orange, CA) is a zinc oxide–eugenol sealer. AH Plus (Dentsply International Inc, York, PA) is an epoxy resin-based sealer, and RealSeal SE (Pentron Clinical Technologies LLC, Wallingford, CT) is a polymethacrylate resin–based sealer. The sealers were prepared according to manufacturers' instructions, weighed, and mixed with crushed amoxicillin (TEVA Pharmaceuticals, Sellersville, PA) at 10% of the sealer's total weight. The antibiotic and amount of amoxicillin was based on the results of Hoelscher et al (20), who mixed several antibiotics with Kerr Pulp Canal Sealer EWT

and found amoxicillin at 10% weight to be the most efficacious. Amoxicillin was also chosen because of its extended spectrum of bactericidal activity against many gram-negative and gram-positive microorganisms. It is also readily available and inexpensive and is generally found to have few side effects.

Microorganism

E. faecalis (American Type Culture Collection 4082) was obtained from the American Type Culture Collection (Manassas, VA). The bacterium was grown and maintained on brain-heart infusion (BHI) broth (Difco, Sparks, MD). To preserve the bacterium and its characteristics, upon receipt, cultures were frozen (−20°C) in vials with glycerol from which new stock cultures were periodically established. A culture of *E. faecalis* was grown overnight at 37°C in BHI broth. Bacterial growth was checked by changes in turbidity at 24 hours.

DCT

The DCT places bacteria in direct contact with a test material, and subsequent bacterial growth is then monitored by changes in absorbance. Indirectly, the effect of an antibiotic molecule is based on turbidometric determinations of bacterial growth. A treated and inoculated 96-well polystyrene microtiter plate (Becton Dickinson and Company, Franklin Lakes, NJ) was placed into a microplate reader to measure absorbance (OD = 630 nm) to determine bacterial growth. The microplate reader (EL800 Universal Microplate Reader; Bio-Tek Instruments, Inc) uses a light source that transilluminates the sample with a predetermined wavelength (selected by an optical filter or a monochromator), and a light detector located on the opposite side of the well measures the quantity of the initial light transmitted through the sample; a less amount of transmitted light (=greater sample absorbance) will typically be related to a greater density of bacterial cells.

Plate preparation involved orienting it vertically and applying a thin layer of equal amounts of fresh sealer on the walls of the well so as to not affect light transmission of the plate reader. Each sealer was mixed fresh according to manufacturer's instructions with and without amoxicillin, making six groups and two control groups. Each group was placed in eight wells. The groups were as follows: group 1: Pulp Canal Sealer EWT, group 2: Pulp Canal Sealer EWT/amoxicillin mix, group 3: AH Plus, group 4: AH Plus/amoxicillin mix, group 5: RealSeal SE, and group 6: RealSeal SE/amoxicillin mix. *E. faecalis* from an overnight culture was then applied directly to the fresh sealer. The negative control (no inoculum) consisted of eight wells, two wells of each sealer without the amoxicillin combination and two wells without any sealers. The positive controls included eight wells without sealers and were inoculated with *E. faecalis*.

In the first test plate, the six test groups and the positive controls were immediately inoculated with 10 μL of the *E. faecalis* cell suspension directly onto the freshly mixed sealers. No *E. faecalis* was placed in the negative controls. The *E. faecalis* cell suspension was allowed to dry in direct contact with the sealer groups. After drying, 250 μL of sterile BHI broth was added into each well of the 6 groups and the positive and negative controls, and the plates were incubated at 37°C. This plate was labeled as fresh, and this test was conducted three separate times. An immediate absorbance reading (OD = 630 nm) was obtained from the microplate reader and hourly for 8 hours. In a pilot DCT study, the turbidometric growth of the *E. faecalis* was measured for 27 hours. Results of the pilot study showed no significant change in absorbance after 8 hours, and, therefore, in the current study each subsequent plate was measured for 8 hours.

Additional plates were prepared, except the six sealer groups were allowed to set for 1 day, 3 days, or 7 days at 37°C before inoculation with the bacteria. After the *E. faecalis* cell suspension dried in direct contact, BHI broth was added, and absorbance readings were obtained as described previously. These tests were conducted two separate times each. Growth of the *E. faecalis* in the different treatments was compared and analyzed statistically for significance.

Results

The growth of *E. faecalis* was measured as absorbance and analyzed with one-way analysis of variance, two-way analysis of variance, and Tukey multiple comparison for statistical significance. The results of the mean growth over 8 hours are presented in Figure 1. The negative control showed no growth. Results in the fresh, 1-day, 3-day, and 7-day set plates revealed that groups with amoxicillin (groups 2 [Pulp Canal Sealer EWT/amoxicillin], 4 [AH Plus/amoxicillin], and 6 [RealSeal SE/amoxicillin]) completely inhibited the growth of *E. faecalis*. Data showed no statistically significant difference in inhibiting growth between groups 2, 4, and 6 when fresh or after setting 1 day, 3 days, or 7 days ($p > 0.05$). The groups without amoxicillin (groups 1 [Pulp Canal Sealer EWT], 3 [AH Plus], and 5 [RealSeal SE]), whether fresh or set 1 day, 3 days, or 7 days, did not differ significantly from each other or the positive control ($p > 0.05$). All three sealers with amoxicillin were statistically different than sealers without amoxicillin whether fresh or set ($p < 0.001$).

Discussion

The results of this study showed that all three sealers with amoxicillin, even after being set for up to 7 days, still maintained antimicrobial properties and completely inhibited the growth of the *E. faecalis*. This study also showed that all three sealers without amoxicillin did not inhibit growth of the *E. faecalis*. In fact, the sealers without added amoxicillin allowed growth similar to the positive control. This is in agreement with Slutzky-Goldberg et al (25) who found that AH Plus and Epiphany SE (identical to RealSeal SE, see SybronEndo.com) did not inhibit growth of the microbes whether fresh or set. In a modified DCT, Zhang et al (22) also showed that after 1 day AH Plus and Ephiphany SE had lost most of their antimicrobial effect.

In contrast, Zhang et al (22) also concluded that fresh iRoot SP, AH Plus, and EndoRez were effective antimicrobial agents against *E. faecalis*. Pizzo et al (27) in a DCT found that freshly mixed AH Plus, Endomethasone, and Pulp Canal Sealer completely inhibited the growth of *E. faecalis*. Other studies in a direct contact method have shown that eugenol-based sealers without antibiotics have antimicrobial properties (21, 26–28). This antimicrobial effect may have been largely caused by a high concentration of eugenol or a function of pH. Another study by Sagsen et al (29) used a time-kill assay to measure the antimicrobial efficiency of sealers. They found that sealers containing eugenol and epoxy resin might be preferred because of their antibacterial effect. The reason why Pulp Canal Sealer EWT, a eugenol-based sealer in the current study tested without amoxicillin, did not have a greater inhibition of growth may be because of the strain of *E. faecalis* or the formulation of the sealer tested. In the present study, the *E. faecalis* (American Type Culture Collection 4082) used was originally isolated from the root canal of a pulpless tooth, whereas several studies have used *E. faecalis* (American Type Culture Collection 29212), which, although originally isolated from urine, is a bacterium used in standard antimicrobial assays (26, 27, 30).

For years antibiotics have been used in dentistry. The local application of antibiotics by a sealer may be a more effective mode for delivery in endodontics (31). Sealers are essential for a well-sealed obturation and can reduce the amount of leakage in obturated canals from 5 to 20 times (32). Grossman's ideal properties of a root canal