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In vitro antimicrobial activity of root canal sealers and calcium hydroxide paste

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Abstract

Aim: To evaluate the *in vitro* antimicrobial activity of different root canal sealers and calcium hydroxide (CH) paste. **Materials and Methods:** The sample was composed of two sealers (Fill Canal® and Sealer 26®), one CH cement (Hydro C®), and a CH paste. The agar diffusion test was performed in Petri dishes inoculated with the following microorganisms: *Streptococcus salivarius*, *Streptococcus oralis*, *Streptococcus mitis*, *Lactobacillus casei*, *Streptococcus mutans*, *Candida albicans*, *Candida krusei*, and *Candida tropicalis*. The diameters of the zones of microbial growth inhibition were measured after 24 h. The tests were performed in triplicate. Data were analyzed statistically by ANOVA and Tukey's test at 5% significance level. **Results:** Fill Canal® exhibited the largest mean zone of microbial growth inhibition against the Candida species and differed significantly from the other groups ($P < 0.001$). When inhibition was observed against *S. mitis* and *S. oralis*, the CH paste presented a larger mean zone of microbial growth inhibition than those of the other materials ($P < 0.05$). Regarding the inhibition of *S. mutans*, a statistically significant difference was observed only between the CH paste and Hydro C® ($P < 0.05$); the paste produced the largest mean zone of microbial growth inhibition against this microorganism. Regarding the inhibition of *S. salivarius*, Fill Canal® presented smaller mean zone of microbial growth inhibition than Sealer 26® and CH paste ($P < 0.05$). **Conclusion:** All the materials presented zones of microbial growth inhibition against all the test bacteria. Fill Canal® presented the largest mean zone of inhibition against the *Candida* species. For the *Streptococcus* cultures, none of the sealers presented inhibition superior to that of the CH paste.

Keywords: *Candida*, microbiological techniques, root canal filling materials

Introduction

A large number of endodontic pathologies are caused by microorganisms present in the root canal system, but occasional reports on the involvement of yeasts and fungi are also found in the literature. The greatest challenge in eliminating endodontic infection is not related to microorganisms present in the lumen of the main canal, but rather to those disseminated in the root canal system ramifications (ie, the dentinal tubules, lateral canals, accessory canals, secondary canals, apical delta ramifications, apical foramen, and apical root cementum surface).[1-3]

Several studies have investigated the antibacterial activity of endodontic materials.[4-10] The goals of root canal sealers are to impede microbial recolonization and multiplication in the root canal system, prevent the growth of residual microorganisms, and neutralize their toxic products in order to create a favorable environment for the healing process to proceed.[4]

The intracanal medication must diffuse through the dentin to be effective against the microorganisms that persist in the root canals and reach sufficient levels for a lethal effect. The efficacy of an intracanal dressing against residual bacteria will depend on the type of root canal instrumentation, the type of vehicle used for preparation of the paste, the polymicrobial nature of the endodontic infection, and the buffering capacity of the dentin.[11] Therefore, canal filling should prevent the growth and survival of resistant pathogens, as well as prevent reinfection in pulp necrosis cases or infection in case of noncontaminated healthy pulps.[12]

Several root canal sealers based on epoxy resin, calcium hydroxide (CH), zinc oxide–eugenol, with and without the addition of paraformaldehyde are currently available. However, few studies have directly compared different types of sealers against endodontic pathogens, especially strict anaerobic species on which there has been an increasing number of reports of infecting root canals.[6]

The agar diffusion method has been widely used to evaluate the antimicrobial activity of dental materials and medications. The advantage of this method is that it allows for a direct comparison of the efficacy of different root canal sealers against the target pathogens, indicating which sealers could potentially eradicate bacteria in the microenvironment of the pulp space.[13] It is important that root filling materials have broad antimicrobial activity and the antimicrobial spectrum of action of these materials should be investigated. The purpose of this study was to evaluate the *in vitro* antimicrobial activity of different root canal sealers.

Materials and Methods

The *in vitro* antimicrobial activity of the following endodontic materials against bacterial and yeast species was evaluated

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by the agar diffusion test (agar-well technique): Fill Canal® (Technew Com. e Ind. Ltda., Rio de Janeiro, RJ, Brazil), Sealer 26® (Dentsply Ind. e Com., Petrópolis, RJ, Brazil), Hydro C® (Dentsply Ind. Com., Petrópolis, RJ, Brazil), and CH paste (CH p.a. mixed with distilled water) (Biodinâmica Quím. e Farm. Ltda, Ibiporã, PR, Brazil). The following bacterial and yeast strains obtained from the American Type Culture Collection (ATCC) were used as indicator microorganisms in the study: *Streptococcus salivarius* (ATCC 7073), *Streptococcus oralis* (ATCC 10557), *Streptococcus mitis* (ATCC 903), *Lactobacillus casei* (ATCC 7469), *Streptococcus mutans* (ATCC 25175), *Candida albicans* (ATCC 10231), *Candida krusei* (ATCC 6538) and *Candida tropicalis* (ATCC 13803).

The bacterial strains were reactivated in Brain Heart Infusion (BHI; Difco, Detroit, MI, USA) broth and seeded in 20 × 10 mm sterile Petri dishes containing Müller–Hinton agar supplemented with 5% blood (Difco, Detroit, MI, USA) using swabs saturated in the bacterial suspension corresponding to the 8 standard of the McFarland scale. The fungal strains were reactivated in Sabouraud agar broth (Difco, Detroit, MI, USA) and seeded in Petri dishes containing Sabouraud agar medium (Difco, Detroit, MI, USA) in the same way as described for the bacterial species.

Fill Canal® (zinc oxide and eugenol) and Sealer 26® (CH) sealers, Hydro C® (CH) and the CH paste were prepared according to the manufacturers' instructions on glass plates using a sterile stainless steel spatula. All the materials were used immediately after mixing.

For the agar diffusion test, after solidification of the seed layer, 6-mm-diameter wells were made in each dish by removal of the agar at equidistant points using a sterile straw, and were immediately filled with the materials. The test was done in triplicate, that is, 3 dishes were used for each test microorganism. The dishes were maintained at room temperature for 2 h for prediffusion of the materials, and were then incubated in aerobiosis—except for the *S. mutans* dishes, which were incubated in microaerophilia (candle jar system)—at 37°C for 24 h. After incubation, the diameter of the zones of microbial growth inhibition formed around the wells was measured in millimeters with a digital caliper (Mitutoyo, Tokyo, Japan) under reflected light. Three measurements were made for each material and the average of the three values was calculated. Data of antimicrobial activity of the endodontic sealers were analyzed statistically by analysis of variance and Tukey's post hoc test at a significance level of 5% using the Graph Pad Prism 4 software (Graph Pad Inc., San Diego, CA, USA).

Results

Table 1 shows the diameters (means and standard deviations) of the zones of microbial growth inhibition (in mm) obtained for the tested materials.

Fill Canal® presented the largest microbial growth inhibition zones against the *Candida* strains and differed significantly from the other groups ($P < 0.001$). Comparing the zones of microbial growth inhibition against *S. mitis* and *S. oralis*, the HC paste presented greater efficacy than the other studied materials ($P < 0.05$). The zones of microbial growth inhibition against *S. mutans* presented significant differences only for the HC paste and Hydro C® ($P < 0.05$), and the largest mean zone of bacterial growth inhibition against this microorganism was produced by the CH paste ($P < 0.05$). Regarding *S. salivarius*, Fill Canal® produced smaller zones of microbial growth inhibition than Sealer 26® and the HC paste ($P < 0.05$).

Discussion

Total elimination of microorganisms from the root canal system is the goal of endodontic treatment.[10] Instrumentation and disinfection of the root canal system as well as tight adaptation of the filling material to the canal space, promoting an adequate seal and preventing bacterial leakage, are key factors for endodontic treatment success.[6] A root canal sealer with antimicrobial activity might better cope with a persistent residual infection and coronal microbial leakage, therefore increasing the chances for a successful endodontic treatment outcome.[10] Nevertheless, no material fulfills all the requirements for an ideal root canal sealer.[12]

Facultative and strict anaerobic bacteria are the most common microorganisms of the endodontic microbiota and cause infections that stimulate periapical bone resorption

Table 1: Diameter of the microbial growth inhibition zones produced by the different materials

Microorganisms	Materials			
	Sealer 26®	Fill Canal®	Hydro C®	Calcium Hydroxide Paste®
Streptococcus salivarius	18.67 ± 1.53	15.00 ± 1.00	16.00 ± 1.00	19.00 ± 1.00
Streptococcus oralis	12.67 ± 0.58	11.67 ± 1.15	12.67 ± 0.58	12.67 ± 2.52
Streptococcus mitis	12.00 ± 1.00	13.00 ± 2.65	12.00 ± 0.00	17.67 ± 2.08
Lactobacillus casei	14.00 ± 1.00	13.00 ± 1.73	14.67 ± 2.89	17.33 ± 1.15
Streptococcus mutans	11.33 ± 0.38	11.33 ± 0.38	10.00 ± 0.00	12.33 ± 1.15
Candida albicans	13.67 ± 0.58	32.00 ± 3.46	15.67 ± 2.52	19.00 ± 3.61
Candida krusei	14.33 ± 0.58	27.00 ± 2.65	14.33 ± 2.52	20.00 ± 1.00
Candida tropicalis	12.33 ± 0.58	27.67 ± 1.15	14.67 ± 0.58	17.00 ± 0.00

Values are expressed as mean ± standard deviation.

and are refractory to endodontic treatment.[14] Facultative microorganisms, such as *E. faecalis*, *S. aureus*, and even *C. albicans* have been considered as the most resistant oral species and possible causes of failure of root canal treatment. [7]

The agar diffusion test used in this study is one of the most frequently used methods for assessing the antimicrobial activity of endodontic materials.[9] A disadvantage of the agar diffusion test is that the result of this method does not depend exclusively on the toxicity of the material for the target microorganism, but it is also highly influenced by the diffusion of the material across the medium.[13] The size of the inhibition zone definitely does not indicate the antimicrobial efficacy of the material.[15]

In the same way as reported by Kopper *et al*.,[12] there was no need of using a positive control group in the present study because microbial viability was assessed on dishes in which microbial growth was observed. Additionally, the sterility test of the tested materials demonstrated that they were not contaminated, which could have interfered with the results of this study.

The materials evaluated in this study were selected because they are routinely used in the Brazilian dental practice. Fill Canal® is a Grossman's cement, whereas Sealer 26® is a resin-based root canal sealer. The other two materials (Hydro C® and CH paste) are based on CH, which has been extensively used in dentistry due to its capacity to stimulate mineralization and its excellent antimicrobial action.[5,16]

The results of the present study revealed that all the materials tested possessed antimicrobial activity, as demonstrated by the formation of growth inhibition zones against all the evaluated strains. The Grossman's cement (Fill Canal®) produced the largest zones of microbial growth inhibition against *C. albicans*, as observed in a previous study.[17] Sealer 26® presented a weaker antimicrobial action, but absence of antimicrobial action of Sealer 26® against *C. albicans* has been reported.[17]

The antimicrobial activity of CH-based materials, such as Sealer 26® may be related to ionization with subsequent release of hydroxide ions and an increase of pH levels, creating an unfavorable environment for microbial growth. [11] Essential functions, such as metabolism, growth, and cellular division require the participation of the cytoplasmic membrane, which is the seat of important enzymatic systems. In this way, changes in physiological activities of microorganisms can be directly influenced by the release of hydroxyl ions, which alter the integrity of the cytoplasmic membrane by means of biochemical injuries to the organic components, interfering in the transportation of nutrients or destroying phospholipids or unsaturated fatty acids, and leading to a saponification reaction. Since the action site of hydroxyl ions released from CH includes the enzymes in the cytoplasmic membrane, this medication has a large scope of action depending on the amount of material, and therefore affects a diverse range of microorganisms, irrespective of their metabolic capacity.[18]

The findings of this study on the antimicrobial activity of Hydro C® is in agreement with those of a previous investigation, although in the present study the HC paste presented greater antimicrobial activity against *S. mutans* than Hydro C®. However, there are reports in the literature that CH presents low solubility in water due to the size of its molecules, which restraints its diffusibility,[19] or due to the lack of solubility of the paste, in order to provide dissociation of calcium and hydroxyl ions, which are responsible for the antiseptic action.[5] Nevertheless, different paste vehicles can change the speed of dissociation and diffusion of CH hydroxyl ions.[20]

In the present study, no statistically significant differences were found on the growth inhibition zones against *L. casei*.

Endodontic treatment must be carried out under aseptic conditions, using a powerful irrigating solution, an intracanal medication when required, a sealer with antimicrobial activity, and an effective coronal seal to prevent coronal microleakage in order to increase the chances for root canal treatment success.[7,13]

Results obtained from *in vitro* antimicrobial efficacy studies do not have direct clinical application, but they do permit comparisons. *In vitro* tests can identify only the materials that have the potential to inhibit microbial growth in the local microenvironment of the root canal.[15] Therefore, further studies are required on the antimicrobial activity of the different root canal sealers available in the market against different microbial cultures.

Conclusion

All materials produced zones of microbial growth inhibition against all the tested microorganisms. Fill Canal® exhibited the largest mean growth inhibition zone against *C. albicans*. None of the evaluated root canal sealers presented larger growth inhibition zones against the streptococcal species than the CH paste.

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