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Antimicrobial activities of different bioceramic root canal sealers on various bacterial species

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Abstract
Objective: The aim of the present study was to evaluate the antimicrobial activity of new bioceramic root canal sealers Smartpaste Bio, MTA Fillapex and AH plus.
Materials and Methods: The antimicrobial activity of sealers was tested by agar diffusion method on the surface of agar plates of *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa*, *Candida albicans* and *Escherichia coli*. The sealers were placed into the prepared well on the agar plates. The diameters of the inhibition zones were measured after incubation for 24, 48, 72 hours. The data were analyzed statistically by ANOVA and Duncan's tests at 5% significance level.
Results: All tested sealers had antimicrobial effect against to test microorganisms. Smartpaste Bio had the biggest inhibition zone and the lowest had by MTA Fillapex and there was statistically difference between Smartpaste Bio and MTA Fillapex ($p<0.05$). The mean of inhibition zones was decreased in order 24, 48, 72 h time intervals for each group. There was statistically significant difference between 24 and 72 h mean of inhibition zone measurements ($p<0.05$).
Conclusion: In comparison, all tested substance exhibited antimicrobial effect on standard strains however Smartpaste Bio had the biggest inhibition zone measurement than the other groups.

Keywords: Smartpaste Bio; MTA Fillapex; AH Plus; Root Canal Sealers; *Enterococcus faecalis*

Introduction
The main purpose of endodontic treatment is the elimination of microorganism from root canal system [1, 2]. Microorganisms and their byproducts are regarded as the primary etiological factors of periapical diseases and pulpal necrosis [3-5]. Microbial reduction obtained by mechanical instrumentation, irrigation, using intracanal medicaments and adequate filling of root canal [6].
The primary reasons of endodontic treatment failure are the persistence of microorganisms in the root canal system due to anatomical complexities such as; apical ramifications, dentinal tubules, lateral canals which cannot be cleaned with chemo-mechanical procedures [7, 8]. Thus, the usage of root canal sealers with antimicrobial properties may reduce the remaining microorganisms or even eradicate the infection completely [9].
There have been several studies that evaluate the antimicrobial activity of sealers [10-12]. AH Plus (Dentsply DE Trey, Konstanz, Germany) is an epoxy resin based sealer and has been shown to be suitable for successful endodontic therapy because of its antimicrobial activity [4, 13, 14]. MTA Fillapex (Angelus, Londrina, PR, Brazil) was introduced as a MTA based root canal sealer. Its chemical composition is, silica nanoparticles, synthetic portland cement, bismuth oxide and butyl ethylene glycol disalicylate. According to manufacturer, it has excellent biocompatibility easy handling, low solubility, adequate working time and radiopacity [15]. There are not many studies about antimicrobial activity of MTA Fillapex and have shown different results against different microorganisms such as *E. Faecalis, S. mutans, E. coli* [1, 4, 16].
Smartpaste Bio (Smart Seal DRFP Ltd, Stamford, England) is a calcium silicate based sealer that has latterly introduced. According to manufacturer Smartpaste Bio is insoluble, injectable hydrophilic, nonresorbable and radiopaque. During the polymerization process it releases calcium hydroxide and hydroxyapatite and very biocompatible once set [17]. There is a shortage of information about this sealer in literature and there is no study about the antimicrobial activity of Smartpaste Bio.

The aim of the current study is to evaluate the antimicrobial activity of Smartpaste Bio, MTA Fillapex and AH Plus against *Candida albicans, Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* at three different time intervals.

Materials and Methods

The composition of the materials that used in the present study (Smartpaste Bio, MTA Fillapex, AH Plus) was showed in Table 1. *E. faecalis* (ATCC 29212), *S. aureus* (ATCC 29213), *C. albicans* (ATCC 10231), *P. aeruginosa* (ATCC 27853) and *E. coli* (ATCC 25922) strains were used (Figure 1).

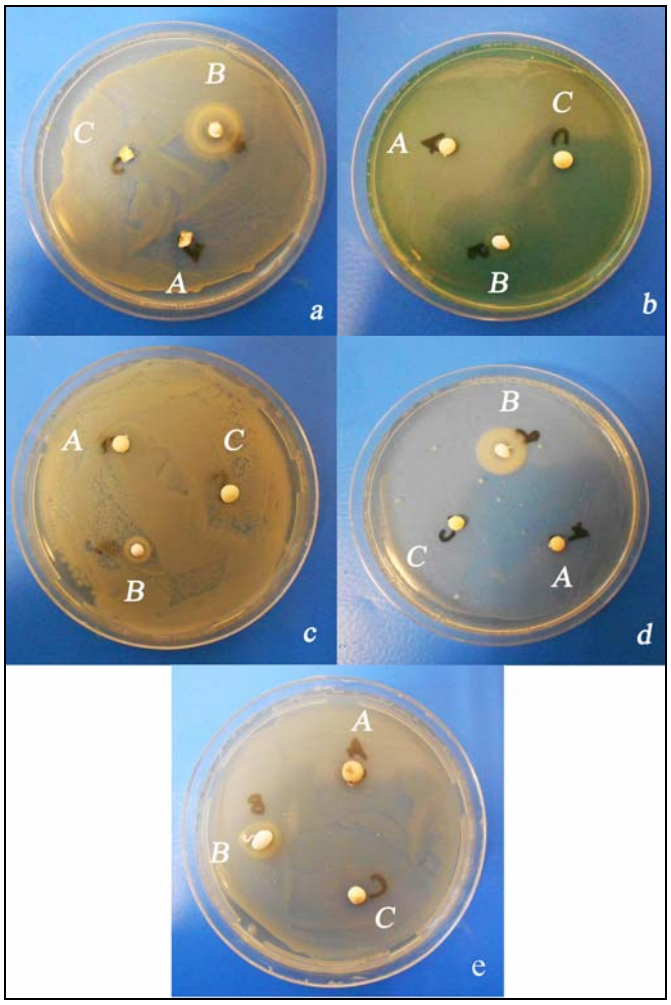

Fig 1: Inhibition zones prometed by sealers. A. MTA Fillapex B. Smartpaste Bio C. AH Plus. *C. albicans* (a), *P. aeruginosa* (b), *S. aureus* (c), *E. faecalis*(d), *E. coli* (e).

The microbiological assays were performed under aseptic conditions inside a class 2 cabinet (Danlaf VFRS 1806, Denmark) previously sterilized. Single operator performed all testing procedures with sterile instruments.

Agar diffusion test (ADT) was used for determination of antimicrobial activities of test materials. The microorganisms inoculated in Brain Heart Infusion (Difco, USA) and incubated for 24 h at 37 °C. After incubation, the broth cultures were adjusted to 0.5 McFarland by using McFarland densitometer. Then, 100 µl aliquots *C. albicans* suspension was streaked onto Sabouraud Dextrose Agar (Merek, Darmstadt, Germany) and other bacterial suspensions were streaked onto Mueller Hinton Agar (Oxoid, UK).

A total of 20 plates were dried at room temperature for 2 h. Three wells (diameter: 3mm, depth: 3mm) were punched in agar and filled with freshly mixed sealers according to manufacturer's instructions. The wells were located in order to create safe distance from each other and the edges to not overlap the inhibition zones around the wells. The plates contains only microorganism strains were used as positive control and distilled water as negative control group.

All plates were kept at room temperature for 2 h for prediffusion of the sealers; afterwards, plate were incubated at 37 °C for 24, 48, 72h. At the end of each incubation period, inhibition zones were measured in millimeters with a digital caliper (Mitutoyo, Japan). Each tests was performed triplicate and the mean value was detected.

Data were analyzed by using One-way ANOVA and Duncan's test. Statistically significant differences were set at $p<0.05$.

Table 1: Chemical composition of the experimental materials

Material	Composition
Smartpaste Bio	Zirconium oxide, calcium slicates, calcium phosphate monobasic, calcium hydroxide, filler and thickening agents.
MTA Fillapex	Salicylate resin, diluting resin, natural resin, bismuth trioxide, nanoparticulated silica, MTA, pigments
AH Plus	Epoxide paste: Diepoxide, calcium tungstate, zirconium oxide, aerosil, pigment Amine paste: 1-adamantane amine, N,N'-dibenzyl-5-oxa-nonandiamine-1,9, TCD-Diamine, calcium tungstate, zirconium oxide, aerosil, silicone oil

Results

Antimicrobial activities were assessed with the measurement of inhibition zones forming around the sealers. The mean values of microbial inhibition zones for each sealer against microorganisms are shown in Table 2. In all positive control group plates bacterial growth detected while in the negative control did not.

All tested sealers had antimicrobial effect against to test microorganisms. At all-time intervals Smartpaste Bio showed significant inhibition on bacterial growth ($p<0.05$), except *P. aeruginosa*. There was significant difference between the antimicrobial activity of Smartpaste Bio and MTA Fillapex ($p<0.05$). There was no statistically significant difference between Smartpaste Bio and AH Plus ($p>0.05$) but Smartpaste Bio showed greatest antimicrobial effect ($p<0.05$).

Smartpaste Bio showed the highest antimicrobial activity and MTA Fillapex showed the lowest and there was statistically difference between all sealers against all microorganism groups except *P. aeruginosa* ($p<0.05$). At *P. aeruginosa* group AH Plus had the greatest antimicrobial effect than others.

Antimicrobial effect was decreased in order 24, 48, 72 h for each group. Each root canal sealer had biggest antimicrobial activity at 24 h and the lowest antimicrobial activity at 72 h.

Table 2: The mean values and standard deviations of inhibition zones provided by the tested materials in 24, 48, 72-hours

Microorganisms	Time (hour)	Smartpaste Bio	MTA Fillapex	AH Plus
E. coli	24	10.05±0.23	6.02±0.24	6.25±0.32
	48	9.45±0.22	5.48±0.22	4.35±0.30
	72	8.51±0.13	4.52±0.24	3.45±0.12
S. aureus	24	11.75±0.36	6.00±0.38	7.25±0.35
	48	10.15±0.33	5.46±0.31	6.35±0.32
	72	9.54±0.26	4.51±0.18	5.45±0.25
P. aeruginosa	24	9.00±0.32	9.25±0.35	26.00±0.23
	48	8.46±0.30	8.42±0.32	19.40±0.21
	72	7.45±0.12	7.55±0.25	18.45±0.13
E. faecalis	24	11.75±0.42	6.00±0.36	7.00±0.44
	48	10.11±0.42	5.35±0.33	6.42±0.40
	72	9.34±0.32	4.40±0.26	5.54±0.24
C. albicans	24	8.00±0.25	6.05±0.18	7.00±0.34
	48	7.48±0.20	5.36±0.11	6.30±0.30
	72	6.51±0.15	4.42±0.28	5.51±0.14

Discussion

Antimicrobial properties of sealers may help to reduce microorganisms after chemo-mechanical procedures. The present study evaluated antimicrobial effect of Smartpaste Bio, MTA Fillapex and AH Plus against to five species of microorganisms by using ADT. This technique has been universally used to evaluate the antimicrobial activities of dental materials. It allows the comparison of sealers against the test microorganisms [18].

It has been stated that several microorganisms play role in the progression of pulpal and periapical diseases and endodontic treatment failures. The microorganisms selected for this study were known as the most common bacteria that are presenting in the oral cavity and infected root canals [19-21]. Facultative anaerobic microorganisms such as *S. aureus, E. faecalis and C. albicans* regarded to have higher resistance in oral cavity and potential cause for endodontic treatment failures [14]. *E. faecalis* was chosen due to be association with persistent periapical infections and difficulty of elimination from root canal. It has been reported that the presence of *C. albicans* in root canals may be associated with periapical pathosis [4, 22] and endodontic treatment failures [23-25]. Therefore the antifungal activity of root canal sealers has an effect on infection control [24].

The present results showed that Smartpaste Bio had more antimicrobial affect than AH Plus and MTA Fillapex against to all test microorganisms except for *P. aeruginosa.* However, root canal sealers used in present study lost their antimicrobial activity depending on time. Similarly a previously study showed that antimicrobial activities of root canal sealers related with the time intervals between mixing and testing [26]. Relating to Smartpaste Bio there is no data available about its antimicrobial activity. However there are studies that evaluated antimicrobial effects of some other calcium silicate phosphate based sealers. Zhang [27] reported that antimicrobial effect of another calcium silicate phosphate based sealer derives from high pH of the sealer depending on calcium hydroxide release during polymerization. According to Wang [28] calcium silicate based sealers long-lasting antimicrobial ability can also be due to the biomineralization process induced by calcium silicates/phosphates from the sealer together with the participation of dentin mineral. In the light of these studies, the antimicrobial activity of Smartpaste Bio against to the microorganisms could be attributed to release of diffusible substances such as calcium silicate, calcium phosphate, and calcium hydroxide and to its high alkali pH [29]. Release of these substances increase pH over 9 and this may reversibly/irreversibly inactivate cell membrane enzymes resulting in a loss of biological activity [30]. Also silica dissolved in a high pH environment may directly inhibit bacterial viability [29].

C. albicans reported as the most commonly isolated fungi from root canals. Dohaithem [31] reported calcium silicate based root canal sealers showed antifungal effect. Similarly current study showed that Smartpaste Bio has better antifungal effect on *C. albicans* than other sealers. This antifungal effect was attributed to its high pH levels.

Several studies assessed the antimicrobial effect of MTA on microorganism associated with endodontic disease [1, 2, 32]. Razmi [5] and Lovato and Sedgley [11] stated that MTA showed antimicrobial effect on *E. faecalis*. Torabinejad *et al*. [32] stated that MTA has some antimicrobial effect on facultative anaerobic bacteria, but no effect on those surely anaerobic. Kuga *et al*. stated that MTA Fillapex showed antimicrobial activity against *S. aureus* and *E. faecalis* [16]. Morgental *et al*. achieved that MTA Fillapex has an antimicrobial activity against *E. faecalis* before setting but sustain the antimicrobial effect 7 days after mixture [1]. Ozcan *et al*. concluded that freshly mixed MTA Fillapex has moderate antifungal effect on *C. albicans* [4]. Similar to previous studies, in this study MTA Fillapex was shown antimicrobial effect on all tested microorganisms at 24, 48, 72 h. This antimicrobial effect was attributed to its hydrophilicity, active calcium hydroxide release and high pH [11, 22, 33].

It has been shown that epoxy resin based sealers have antimicrobial effect. In a previous study AH Plus on the freshly mixed condition showed inhibitory effect on microorganisms [27, 34]. Another study stated that AH Plus exerted a strong antimicrobial effect and led to a significant decrease of cell proliferation [35]. Saleh *et al*. reported that AH Plus killed all bacteria in dentinal tubules [36]. In present study, AH Plus has antimicrobial effect on all test microorganisms. This effect might be associated with bisphenol-A-diglycidyl ether [3, 13] or the minimal amount of formaldehyde release [10, 14].

Conclusion

The present findings indicate that Smartpaste Bio displayed a higher antimicrobial effect than other testing materials. Additionally the date presented in this study relate to in vitro conditions. In vivo studies recommended evaluating the antimicrobial activity of Smartpaste Bio. Also long term follow-up of patients treated with Smartpaste Bio will be helpful for antimicrobial evaluation.

Acknowledgement
The authors deny any conflicts of interest related to this study.

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