

Antimicrobial Activity and Flow Rate of Newer and Established Root Canal Sealers

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Endodontic sealers that possess both optimum flow ability and antimicrobial properties may theoretically assist in the elimination of microorganisms located in confined areas of the root canal system. The antimicrobial effects and the flow rate of the following sealers were investigated and compared: Kerr Pulp Canal Sealer EWT, Grossman's Sealer, ThermaSeal, Sealer 26, AH Plus, and Sealer Plus. The agar diffusion test was used to assess the antimicrobial activity of the sealers. In the flow assay, the sealers were placed between two glass slabs and a weight of 500 g was placed on the top of the glass. The diameters of the formed discs were recorded. All root canal sealers tested showed some antimicrobial activity against most of the microorganisms. There were no significant differences between the materials tested ($p > 0.05$). All root canal sealers also flowed under the conditions of this study. Statistical analysis of the results revealed that AH Plus and Kerr Pulp Canal Sealer EWT had flow values significantly superior to the other sealers tested ($p > 0.05$). Taken together, these findings suggest that these sealers have the potential to help in the microbial control in the root canal system.

Endodontics is essentially a clinical discipline concerned with the prevention and control of the root canal infection. Cleaned and shaped root canals must be three-dimensionally filled, eliminating the empty space, which has the potential to be infected or reinfected. In addition, by creating a fluid-tight apical, lateral, and coronal seal, root canal fillings may confine residual irritants within the root canal system, impeding their egress to the periradicular tissues. A fluid-tight seal of the root canal system also prevents both coronal recontamination by saliva and seeping of periradicular tissue fluids into the root canal, denying a nutrient supply to the remaining microorganisms. Therefore, root canal obturation plays an important role in both the prevention and control of endodontic infections (1).

Most root canal filling techniques use core materials associated with endodontic sealers. Core obturating materials, such as gutta-percha, usually occupy space, whereas the endodontic sealers enhance the possible attainment of an impervious seal by serving as a filler for canal irregularities and minor discrepancies between the root canal wall and the core material. Several properties are required to an ideal endodontic sealer (2). Among them sealing ability, biocompatibility, and antimicrobial activity probably influence the success of the root canal treatment (1).

To create and maintain a three-dimensional seal of the entire root canal system, sealers should have adhesiveness, be dimensionally stable, be insoluble to oral and tissue fluids, and have an adequate flow rate. This latter property allows the material to penetrate into irregularities, isthmi fins and ramifications, which increases the likelihood of obtaining an adequate seal of the root canal system. Moreover, sealers that possess both optimum flow ability and antimicrobial properties might theoretically eliminate microorganisms located in such confined areas of the root canal system.

Sealers should have microbicidal activity or, at a minimum, they should not encourage microbial growth. Studies have reported that several endodontic sealers have antimicrobial effects (3–6). Sealers having antimicrobial effects may help to eliminate residual microorganisms unaffected by the effects of both chemomechanical preparation and intracanal medication. In addition, they may limit the ingress of microorganisms from saliva, impeding or at least retarding the complete recontamination of the root canal after saliva challenge.

Given the importance of the control and prevention of endodontic infections in the success of the root canal therapy, the purpose of this study was to investigate and compare the antimicrobial effects and the flow rate of some newer and established root canal sealers.

MATERIAL AND METHODS

Root Canal Sealers

The endodontic sealers used in the present study were Kerr Pulp Canal Sealer EWT (Kerr, Romulus, MI), Grossman's Sealer (Fill Canal, Dermo Laboratórios, Rio de Janeiro, Brazil), ThermaSeal (Tulsa Dental Products, Tulsa, OK), Sealer 26 (Dentsply, Petrópolis, Brazil), AH Plus (Dentsply DeTrey, Konstanz, Germany), and

Sealer Plus (Dentsply, Petrópolis, Brazil). All materials were prepared according to the manufacturers' directions immediately before testing.

Antimicrobial Assay

The microorganisms used in this assay included two obligate anaerobic microorganisms, eight aerobic or facultative anaerobic microorganisms, and a mixed culture. The obligate anaerobes were *Prevotella nigrescens* (ATCC 33563) and *Porphyromonas gingivalis* (ATCC 33277). The aerobic or facultative bacteria included *Streptococcus mitis* (clinical isolate), *Streptococcus bovis* (clinical isolate), *Enterococcus faecalis* (ATCC 29212 and a clinical isolate), *Pseudomonas aeruginosa* (ATCC 15442), *Lactobacillus casei* (ATCC 4646), and *Escherichia coli* (ATCC 11229). *Candida albicans* (ATCC 10231), a yeast, and a sample of whole saliva obtained from one of the authors were also used in the antimicrobial test. After activation from stock cultures, lyophilized or frozen in skim milk, microorganisms were maintained in appropriated culture media until used.

Overnight cultures of the microorganisms were used. The yeast and aerobic or facultative anaerobic bacteria were grown in trypticase-soy broth (Difco, Detroit, MI), and anaerobes were grown in prereduced anaerobically sterilized brain heart infusion broth (Difco) supplemented with hemin (5 µg/ml) and vitamin K₁ (1 µg/ml). Saliva was collected immediately before the experiment. Turbidity of the inocula, prepared in prereduced anaerobically sterilized brain heart infusion broth for anaerobes or in trypticase-soy broth for the other microorganisms, was adjusted to the turbidity of a 0.5 McFarland BaSO₄ standard (~1.5 × 10⁸ colony-forming units/ml).

Petri dishes containing trypticase-soy agar (Difco) enriched with 5% defibrinated sheep blood and supplemented with hemin and vitamin K₁ were seeded with the anaerobic bacteria or the mixed culture (saliva). Plates containing *Mitis salivarius* agar were seeded with the test strains of *Streptococcus* and *Enterococcus*. The other microorganisms were inoculated onto the surface of trypticase-soy agar plates. Seeding was done using sterile cotton-tipped applicators that were brushed across the agar surfaces. Six wells of 5 mm depth and 6 mm diameter were punched in each agar plate and filled with the freshly prepared sealers. Plates were then left at room temperature for ~10 min to allow the absorption of the inoculum. All the procedures were done in duplicate.

Agar plates inoculated with the mixed culture or the anaerobic bacteria were incubated into anaerobic jars at 37°C for 5 days. Anaerobic conditions were obtained by using the GasPak Plus generators (BBL, Becton-Dickinson Microbiology Systems, Cockeysville, MD). Agar plates inoculated with the other microorganisms were incubated aerobically at 37°C for 24 to 48 hr. The antimicrobial effects of each material were determined by measuring the diameter of zones of inhibition in millimeters. The diameter of 6 mm served as the cut-off value.

Flow Assay

The flow test was conducted as described previously by Benatti et al. (7) and Siqueira et al. (8). Briefly, 0.5 ml of each sealer was prepared and placed between two glass slabs, and a weight of 500 g was placed on top of the glass for 1 min. The weight of the glass was 20 g. Hence the weight that was acting on the specimen was

520 g. Three different diameters were measured from the formed discs, and the arithmetical average was calculated. Six samples of each sealer were used.

Statistical Analysis

To allow a general view about the antimicrobial effectiveness of the test materials, data were analyzed using the Kruskal-Wallis test with tied ranks. Results from the flow test were statistically analyzed by means of the ANOVA and Tukey's test. Significance levels were always established at 5% ($p < 0.05$).

RESULTS

Antimicrobial Assay

All root canal sealers tested showed some antimicrobial activity against most of the microorganisms used. Regardless of the material, the inhibitory effects were discrete against most of the microbial strains tested. General analysis of the data showed no significant differences between the materials tested ($p > 0.05$). The assessment of the sum of the ranks attributed to each value of zones of inhibition demonstrated that, in general, the most resistant microorganisms tested were *P. aeruginosa*, *E. faecalis* (ATCC strain), and *E. coli*. The means of the diameters of the zones of microbial inhibition for each sealer against each microorganism are given in Table 1.

Flow Assay

The average and range of the mean diameters of the discs are summarized in Table 2. All root canal sealers flowed under the conditions of this study. Statistical analysis of the results revealed that AH Plus and Kerr Pulp Canal Sealer EWT had flow values significantly superior to the other sealers tested ($p < 0.05$). There was no significant difference when comparing these two sealers ($p > 0.05$). Tukey's test failed to show any other significant differences between the materials ($p > 0.05$).

DISCUSSION

The agar diffusion test has been widely used to evaluate the antibacterial activity of dental materials (3–6, 9, 10). A great disadvantage of this method is that it does not distinguish between microbistatic or microbicidal properties of the materials (9). In addition, the results of the agar diffusion test does not depend on only the toxicity of the material for the particular microorganism, but are also highly influenced by the diffusibility of the material across the medium (10). A material that diffuses more easily will probably provide larger zones of inhibition. Thus, in addition to direct cytotoxicity, the different diffusion rates of the different sealers may have influenced the results. Others variables such as inoculum size, incubation time, and the good material/agar contact may also interfere with the results. Nevertheless, if most of these variables are carefully controlled, consistent and reproducible results may be obtained (9). This method permits direct comparisons between materials and also indicates which sealers are likely to have antimicrobial activity within the root canal system. Because

TABLE 1. Means of the diameters of the zones of microbial inhibition provided by sealers (in mm)

	Sealer Plus	Sealer 26	ThermaSeal	KPCS	Fill Canal	AH Plus
<i>P. nigrescens</i>	4	10	6.5	9	6.5	NT
<i>P. gingivalis</i>	8	20.5	16	3	7	4.5
<i>S. mitis</i>	1	9	7.5	12.5	NT	4.5
<i>P. aeruginosa</i>	4.5	2.5	2	2.5	1	5
<i>E. faecalis</i> ATCC	4	0	7	0	3.5	4
<i>E. faecalis</i> c.i.	3	16.5	13.5	8.5	NT	5.5
<i>L. casei</i>	5.5	10	10.5	3.5	4	5.5
<i>E. coli</i>	4	0	6	7.5	8	5
<i>S. bovis</i>	5.5	9	11	3	5.5	5.5
<i>C. albicans</i>	8	0	0	9.5	13.5	NT
Saliva	2	18	14	3	2	5.5

c.i. = clinical isolate; NT = not tested; KPCS = Kerr Pulp Canal Sealer.

of the obvious limitations of in vitro studies, clinical inferences should be drawn with strict caution.

In addition to the main root canal and dentinal tubules, the root canal system may also possess several anatomical variations such as ramifications, fins, deltas, culs-de-sacs, and isthmi. This morphologically complex system may be entirely colonized by microorganisms after pulpal necrosis. Mechanical debridement cannot reach microorganisms within those complexities, and chemical agents used in irrigation will also have difficulty in reaching the microorganisms. Thus, infections that propagate to the entire root canal system may present a problem for both the host and the therapist. Intracanal medications are usually needed to eliminate microorganisms in confined areas (1). In addition, it seems logical to assume that the expression of an endodontic sealer that has both antimicrobial activity and a good flow rate through complexities may also assist in the effective microbial control within the root canal system.

Several factors may influence the penetration of endodontic sealers within confined areas of the root canal system. Among them, the obturation technique used, the contact area, the dimension of irregularities, accessibility to the complexities, and the sealer's flow rate seem to play an important role in allowing sealer penetration. Data from the flow test showed that all sealers flowed under the conditions of this study. However, AH Plus and Kerr Pulp Canal Sealer EWT had flow values significantly superior to the other sealers tested. Undoubtedly, this is a very important property of an endodontic sealer.

The findings from the antimicrobial test revealed that all sealers tested displayed some antimicrobial effect during setting. The inhibitory effects of the sealers on most of the microorganisms tested were considered discrete. In addition, sealers showed varying effectiveness on the microbial strains tested. Taken together, these results suggest that the clinical efficiency of sealers will depend on the accessibility to confined areas, number of remaining

microorganisms, and the microbial species infecting each root canal system.

The endodontic microbiota of untreated teeth is dominated by strict anaerobic bacteria (11). Although aerobic and facultative microorganisms (bacteria and fungi) are usually minor constituents of primary infections, they have been found in cases in which the treatment had been protracted, in flare-ups, and associated with endodontic failures (12–15). These microorganisms can enter the root canal system during the treatment, survive the treatment procedures, and persist after obturation. They then cause secondary infections by using opportunities created by the removal of the members of the primary infection and by surviving in the low-nutrient environment of the treated root canal (15). Therefore, in addition to anaerobic bacteria, it also seems important to evaluate the antimicrobial activity of endodontic materials against aerobic and facultative microorganisms. In general, our results revealed that the most resistant microorganisms tested were *P. aeruginosa*, *E. faecalis* (ATCC strain), and *E. coli*. Not coincidentally, strains of these bacterial species have been reported to be resistant to several antimicrobial agents (16). Because of such a feature, they have been found associated with serious human infectious diseases, with increased morbidity and mortality (16, 17). *Pseudomonas* and enterococci species have also been involved in some persistent and therapy-resistant endodontic infections (15, 18, 19).

Most endodontic sealers possess antimicrobial components that need to be released from the sealer matrix to be effective. Several compounds may have been responsible for the antimicrobial effects of the sealers used: eugenol and zinc oxide (Fill Canal and Kerr Pulp Canal Sealer), silver (Kerr Pulp Canal Sealer), hexamethylenetetramine (Sealer 26 and ThermaSeal), calcium oxide (Sealer Plus), calcium hydroxide (Sealer 26), and epoxide resin components (Sealer 26, ThermaSeal, AH Plus, and Sealer Plus). Because the antimicrobial components of endodontic sealers do not have selective toxicity against microorganisms, they usually exert toxic effects on the host tissues. However, discounting paraformaldehyde-containing endodontic materials, the endodontic sealers exhibit toxicity when freshly mixed, which is greatly reduced on setting (20). Likewise, the antibacterial effects of the test sealers might be short-lived and temporary. Further studies addressing the biocompatibility of the newer sealers are necessary. In addition, long-term studies are also needed to evaluate the antibacterial activity of set materials against bacterial species commonly isolated from infected root canals.

Taken together, the findings of the present study indicate that all sealers tested had both antimicrobial activity and a good flow rate.

TABLE 2. Mean flow values provided by sealers

Sealers	Mean (cm)	Range (cm)
Sealer Plus	3.5	3.5–3.6
Sealer 26	3.7	3.2–4.0
ThermaSeal	3.7	3.5–4.2
KPCS	4.2	3.7–4.7
Fill Canal	3.5	3.4–3.5
AH Plus	4.6	4.1–4.9

KPCS = Kerr Pulp Canal Sealer.

AH Plus and Kerr Pulp Canal Sealer EWT showed superior flow rate when compared with the other sealers. Further *in vitro* and *in vivo* studies may help to elucidate if sealers that have both properties really assist in root canal disinfection.

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