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Original

In vitro antimicrobial activity of endodontic sealers, MTA-based cements and Portland cement

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Abstract: The aim of this study was to evaluate the antimicrobial activity of different root-end filling materials – Sealer 26, Sealapex with zinc oxide, zinc oxide and eugenol, white and gray Portland cement, white and gray MTA-Angelus, and gray Pro Root MTA – against six different microorganism strains. The agar diffusion method was used. A base layer was made using Müller-Hinton agar (MH) and wells were formed by removing the agar. The materials were placed in the wells immediately after manipulation. The microorganisms used were: *Micrococcus luteus* (ATCC9341), *Staphylococcus aureus* (ATCC6538), *Escherichia coli* (ATCC10538), *Pseudomonas aeruginosa* (ATCC27853), *Candida albicans* (ATCC 10231), and *Enterococcus faecalis* (ATCC 10541). The plates were kept at room temperature for 2 h for prediffusion and then incubated at 37°C for 24 h. Triphenyltetrazolium chloride 0.05% gel was added for optimization, and the zones of inhibition were measured. Data were subjected to the Kruskal-Wallis and Dunn tests at a 5% significance level. The results showed that all materials had antimicrobial activity against all the tested strains. Analysis of the efficacy of the materials against the microbial strains showed that Sealapex with zinc oxide, zinc oxide and eugenol and Sealer 26 created larger inhibition halos than the MTA-based and Portland cements ($P < 0.05$). On the basis of the methodology used, it may be concluded that all endodontic sealers, MTA-based and Portland cements evaluated in this study possess antimicrobial activity, particularly the endodontic sealers. (J. Oral Sci. 49, 41-45, 2007)

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Introduction

The aim of retrograde filling is to seal the root canal system by means of an apical cavity preparation, which is then filled using a material with adequate physical, chemical and biological properties. The ideal root-end filling material must possess certain characteristics, including biocompatibility, adequate marginal sealing, ability to allow or induce bone repair, and antimicrobial activity (1,2).

In the search for ideal root-end filling materials, various alternatives have been studied. Gerhards and Wagner (3) studied Diaket, an epoxy resin-based sealer used in retrograde fillings, and obtained satisfactory apical seals. Regan et al. (4) observed good periapical repair in dogs following retrograde filling with Diaket. Sealer 26 is also an epoxy resin-based endodontic sealer, composed of bismuth oxide, calcium hydroxide, and epoxy resin. A greater proportion of powder/resin is used to obtain a thicker consistency for use in retrograde fillings. Sealer 26 is known for its excellent sealing properties when used as a root canal sealer (5) or in root-end fillings (6). This material has also been reported to show satisfactory biocompatibility when used for root-end filling in dogs (7).

Sealapex is a root canal filling material with good biological properties (8,9). The antimicrobial activity of this material as a root canal sealer has already been reported

(10,11). When Sealapex is used as a root-end filling material, zinc oxide is added to improve its consistency and facilitate its clinical use (12). Another well-known material is mineral trioxide aggregate (MTA), which has good sealing capacity (1,13). Torabinejad et al. (14) demonstrated better repair with MTA than with amalgam for retrograde filling of teeth in dogs.

Portland cement has a composition similar to MTA except for absence of bismuth oxide, which is added to MTA to enhance its radiopacity (15,16). MTA-Angelus (Angelus, Londrina, Paraná, Brazil) is produced from Portland cement, with bismuth oxide added to increase its radiopacity. Its properties, in terms of increasing the pH and releasing calcium ions, are similar to those of Pro Root MTA (Dentsply Tulsa Dental, OK, USA), as demonstrated previously by Duarte et al. (17). White Portland cement and white MTA have identical compositions, differing only in the presence of bismuth oxide in the MTA (18).

The goal of the present study was to evaluate the antimicrobial activity of different root-end filling materials derived from endodontic sealers, or based on MTA and Portland cements.

Materials and Methods

The sources of the various root-end filling materials are presented in Table 1. The morphotype and source of the strains used as indicators of antimicrobial activity are presented on Table 2. The materials were evaluated in triplicate for antimicrobial activity using the agar diffusion method.

The well method was conducted on double-layered plates. The base layer was composed of 10.0 ml sterilized Müller-Hinton agar (MH; Difco, Detroit, MI, USA) poured into 20×100 mm sterilized Petri dishes. After solidification, a 5.0-ml seed layer, obtained by addition of the inoculum at a concentration of 10^6 colony-forming units/ml to 5.0

Table 1 Root-end filling materials used in the study

Material	Manufacturer
Sealer 26	Dentsply - Industria e Comércio Ltda., Petrópolis, RJ, Brazil
Sealapex* + zinc oxide**	*SybronEndo, Glendora, CA, USA **S.S.White Artigos Dentários Ltda., Rio de Janeiro, RJ, Brazil
Zinc oxide and eugenol	S.S.White Artigos Dentários Ltda., Rio de Janeiro, RJ, Brazil
Gray Portland cement	Cimento Portland Itaú, Votorantin Cimentos Ltda., Itaú de Minas, MG, Brazil
White Portland cement	Irajazinho, Votorantin, Rio de Janeiro, RJ, Brazil
Gray MTA	Angelus, Londrina, PR, Brazil
White MTA	Angelus, Londrina, PR, Brazil
Gray Pro Root MTA	Tulsa Dentsply, USA

Table 2 Strains used as indicators of antimibrobial activity, their source, and morphotype

Microrganisms	Source	Morphotype
Micrococcus luteus	ATCC 9341	cg+
Staphylococcus aureus	ATTC 6538	cg+
Escherichia coli	ATCC 10538	bg-
Pseudomonas aeruginosa	ATCC 27853	bg-
Candida albicans	ATCC 1023	ye
Enterococcus faecalis	ATCC 10541	cg+

cg+: Gram-positive cocci; bg-: Gram-negative bacilli; ye: yeast ml of MH, was added. Thereafter, eight 4-mm wells (one for each material) were made by removal of the agar at equidistant points and then filled immediately with the materials to be evaluated.

The root canal sealers were manipulated as recommended for use as root-end filling materials. Accordingly, Sealer 26 was used as proposed by Tanomaru-Filho et al. (7), zinc oxide and eugenol cement according to Bernabé et al. (19), and Sealapex with zinc oxide was mixed using the proportions recommended by Tanomaru-Filho et al. (7). Portland cement and MTA-based materials were manipulated in accordance with the manufacturer's instructions.

The plates were maintained at room temperature for 2 h to allow prediffusion of the materials, and then incubated at 37°C for 24 h. Aliquots of 5.0 ml prepared with 1% agar (Difco) and 0.05% triphenyltetrazolium chloride (Sigma, St. Louis, MO, USA) were added for optimization. After solidification, they were incubated at 37°C for 30 min. This technique allows differentiation between areas of microbial growth (red areas) and diffusion zones. The inhibition zones around the wells were then measured with a millimeter ruler with accuracy of 0.5 mm. Data were subjected to the Kruskal-Wallis and Dunn tests at a 5% significance level.

Results

Figure 1 shows the inhibition zone after use of TTC gel. Table 3 shows the antimicrobial activity of the materials tested and the mean diameter of the inhibition zones in millimeters. The results demonstrated that all microbial species used in the study were inhibited by the root-end filling materials evaluated. Analysis of the efficacy of the materials against the whole set of microbial strains tested using the Kruskal-Wallis and Dunn tests demonstrated that Sealapex with zinc oxide, zinc oxide and eugenol, and Sealer 26 presented larger inhibition halos than the MTA-based and Portland cements ($P < 0.05$).

Discussion

The agar diffusion method used in the present study is

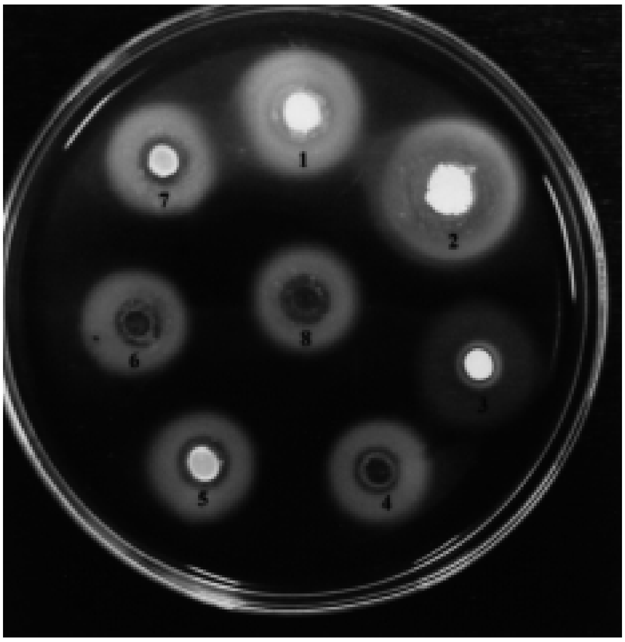

Fig. 1 Inhibition zone for *Staphylococcus aureus* after optimization of the medium with TTC gel. 1: Sealer 26, 2: Sealapex + zinc oxide, 3: Zinc oxide and eugenol, 4: Gray Portland cement, 5: White Portland cement, 6: Gray MTA-Angelus, 7: White MTA-Angelus, 8: Gray Pro Root MTA.

Table 3 Means of the inhibition zones (in mm)*

Microrganisms materials	*M. luteus*	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*E. faecalis*
Sealer 26	24	21.5	12.5	17	23	18.5
Sealapex + zinc oxide	28.5	25.5	21.5	19	28	18.5
Zinc oxide and eugenol	29.5	21	27.5	12	25	16.5
Gray Portland cement	19	17.5	10	14.5	21	17.5
White Portland cement	19	17.5	10	14.5	21	17.5
Gray MTA	18.5	20.5	9.5	14	20	16
White MTA	18	18.5	9.5	14	20	16
Gray Pro Root MTA	18	17.5	11	14	21.5	16

*Means of the triplicate assays

one of the most commonly employed techniques for evaluation of antimicrobial activity (20,21). The prediffusion period, which consists of maintaining the innoculated culture medium at room temperature for 2 hours, is an important step for demonstrating the antimicrobial activity of calcium hydroxide-based materials, such as Sealer 26, Sealapex, and that of MTA-based cements. Our results were in accordance with those obtained by Leonardo et al. (10), who employed similar methodology.

Dental materials/cements may show different degrees of diffusion in agar. Since in this situation the extent of the inhibition zone does not necessarily reflect the strength of the antimicrobial agent, the data corresponding to the antimicrobial activity of each material were analyzed statistically using the Kruskal-Wallis and Dunn tests, as advised by Siqueira et al. (22).

Optimization of the culture medium with 0.05% TTC gel after the incubation period was performed to allow differentiation of growing bacterial colonies. This procedure changes the color of the medium, allowing examination of the bacterial growth inhibition zone, which could otherwise be misinterpreted as being the material diffusion zone (23), as reported in other studies (10,24).

The microorganisms utilized in this study included facultative bacteria and a yeast. These microorganisms are predominant in persistent or refractory periapical lesions in teeth subjected to periapical surgery (25,26).

Our results revealed that all of the materials tested possessed antimicrobial activity, substantiated by the formation of growth inhibition zones in all strains evaluated. The antimicrobial activity of calcium hydroxide-based materials such as Sealapex and 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 (10,27).

The antimicrobial activity of MTA was evaluated by Torabinejad et al. (28), who demonstrated its effectiveness against some facultative bacteria. MTA-based materials and Portland cement contain calcium oxide, which when mixed with water, forms calcium hydroxide, inducing an increase of pH by dissociation of calcium and hydroxide ions, as demonstrated by Duarte et al. (17) for two MTA-based materials: Pro Root MTA and MTA-Angelus.

Therefore, the antimicrobial activity of MTA seems to be associated with elevated pH. Torabinejad et al. (28) observed an initial pH of 10.2 for MTA, rising to 12.5 in 3 h. It is known that pH levels in the order of 12.0 can inhibit most microorganisms, including resistant bacteria such as *Enterococcus faecalis* (29).

The results obtained for Portland cement and MTA by Holland et al. (30) demonstrate that both materials have similar modes of action regarding the mineralization process. In this study, MTA-based and Portland cements had similar antimicrobial activity, suggesting that the addition of a radiopacifying agent to Portland cement during the manufacture of MTA-based material does not hinder its antimicrobial action.

Using similar methodology, Sipert et al. (11) observed in vitro antimicrobial activity for Sealapex, Fill Canal, Pro Root MTA, and Portland cements. However, they did not observe antimicrobial activity for MTA and Portland cement against *E. coli*. This activity was demonstrated in our study, even though these cements produced smaller inhibition areas in comparison to Sealapex with zinc oxide and zinc oxide and eugenol.

The antimicrobial activity of MTA-based materials against Candida albicans observed in the present study can be explained by the sensitivity of this strain to high pH. Al-Nazhan and Al-Judai (31) demonstrated that at a stable concentration of 50 mg/ml, white MTA was able to eliminate *C. albicans in vitro* for up to three days.

According to the methodology proposed, and on the basis of the present results, it may be concluded that all the tested microbial strains were inhibited by the endodontic sealers, Portland and MTA-based cements. Among the evaluated materials, the endodontic sealers produced the largest inhibition halos and differed from the other materials ($P < 0.05$).

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