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Comparison of antimicrobial activity of three root canal sealers: An *in vitro* study

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Abstract
Aim and Objectives: To evaluate the antimicrobial activity of three endodontic sealers AH Plus, Apexit plus and MTA Fillapex on three types of isolated root canal microorganisms: Enterococcus Faecalis, Staphylococcus Aureus and Candida Albicans using agar diffusion test.
Material and Method: Three endodontic sealers were evaluated in this study: AH Plus (Dentsply, Rio de Janeiro, RJ, Brasil), Apexit Plus (Vivadent; Schaan, Liechtenstein) and MTA Fillapex (Angelus, Londrina, PR, Brazil). The following reference strains were included in this study: Candida albicans, Enterococcus faecalis, and Staphylococcus aureus.
Antimicrobial activity of the materials was evaluated by the method of agar diffusion. A base layer of 10 ml of Mueller Hinton agar was poured in Petri plates (90 x 15 mm).
Inhibition halos formed around the wells were measured with the aid of a millimetric rule with 0.5 mm of accuracy. All the experiments were repeated three times in different moments.
Result: In inter group comparison, antimicrobial action of AH Plus was superior to that of Apexit Plus and MTA Fillapex against all the tested microorganisms $p < 0.05$). In intra group comparison AH Plus was significantly more effective against Candida, followed by Staphylococcus aureus and Enterococcus faecalis ($p < 0.05$)
Conclusion: AH Plus showed maximum antimicrobial activity followed by MTA Fillapex against all the tested microorganisms.

Keywords: Endodontic sealer, endodontic microbiology, culture media

Introduction
Microorganisms and their products are the main etiologic factors in dentinal, pulpal, and periapical pathology [1-2]. One of the fundamental steps needed for periapical repair to occur after root canal treatment is the removal of microorganisms and toxins from inside the canal [3]. Instrumentation, irrigation, and endodontic medications are the means to achieve this goal. Nevertheless, the complexity of the internal anatomy of the tooth, [4] particularly at the apical two thirds of the root canals, makes the complete removal of microorganisms a difficult task. Sometimes microorganism’s persistence and reproduction in these areas may even result in a complete failure of the treatment, particularly in the long term. Improvement of the overall success rate of endodontic treatment can be achieved by sealing materials that exhibit both excellent sealing and antibacterial properties.
Endodontic sealers with antimicrobial properties can be advantageous as they would cope better with persisting residual infection and bacteria reentering from the oral cavity [5] It has been shown that several endodontic sealers possess antimicrobial activity [6]. Since it has also been shown that different bacteria may vary in their sensivity to the same material, it was important to use more than one type of the bacteria in the evaluation of antibacterial activity of the sealers [7] Although aerobic and facultative microorganisms 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 [8-10]. 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, Pseudomonas aeruginosa, Enteroccus faecalis and Candida albicans strains have been reported to be resistant to several antimicrobial agents and have also been involved in some persistent and therapy

resistant endodontic infections [11, 12].

Today, numerous root canal sealers are available based on various formulas, such as epoxy resin sealers, calcium-hydroxide-based materials, zinc oxide eugenol cements and newly introduced Mineral trioxide aggregate (MTA) based sealers.

At present epoxy resin based sealers have good physical properties and ensure an adequate biological performance. Excellent apical sealing has been found with epoxy resin based sealers. These sealers are shown to have cytocompatibility, biocompatibility, good tissue tolerance, long term dimensional stability and good sealing ability [13].

The calcium hydroxide based sealers are also widely used in endodontics. These have a good antimicrobial action and also have the ability to stimulate the deposition of hard tissue at the root apex forming a biological seal that would be advantageous to the root canal therapy [14].

Mineral trioxide aggregate (MTA) is a biomaterial that has been investigated for endodontic applications since the early 1990s[15]. MTA is widely accepted for its biocompatibility and excellent sealing capacity [16, 17]. However, despite favourable characteristics, MTA has physical properties that hinder its use for root canal filling [15]. The need for a biocompatible material that induces the formation of mineralized tissue, and also has suitable flow rate and manipulation, led to the development of MTA-based root canal sealers.

The aim of the present study was to evaluate the antimicrobial activity of three endodontic sealers AH Plus, Apexit plus and MTA Fillapex on three types of isolated root canal microorganisms: Enterococcus Faecalis, Staphylococcus Aureus and Candida Albicans using agar diffusion test.

Materials and Method

Three endodontic sealers were evaluated in this study: AH Plus (Dentsply, Rio de Janeiro, RJ, Brasil), Apexit Plus (Vivadent; Schaan, Liechtenstein) and MTA Fillapex (Angelus, Londrina, PR, Brazil). The following reference strains were included in this study: Candida albicans, Enterococcus faecalis, and Staphylococcus aureus. Firstly, four isolates were plated in Petri plates containing Mueller Hinton agar (Difco, Detroit, USA) and incubated at 37 °C for 24 h. Then, standardized suspensions (10^6 cells/ml) of each microorganism in sterile saline solution (NaCl 0.9%) were obtained by spectrophotometry (Micronal S/A – SaoPaulo, SP, Brasil).

Antimicrobial activity of the materials was evaluated by the method of agar diffusion. A base layer of 10 ml of Mueller Hinton agar was poured in Petri plates (90 x 15 mm). After the solidification of the agar, a second layer or seed layer containing 10 ml of Mueller Hinton agar and 200 µl of the microbial standardized suspensions was poured on the first layer.

After solidification of the second layer, three wells of 6 mm of diameter (one for each material) were obtained by removing a standardized portion of the agar in equidistant points with the aid of sterilized plastic straw. The wells were immediately filled with the sealers to be evaluated. The endodontic sealers AH Plus, Apexit Plus and MTA Fillapex were prepared according to the manufacturer's instruction.

Inhibition halos formed around the wells were measured with the aid of a millimetric rule with 0.5 mm of accuracy. All the experiments were repeated three times in different moments.

Results

Mean and standard deviation of the inhibition halos (in mm) obtained for each endodontic sealer tested after the period of 24 h are described in Table 1. *P* values are obtained by Kruskal Wallis Test.

Table 1: Mean and standard deviation of the inhibition halos

Test material	Enterococcus faecalis		Staphylococcus aureus		Candida albicans	
	Mean	SD	Mean	SD	Mean	SD
AH Plus	11.6	0.17	12.8	0.60	15.1	5.77
Apexit Plus	0		0		0	
MTA Fillapex	10.2	1.04	10.6	0.73	9.7	0.89

In inter group comparison, antimicrobial action of AH Plus was superior to that of Apexit Plus and MTA Fillapex against all the tested microorganisms $p<0.05$). In intra group comparison AH Plus was significantly more effective against Candida, followed by Staphylococcus aureus and Enterococcus faecalis ($p<0.05$). MTA Fillapex did not show significant difference in intra group comparison and almost equally inhibited all microorganisms. Apexit Plus did not show inhibition halos against any tested microorganism.

Discussion

Endodontic sealers are essential to achieve the satisfactory marginal sealing and the criteria of a good sealer are to be biocompatible, dimensionally stable, show good flow, low solubility and disintegration, besides having antimicrobial activity[18]. Antimicrobial activity of sealers might help to eliminate residual microorganisms that have survived the chemomechanical instrumentation and thereby improve the success rate of endodontic treatment [19]. Many studies have been performed to evaluate the antimicrobial activity of different endodontic sealers and the most frequently used method to evaluate this activity is the diffusion in agar [20-25].

The agar diffusion method has been widely employed to investigate the antimicrobial activity of dental materials. However, this procedure is influenced by two factors: the materials microbial toxicity as well as the materials, diffusion and affinity in the culture medium [26]. A material that easily diffuses will produce larger zones of inhibition of bacteria [27, 28-33]. In addition, a disadvantage of this method is that it cannot distinguish between bacteriostatic or bacteriocidal properties of the materials. Standardization of these factors allows us to reach more conclusive results and exclude the numerous variables existing *in-vivo*. In this study, the freshly processed root canal sealers were immediately placed into agar plates.

Because of various temporary or permanent by-products, dental materials should be tested immediately after mixing and when final chemical setting stage has been reached. Root canal sealers are used in patients when freshly mixed; thus it is likely that after their clinical application local responses are provoked by leaching components that have partially set or not set at all. However, after setting, toxic ingredients may still be released from the materials. The difference in antimicrobial patterns of various materials may be related to the degree or time taken to set.

The sensitivity of antibacterial properties depends on different factors i.e. type of materials, inoculated bacteria, test method, and interval times. The sealers evaluated in this study showed different inhibitory effects depending on the type of root canal sealers and bacterial strains tested. Overall, AH plus showed the greatest inhibition halo in relation to the other sealers tested. AH Plus showed the maximum inhibition against candida albicans followed by staphylococcus aureus and least against enterococcus faecalis. The antimicrobial activity showed by AH Plus seems to be correlated to the components epoxy resin and amines that are present in this sealer [35]. Pizzo *et al.*, [36] reported that in Direct contact test (DCT) only fresh AH Plus showed antibacterial activity, whereas 24-hour and 7-day-old samples did not show antibacterial effect against E. faecalis. Similar results were reported by Kayaoglu *et al*. [37]. Estudo de Zhang *et al*. [38] also showed that fresh AH Plus had significant antibacterial effect, whereas set samples did not show antimicrobial activity. Apexit plus, a calcium hydroxide based material, was least effective against all organisms and exhibited no activity.

Antibacterial activity of calcium hydroxide based materials depends on ionization that releases hydroxyl ions causing an increase in the pH and has antioxidant effect. A pH > 9 may reversibly or irreversibly inactivate cellular membrane enzymes of microorganisms resulting in a loss of biological activity of the cytoplasmic membrane or leading to the destruction of phospholipids or nonsaturated fatty acids that results in a loss of cytoplasmic membrane integrity [39, 40]. Using the agar diffusion method, the inefficiency of some calcium hydroxide based materials in inhibiting some facultative anaerobic may be because this substance has low solubility and may slowly diffuse in agar [41, 42]. These results are similar to the results of Eldeniz *et al.*, who did not find any antibacterial activity in the Agar Diffusion Test because this substance has low solubility and might diffuse slowly in agar.

MTA fillapex also showed some antibacterial activity against all three microorganisms. Antimicrobial activity of Fillapex can be explained by presence of MTA.MTA based materials contain calcium oxide, which when mixed with water, forms calcium hydroxide, inducing an increase of pH by dissociation of calcium and hydroxide ions [43]. In regard to MTA Fillapex, there are no other data available about its antimicrobial effect.

Conclusion

- AH Plus showed maximum antimicrobial activity followed by MTA Fillapex against all the tested microorganisms.
- No antimicrobial activity was observed for Apexit Plus sealer.
- AH Plus showed the maximum inhibition against Candida albicans followed by Staphylococcus aureus and least against Enterococcus faecalis
- Using the Agar Diffusion Test, the inefficiency of some calcium hydroxide based sealers might be related to low solubility and diffusibilty of these substances in agar
- When new materials are in test, more than one method should be employed.
- To improve the assessment of the antimicrobial activity of root canal sealers, new methods should be developed where there is no interference from the diffusivity and solubility of the material in the culture medium.

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