Antibacterial Properties of Four Endodontic Sealers

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Abstract

The purpose of this study was to evaluate the antimicrobial effects of root canal sealers. The direct contact test (DCT) was used to assess the antibacterial properties of AH plus, Apexit Plus, Epiphany SE, and RoekoSeal when in contact with *Enterococcus faecalis.* The materials were examined immediately after setting and 1, 2, 7, and 14 days after aging in phosphate-buffered-saline. Statistical analysis with two-way analysis of variance, one-way analysis of variance, and Tukey multiple comparison was applied to the data. Apexit Plus had a short-term antibacterial effect of 1 day on *E. faecalis*, whereas Epiphany SE enhanced bacterial growth for at least 7 days. AH plus and RoekoSeal were ineffective. *(J Endod 2008;34:735–738)*

Key Words

Antibacterial properties, direct contact test, *E. faecalis*, endodontic sealers

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One of the main goals of endodontic treatment is to eliminate microorganisms from the root canal system. This is achieved through mechanical cleaning and shaping, supplemented by antibacterial irrigants, adequate filling of the empty space, and the possible use of antimicrobial dressings between appointments (1, 2). However, treatment might reduce, but not necessarily eliminate, root infection (3–5). Thus, the root filling should prevent coronal reinfection (6) and entomb remaining bacteria within the canals (7). A possible treatment modality is the use of root canal sealers with antimicrobial properties to improve the outcome of endodontic treatment (8).

Enterococcus species constitute only a small proportion of the initial flora in untreated root canals (9); this genus is the most commonly recovered one from the root canals of teeth with failed root treatment (10, 11).

Grossman (8) advocated that the ideal root canal filling material should be bacteriostatic, and indeed it has been shown that several endodontic sealers possess antimicrobial activity. In the past, the antimicrobial activity of root canal sealers was assessed by using the agar diffusion test (ADT). However, this technique is relatively insensitive, and the results are dependent on the diffusion and physical properties of the tested materials (12). The direct contact test (DCT) was designed to overcome these limitations. It has been used to evaluate the in vitro antibacterial activities of numerous endodontic sealers such as zinc oxide–eugenol (ZOE), resin-based sealers, and those containing calcium hydroxide (13).

A new thermoplastic-filled polymer obturation system, Resilon/Epiphany (Pentron, Wallingford, CT), was recently introduced as an alternative to gutta-percha. A possible advantage for the use of this system over the conventional root filling technique is the formation of a "monoblock" without the typical gaps in gutta-percha fillings (14). Yet, this fact has not been confirmed by other studies that demonstrated that root canals filled with Resilon did not show less microbial leakage than gutta-percha (15, 16). Furthermore, Resilon was found to be susceptible to alkaline hydrolysis (17).

The objective of the present study was to compare the antimicrobial effect of Epiphany to 3 other root canal sealers against *Enterococcus faecalis* by using the DCT.

Materials and Methods

Tested Materials

Four root canal sealers were tested; representing 4 groups of commonly used sealers. An epoxy resin–based material, AH plus (Dentsply International Inc, York, PA); a calcium hydroxide–based material, Apexit Plus (Vivadent; Schaan, Liechtenstein); a resin-based material, Epiphany SE (Pentron Clinical Technologies LLC, Wallingford, CT); and a silicon-based material, RoekoSeal (Coltène/Whaledent, Altstätten, Switzerland) were tested.

Test Microorganism and Growth Conditions

The DCT is based on turbidimetric determination of bacterial growth in 96-well microtiter plates (Nunclon; Nunc, Copenhagen, Denmark). The kinetics of the outgrowth in each well were recorded every 30 minutes for 16 hours by using a temperature-controlled spectrophotometer set at 37°C (VersaMax; Molecular Device Corp, Menlo Park, CA).

Clinically isolated *E. faecalis* was grown aerobically from frozen stock cultures in brain heart infusion broth (Difco Laboratories, Detroit, MI) containing 1.25% streptomycin at 37°C. *E. faecalis* is naturally resistant to streptomycin; therefore, this antibiotic was added to the growth media and buffers to prevent microbial contamination. Automixing before each reading ensured a homogeneous bacterial cell suspension.

TABLE 1. Bacterial Growth Rate: *E. faecalis* Growth Rate According to the Slope of the Linear Portion of the Growth Curve.

Material	Fresh material	1 Day	2 Days	7 Days	14 Days
Apexit Plus	0.0017 ± 0.0012	0.0004 ± 0.00003	0.091 ± 0.0252*	0.114 ± 0.0094*	0.0718 ± 0.0087*
AH plus	0.079 ± 0.0064*	0.0658 ± 0.0291*	0.0581 ± 0.0072*	0.1028 ± 0.0256*	0.0742 ± 0.0172*
RoekoSeal	0.0698 ± 0.0039*	0.0544 ± 0.0132*	0.0531 ± 0.0288*	0.082 ± 0.0043*	0.051 ± 0.0057*
Control	0.0893 ± 0.0055*	0.073 ± 0.004*	0.0845 ± 0.0007*	0.099 ± 0.0101*	0.091 ± 0.0371*
Epiphany SE	0.087 ± 0.0069*	0.0905 ± 0.0204*	0.2335 ± 0.0954	0.1924 ± 0.019	0.0902 ± 0.0379*
Significance	$P < .0001$	$P < .0001$	$P < .0001$	$P < .001$	None

NOTE. Each number in the table is the average optical density (log) ± standard deviation of the slope of bacterial growth in eight separate wells in the same microtiter plate.

*Values that do not differ significantly (Tukey's comparison).

The experimental set-up of the DCT has been described in detail in the literature (13, 18, 19) A 96-well flat-bottom microtiter plate was held vertically, and the sidewalls of 8 wells were coated evenly with an equal amount (surface area of 19.67 ± 0.03 mm^2) of the tested material. The materials were mixed in strict compliance with the manufacturers' recommendations.

Eight uncoated wells in the same microtiter plate served as a positive control. An identical bacterial inoculum was placed on the sidewall of the uncoated wells and processed as in the experiment wells.

The negative control consisted of a set of wells coated with the tested material, as in the experimental wells, and contained equal volumes of uninoculated medium. All the data presented were calculated after deducting the value obtained from the negative control.

The plate was then incubated at 37°C in the VersaMax microplate reader, and the optical density in each well was followed at OD_{650} for 16 hours. The absorbance measurements were then plotted, providing bacterial growth curves for each well in the microplate. The linear portion of the logarithmic growth curve, which correlates with bacterial growth rate, was used for statistical analysis. The results are expressed by 2 variables: the slope (a) and the constant (b) of the linear function $ax + b = y$. The slope and the constant correlate with growth rate and the initial number of bacteria, respectively. The data were analyzed with two-way analysis of variance (ANOVA), one-way ANOVA, and the Tukey multiple comparison test.

To allow interexperimental comparison, each microplate also included a set of wells for calibration of bacterial outgrowth (13, 18, 19). For this purpose, bacteria were diluted by a factor of 5. The calibration growth curve allows an estimation of the number of viable bacteria at the end of the direct contact incubation period.

Similar experiments were carried out in which the tested materials were allowed to age for 1, 2, 7, and 14 days. Aging was performed in the presence of phosphate-buffered saline containing streptomycin, which was replaced every 48 hours.

Results

The DCT was applied to 8 specimens of each of the 4 materials tested. A regression line was drawn of the linear segment of the curve in group A wells, which represents the logarithmic phase of growth. The R^2 of all the growth curves ranged between 0.90 and 0.99. Two-way ANOVA, performed in all the experiments, indicated a significant difference in bacterial growth rate (slope) as a function of time and material ($P < .001$).

When testing the growth of *E. faecalis* immediately after placement of the materials, Apexit Plus was the only material that exhibited potent antibacterial properties in group A wells. Apexit Plus had a bacteriostatic effect, because bacterial growth in group B wells was similar to that in the control. The other materials did not exhibit any antibacterial effect (Table 1, Fig. 1).

After aging the materials for 1 day, Apexit Plus retained its antibacterial property (Table 1). Apexit Plus, AH plus, and RoekoSeal did not differ from the control after aging the materials for 2 days, whereas

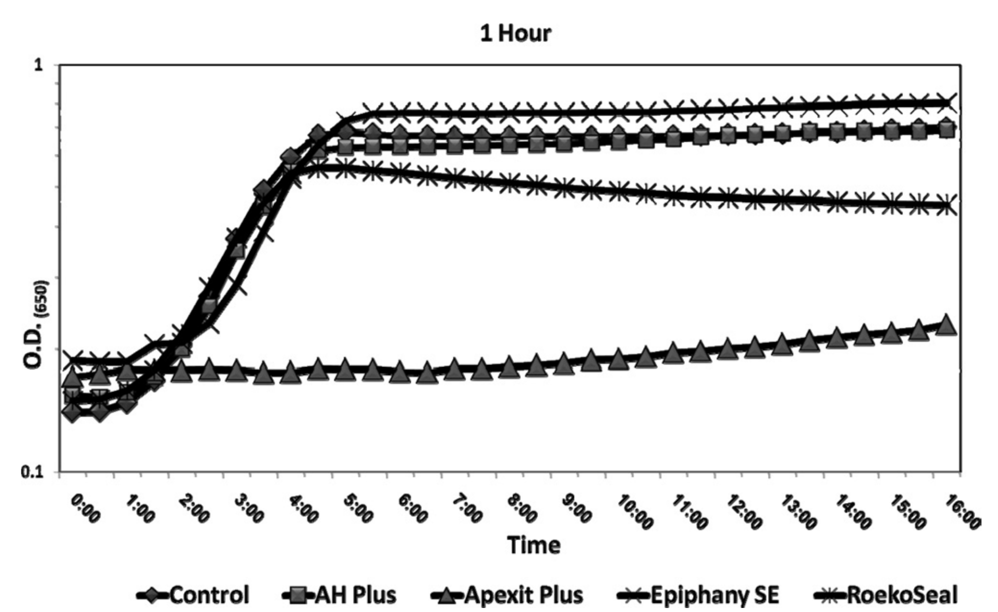

Figure 1. *E. faecalis* growth after direct contact with fresh material. Each point on the curve is the average optical density (OD_{650}) on a logarithmic scale measured in 8 separate wells at the same time.

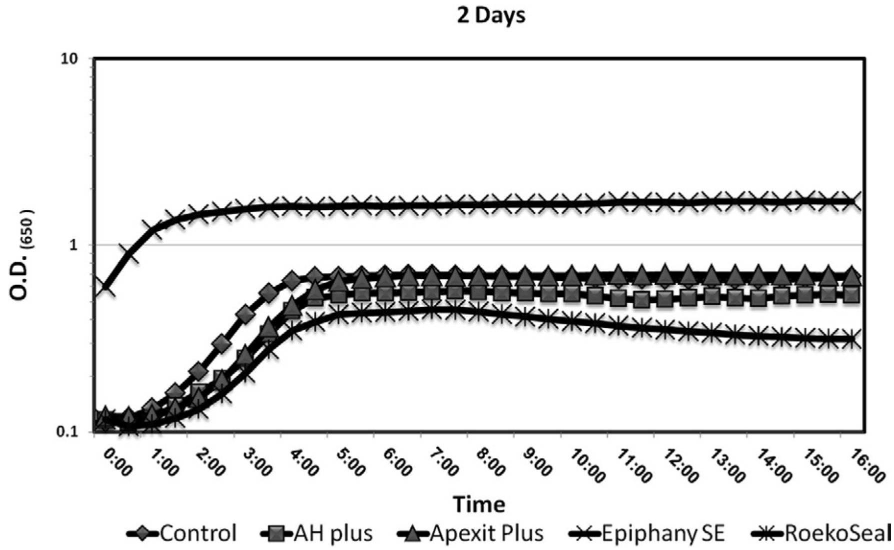

Figure 2. *E. faecalis* growth after direct contact with material that was aged for 2 days in phosphate-buffered saline. Each point on the curve is the average optical density (OD_{650}) on a logarithmic scale measured in 8 separate wells at the same time.

Epiphany SE enhanced bacterial growth (Table 1, Fig. 2). After aging the materials for 7 days, Epiphany SE was still able to enhance bacterial growth; all the other materials were similar to the control (Table 1). After aging the materials for 14 days, there was no longer any bacterial-material reaction (Table 1).

Discussion

Numerous root canal sealers are available, which are based on various formulas. The ideal root canal sealer should be inert, dimensionally stable, and possess good antimicrobial activity and low toxicity toward the surrounding tissue (8, 20).

E. faecalis, which is often associated with persistent apical periodontitis and might be difficult to eliminate from root canals, was chosen as the test organism for this study (3). Therefore, the antibacterial activity of root canal sealers against the facultative anaerobic microorganism , *E. faecalis,* might assist in controlling infections.

Apexit Plus is a calcium hydroxide–based material. Root canal sealers with integrated calcium hydroxide have enhanced antibacterial activity (21, 22). The antimicrobial effect of this sealer stems from the release of hydroxide ions, which raise the pH to above 12.5. As the calcium hydroxide sealer sets, the pH declines to about 9.14, resulting in loss of the sealer's effectiveness (23). Apexit Plus exhibited a higher antibacterial activity and was more effective against the tested microorganism than the other materials, either freshly mixed or after 2 days. These results are similar in part to the results of Eldeniz et al (24), who did not find any antibacterial activity in the ADT but did observe an antibacterial effect in the DCT, when the material was freshly mixed or after 24 hours. These findings support the notion that the ADT is not a reliable method because this substance has low solubility and might diffuse slowly in agar.

The antibacterial effect of epoxy resin–based sealers might be related either to bisphenol-A diglycidyl ether, which was previously identified as a mutagenic component (25), or to the release of formaldehyde during the polymerization process (26). In a previous study, AH plus showed some antibacterial effect against *E. faecalis* (27). The low antimicrobial effect of AH plus against *E. faecalis* might be ascribed to the minimal amount of formaldehyde released over time (26). This is in accordance with previous results showing that AH plus has no antimicrobial effect against *E. faecalis* (28).

Recently, a thermoplastic synthetic material based on polyester (a copolymer of polycaprolactone and urethane dimethacrylate; Resilon) was developed as an endodontic root filling material. The Epiphany obturation system uses Resilon points and a dual-curing resin-based sealer, which is bonded to both the root dentin and the Resilon points (14). The developers state that this root canal sealer is a dual-curing resin that is nonmutagenic, noncytotoxic, biocompatible, resolvable, and less irritating than epoxy resin or ZOE sealers. In a study with the ADT, Epiphany exhibited less antimicrobial activity than 4 other sealers (Endomethasone, Sultan, Sealapex, and Diaket), except for AH 26. The authors suggested that the minimal antibacterial effect of Epiphany results from its hydrophilic resin form (29). However, our DCT results are substantially different. Not only was this material not antibacterial, but it promoted bacterial growth. When the solubility characteristics of several endodontic sealers were tested, Epiphany exhibited the highest apparent water sorption (8%), compared with EndoREZ (3.0%) and AH plus (1.1%) (30). The American Dental Association specifications require $<$3% solubility for endodontic sealers. In the oral environment, these materials can absorb water and release free (unreacted) monomers (31). Their release from resin composites might stimulate the growth of bacteria (31–33), a possible explanation to the thriving of *E. faecalis* in the presence of Epiphany.

RoekoSeal is a silicon-based root canal sealer. In the present study RoekoSeal did not exhibit any bacterial growth inhibition. These results are only in partial accordance with the findings of Cobankara et al (34), who tested the antibacterial properties of RoekoSeal by using both ADT and DCT; they showed that in the ADT, RoekoSeal had no antibacterial activity, whereas other sealers, including AH plus, did inhibit bacterial growth.

Different sealers were found to have different effects on bacterial growth, on the basis of their formula, the assessment test, and time. Our results indicate that the use of Epiphany might be challenging, and that the use of sealers with antibacterial material might be advantageous. Nevertheless, such results should be considered with caution, and other properties must be weighed, for example, the sealing ability of these materials over time. Materials that possess some antimicrobial properties might gradually lose their volume, thus impairing the quality of the seal (23). Furthermore, the data presented here relate to in vitro conditions, and testing the antibacterial activity in vivo might lead to different results, because the amount of liquid in the root canal is limited, and the antibacterial effect of the sealer might depend on the ability of the material to penetrate the dentinal tubuli after infected smear layer removal and to reach the harboring bacteria.

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