

Antibacterial activity of two MTA-based root canal sealers

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Abstract

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Aim To evaluate the pH and antibacterial activity of Endo CPM Sealer and MTA Fillapex by two different methods, using white MTA and Endofill as references for comparison.

Methodology Antibacterial activity was evaluated against *Enterococcus faecalis* (ATCC 29212). The agar diffusion test (ADT) was performed to evaluate the effect before setting. The materials were placed in four equidistant wells made in ten agar plates. After incubation at 37 °C for 48 h, the inhibition zones were measured using a digital paquimeter. The direct contact test (DCT) was performed to assess the antibacterial effect after setting. Suspensions of crushed materials were prepared and mixed with *E. faecalis*. After different periods of time (1, 6, 15 and 60 min), the survival of bacteria was assessed by using 10-fold serial dilution and cultivated on agar plates in tripli-

cate. Colony-forming units (CFU) mL⁻¹ were calculated after incubation. pH values were also measured in triplicate. Comparison between sealers in the ADT and DCT was performed by the Kruskal–Wallis test.

Results In the ADT, inhibition zones were found with MTA Fillapex and Endofill. They were similar to each other and greater than the other sealers ($P < 0.05$). None of the tested sealers demonstrated antibacterial activity in the DCT, and thus, all sealers had similar bacterial counts compared with the negative control group ($P > 0.05$). White MTA and Endo CPM Sealer suspensions had pH values >11 , whilst MTA Fillapex and Endofill had lower values.

Conclusions MTA Fillapex and Endofill had an antibacterial effect against *E. faecalis* before setting, but none of the sealers maintained antibacterial activity after setting, despite the high pH of the MTA-based materials.

Keywords: antibacterial activity, Endo CPM Sealer, Endofill, MTA Fillapex, White MTA.

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Introduction

Mineral trioxide aggregate (MTA) is a biomaterial that has been investigated for endodontic applications since the early 1990s (Roberts *et al.* 2008). First, it was suggested to treat root perforations and in root-end fillings (Lee *et al.* 1993, Torabinejad *et al.* 1993).

Currently, it is being used also in conservative pulpal treatments, repair of root resorption and apexification procedures (Menezes *et al.* 2004b, Jacobovitz & Lima 2008, 2009). MTA is widely accepted for its biocompatibility and excellent sealing capacity (Torabinejad & Chivian 1999, Scarparo *et al.* 2010).

However, despite favourable characteristics, MTA has physical properties that hinder its use for root canal filling (Roberts *et al.* 2008). The need for a biocompatible material that induces the formation of mineralized tissue, and also has suitable flow rate and

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manipulation, led to the development of MTA-based root canal sealers. Thus, a new formulation was created: Endo CPM Sealer (EGEO, Buenos Aires, Argentina). The powder consists of fine hydrophilic particles that form a colloidal gel in the presence of moisture, similar to the original MTA (Orosco *et al.* 2008, Gomes-Filho *et al.* 2009).

Another MTA-based root canal sealer with enhanced consistency, MTA Fillapex (Angelus, Londrina, PR, Brazil), is now available. It has resinous components and its manufacturer claims that it has excellent radiopacity, easy handling and great working time. However, there is a lack of scientific information about this new sealer.

Numerous studies have evaluated the effect of MTA on microorganisms associated with endodontic disease with divergent methodologies and results (Torabinejad *et al.* 1995, Estrela *et al.* 2000, Al-Hezaimi *et al.* 2009, Ribeiro *et al.* 2010). There are few investigations about the antimicrobial activity of Endo CPM Sealer (Tanoamaru *et al.* 2008) and none about MTA Fillapex.

The persistence of microorganisms in dentinal tubules, lateral canals and apical ramifications after root canal treatment has been reported (Sjögren *et al.* 1997, Peters *et al.* 2001, Nair *et al.* 2005). If the filling provides a good seal, it will only impair the exit of bacteria entrapped in the root canal system. However, to eradicate the remaining microorganisms, the antimicrobial activity of the sealer could play an important role (Spangberg *et al.* 1973, Nawal *et al.* 2011).

The agar diffusion test (ADT) is used extensively to assess the antimicrobial effect of endodontic sealers, despite its well-known limitations (Cobankara *et al.* 2004). Its results are influenced by the solubility and diffusibility of the material in the culture medium. Also, this test cannot distinguish the microbiostatic and microbicidal properties of the material (Tobias 1988).

On the other hand, the direct contact test (DCT) does not have these disadvantages and it can be used to assess the antimicrobial effect of water-insoluble materials, providing quantitative and reproducible results (Weiss *et al.* 1996, Zhang *et al.* 2009).

Enterococcus faecalis is often used in research that aims to evaluate the antimicrobial properties of endodontic materials. It seems to play a significant role in the aetiology of persistent periradicular lesions (Gomes *et al.* 2006). *Enterococcus faecalis* possesses several virulence factors that contribute to its ability to survive the effects of conventional root canal therapy (Kayaoğlu & Ørstavik 2004). Besides, this Gram-positive facultative anaerobe is able to invade dentine tubules and bind to collagen (Love 2001).

The aim of this study was to evaluate the effect of two MTA-based root canal sealers (Endo CPM Sealer and MTA Fillapex) against *E. faecalis* by two different methods: the ADT and the DCT to assess the antibacterial activity before and after setting, respectively. White MTA and Endofill were used as references for comparison. The pH values were also recorded and correlated to the antibacterial activity results.

Materials and methods

Materials

White MTA, Endo CPM Sealer, MTA Fillapex and Endofill were tested and compared (Table 1). The materials were prepared in accordance with the manufacturer's recommendations.

Agar diffusion test

The microbiological assays were carried out under aseptic conditions in a laminar flow chamber (Quimis,

Table 1 Tested materials and their composition

Materials	Composition	Manufacturer
White MTA	Powder: tricalcium silicate, tricalcium oxide, tricalcium aluminate and other oxides Liquid: distilled water	Angelus, Londrina, PR, Brazil
Endo CPM Sealer	Powder: tricalcium silicate, tricalcium oxide, tricalcium aluminate and other oxides Liquid: saline solution and calcium chloride	EGEO, Buenos Aires, Argentina
MTA Fillapex	Salicylate resin, diluting resin, natural resin, bismuth trioxide, nanoparticulated silica, MTA, pigments	Angelus, Londrina, PR, Brazil
Endofill (positive control)	Powder: zinc oxide, hydrogenated resin, bismuth subcarbonate, barium sulphate, sodium borate Liquid: eugenol, sweet almond oil	Dentsply, Petrópolis, RJ, Brazil

Diadema, SP, Brazil). The antibacterial activity was evaluated using a standard strain of *E. faecalis* (ATCC 29212). The microorganisms were cultivated in Brain Heart Infusion – BHI broth (Merck, Darmstadt, Germany) at 37 °C for 18 h. Then, a bacterial suspension was prepared with 0.85% saline solution to match the turbidity equivalent to 1.0 McFarland standard tube, corresponding to 3×10^8 CFU mL⁻¹.

Ten replica plates containing BHI agar were spread with 0.1 mL of the bacterial suspension, using a Drigalsky's loop. Thereafter, four wells of 6 mm in diameter and 4 mm in depth (one for each material) were made with a punch by removing the agar at equidistant points and then filled immediately with the materials to be evaluated. Two plates did not receive the bacterial suspension; one did not receive the sealers and aimed to control the sterilization of the culture medium, whilst the other received the sealers and aimed to control their contamination.

All plates were maintained at room temperature for 2 h for prediffusion of the materials and then incubated at 37 °C for 48 h under aerobic conditions. The inhibition zones around each one of the wells were then measured in millimetres using a digital paquimeter (Digimess, São Paulo, SP, Brazil).

Direct contact test

The methodology used was adapted from Zhang *et al.* (2009). The endodontic sealers were manipulated according to manufacturers' instructions and inserted in a glass device with four orifices of 5 mm in diameter and 5 mm in depth (one for each material). They were allowed to set at 37 °C in 100% humidity for 7 days. Next, the blocks of set sealers were crushed to powder with ceramic mortar and pestle (CoorsTek, Golden, CO, USA). The powder was sterilized by ethylene oxide gas (Esterilplus LTDA, Porto Alegre, RS, Brazil). Suspensions of each crushed material were prepared with saline solution at concentrations of 50 mg mL⁻¹.

Bacterial suspensions were prepared as described for the ADT and mixed with sealers suspensions in equal volumes (500 µL) inside polypropylene microtubes. Saline solution without sealers served as a negative control. After incubation at room temperature for 1, 6, 15 and 60 min, the survival of the bacteria in the solutions was assessed by 10-fold serial dilutions to 10⁻⁹ and culture on BHI agar plates. After incubation at 37 °C for 48 h, colonies on the plates were counted and CFU mL⁻¹ was determined. All experiments were made in triplicate.

Measurements of pH

Suspensions of each crushed material were prepared with deionized water at concentrations of 50 mg mL⁻¹. The pH of the supernatant of each sealer suspension was measured with a pH meter (Digimed, São Paulo, SP, Brazil) previously calibrated at room temperature (25 °C). Before the measurements, the suspensions were mixed by vortexing for 30 s and centrifuged for 30 s to allow measurement of the clear supernatant. The pH values were evaluated 1, 6, 15 and 60 min after preparing the suspensions. Deionized water was used as a control. All experiments were performed in triplicate. The mean values of pH with the standard deviation were calculated.

Statistical analysis

To analyse data obtained in the ADT and for comparisons amongst sealers at each experimental period in the DCT, Kruskal–Wallis and Dunn tests were applied. For comparisons between experimental periods at each sealer in the DCT, Friedman test was applied. The level of significance was established at 5%. Data from pH analysis were submitted to descriptive statistics. Statistical analysis was performed with the software BioEstat 5.0 (CNPq, Brasília, DF, Brazil).

Results

Table 2 shows data obtained in the ADT for each sealer. Endofill (positive control) had the largest inhibition zone (Fig. 1a,c), similar to MTA Fillapex (Fig. 1a) and greater than the other sealers ($P < 0.05$). White MTA (Fig. 1a,b) and Endo CPM Sealer (Fig. 1a) themselves resulted in diffusion in the agar, but they were not able to inhibit *E. faecalis*. There was no bacterial growth on the two control plates.

Table 2 Mean values (in mm), standard deviation and the ranks average of the bacterial inhibition zones for each sealer in the ADT

Materials	Inhibition zones	Ranks average
White MTA	0 ± 0	10.50 ^a
Endo CPM Sealer	0 ± 0	10.50 ^a
MTA Fillapex	7.32 ± 0.33	25.50 ^b
Endofill (positive control)	9.55 ± 0.19	35.50 ^b

Different small letters indicate statistically significant difference between sealers according to the Dunn test ($P < 0.05$).

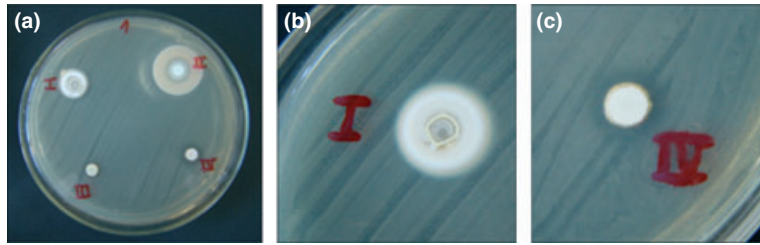


Figure 1 Agar diffusion test: (a) Diffusion of White MTA (I) and Endo CPM Sealer (II) and inhibition zones for MTA Fillapex (III) and Endofill (IV); (b) Diffusion of White MTA (I), increased image; (c) Inhibition zone for Endofill (IV), increased image.

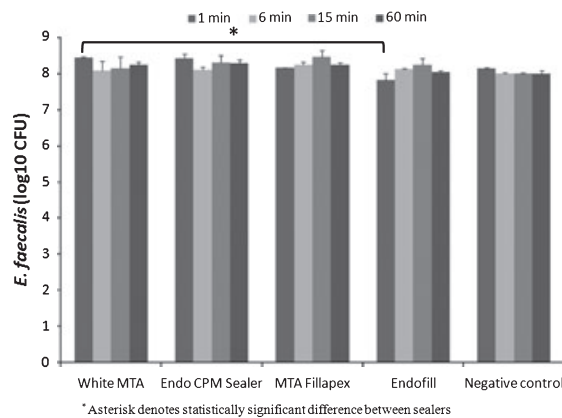


Figure 2 Survival of *Enterococcus faecalis* after incubation with sealer suspensions at different experimental periods in the direct contact test.

The results of the DCT are showed in Fig. 2. None of the set materials had antimicrobial activity against *E. faecalis*. There was a significant difference between the bacterial counts of white MTA and Endofill in the first-minute period ($P < 0.05$). However, the bacterial counts of all sealers were similar to the negative control group in all experimental periods ($P > 0.05$). Moreover, there were no significant differences throughout the experimental periods for any sealers ($P > 0.05$).

The mean pH values for the sealers suspensions are described in Table 3. White MTA and Endo CPM Sealer suspensions presented pH values >11 at all experimen-

tal periods. MTA Fillapex and Endofill had lower but also alkaline values. Deionized water had a neutral pH.

Discussion

This study investigated the pH and antibacterial activity of different endodontic sealers against *E. faecalis*. In the ADT, it was possible to observe inhibition zones with MTA Fillapex and Endofill. This fact could be explained by the presence of resin and eugenol, respectively. In regard to MTA Fillapex, there are no other data available about its antimicrobial effect. On the contrary, zinc oxide and eugenol-based sealers, such as Endofill, have been investigated extensively and have been used as positive controls in antimicrobial activity assays (Gomes *et al.* 2004, Tanomaru *et al.* 2008, Pinheiro *et al.* 2009).

White MTA and Endo CPM Sealer did not have inhibition zones against *E. faecalis*. Similar results were reported previously by Estrela *et al.* (2000), who used grey MTA. On the other hand, Tanomaru *et al.* (2008) verified that white MTA and Endo CPM Sealer had inhibition zones of 15 and 12 mm against *E. faecalis*, respectively. These two sealers showed visible diffusion in the agar medium, which could lead to misinterpretation of their antibacterial activity.

None of the set sealers had antibacterial activity in the DCT. White MTA and Endo CPM Sealer allowed the survival of *E. faecalis*, despite their high pH. According to McHugh *et al.* (2004), *E. faecalis* is unable to live at the pH of 11.5 or greater. As the pH shown by the

Table 3 Mean values of pH for each sealer at different experimental periods

Time (minutes)	White MTA	Endo CPM Sealer	MTA Fillapex	Endofill	Control
1	11.64 ± 0.17	11.39 ± 0.31	10.49 ± 0.07	8.22 ± 0.29	6.33 ± 0.04
6	11.83 ± 0.18	11.23 ± 0.12	10.5 ± 0.02	8.56 ± 0.26	7.82 ± 0.42
15	11.84 ± 0.2	11.36 ± 0.24	10.46 ± 0.02	8.63 ± 0.35	7.79 ± 0.54
60	11.84 ± 0.2	11.19 ± 0.09	10.14 ± 0.21	8.27 ± 0.54	7.07 ± 0.37

above-mentioned sealer was between 11 and 12, it can be assumed that its alkalinity was not enough to make the environment unsuitable for the survival of that microorganism. Its proton pump is probably the key factor in its resistance to alkaline agents (Stuart *et al.* 2006).

These findings contrasts with those by Zhang *et al.* (2009), who reported a significant decrease in bacterial viability within 6 min of contact with grey MTA powder. White MTA powder was employed in the present study; thus, the results could not be directly compared. Holt *et al.* (2007) reported that grey MTA showed greater *E. faecalis* growth inhibition than white MTA and this could explain the divergence.

An important goal of root canal treatment is to eliminate or prevent the introduction of microorganisms into the root canal system (Siqueira & Roças 2008). It is well known, however, that chemomechanical preparation is not able to completely eradicate endodontic infections (Nair *et al.* 2005). Residual bacteria may remain untouched by instruments, irrigants and medicaments (Sjögren *et al.* 1997). To prevent new bacteria growth, filling materials and sealers should have antimicrobial properties upon contact with microorganisms and biofilms especially before setting. After this period, the most important property of the endodontic sealer should be its sealing ability.

Enterococcus faecalis was chosen as the target microorganism because of its high prevalence in persistent endodontic infections, ranging from 24% to 77% (Stuart *et al.* 2006). *Enterococcus faecalis* can compete with other microorganisms and adapt to adverse conditions, such as nutritional deprivation (Kayaoglu & Ørstavik 2004). This microorganism is resistant to several irrigants and intracanal medicaments used in endodontics (Menezes *et al.* 2004a, Zehnder & Guggenheim 2009). Therefore, the antibacterial activity of endodontic sealers against *E. faecalis* is important in clinical practice.

Historically, two different assays have been used to test the antimicrobial characteristics of endodontic sealers: the ADT and the DCT. In this study, the first test was used, despite its limitations, to evaluate fresh sealers immediately after their manipulation. The second test was performed to analyse set sealers, 7 days after their mixture. In the ADT, the size of the inhibition zones from a certain substance depends on its diffusibility in the culture medium used. This fact is the main disadvantage of this semi-quantitative method (Nawal *et al.* 2011). However, the ADT is suitable to indicate the activity of freshly mixed materials, and its

inclusion is interesting for comparative reasons with previous studies.

In turn, the DCT relies on direct contact between the microorganism and the tested material. This method is virtually independent of the diffusion and solubility properties of both the material and the media (Weiss *et al.* 1996). In contrast with the ADT, the DCT is capable of showing the antibacterial activity of insoluble components. When new materials are in test, more than one method should be employed (Nawal *et al.* 2011).

To improve the assessment of the antibacterial 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.

Conclusion

MTA Fillapex and Endofill had an effect against *E. faecalis* before setting, but they did not maintain the antibacterial activity 7 days after mixture. Despite their alkaline pH, white MTA and Endo CPM Sealer did not have antibacterial activity either before or after setting.

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