Original Study

IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF THREE BIOCERAMIC ENDODONTIC SEALERS AGAINST ENTEROCOCCUS FAECALIS AND STAPHYLOCOCCUS AUREUS

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ABSTRACT

Aim: Many microorganisms remain in root canal and dentinal tubules even after debridement and root canal preparation. This confirms the need for the use of appropriate antimicrobial endodontic sealers. Recently, bioceramic sealers have been introduced that can be used as a successful sealer in endodontic treatments due to their well-known biological properties. The aim of this study was to determine the antibacterial effect of Endoseal-MTA, MTA-fillapex and SureSeal against Enterococcus faecalis and Staphylococcus aureus by agar diffusion test.

Materials & Method: E. faecalis and S. aureus were cultured on a Muller Hinton Agar medium, which was prepared in 10 cm plates. Endoseal-MTA, MTA-fillapex and SureSeal were prepared according to the manufacturer in sterile conditions and transferred to the wells that prepared using a punch, at a distance of 2-3 cm from each other in the plate. The prepared plates were incubated for 72 hours and the inhibition zones around each well were measured. This test was done for each sealer with 16 replicates and data were analyzed using SPSS.

Results: The mean diameter of the inhibition zones of S. aureus for the MTA-Fillapex, Sureseal and Endoseal-MTA sealers were 11.56, 11.62, 13.68 mm, respectively (p=0.00). Moreover, the mean inhibition zones of E. faecalis for MTA-Fillapex was 13.65 mm and zero for two other sealers (p=0.00).

Conclusion: We found that all sealers had antibacterial activity against S. aureus, while Endoseal-MTA and Sureseal sealers had no effect on E. faecalis and just MTA-Fillapex showed an appropriate antibacterial effect against E. faecalis.

Key words: Antibacterial activity, Bioceramic sealers, Enterococcus faecalis, Staphylococcus aureus.

Introduction

Microorganisms are the main etiological causes of pulpitis.[1] Successful treatment of endodontic diseases depends on complete removal of the microbial load by chemo-mechanical preparation of root canal.[2] Previous studies have shown that microorganisms may remain in the canal and dentin tubules even after treatment.[2,3] Also, in inappropriate aseptic conditions, the bacteria in the oral cavity may penetrate to the root canal during root canal treatment procedure and can cause infection.[4] Various microorganisms such as Enterococcus faecalis, Staphylococcus aureus and Candida albicans are the most resistant species in the oral cavity which consider as the failure etiologies of the root canal treatment.[5] Most of root canal treatments have a central material together a sealer. The central material occupies space and the sealers fill the canal irregularities.[6-8] Therefore, when pulp necrosis and apical periodontitis exist, choosing sealers with antimicrobial activities can be helpful for reducing and avoiding the growth of the remaining microorganisms.[9]

Sealer' antimicrobial activity increases the success rate of root canal therapy by eliminating infections of the root canal that remains during or after treatment.[9-11] Thus, choosing sealers with high antimicrobial properties can be helpful to treat endodontic infections, especially recurrent infections by high resistance bacteria. There are several types of sealers with different bases and constituents that provide different antimicrobial properties. A new group of sealers which have only been available for use in endodontics in the last 30 years is bioceramic based [10] and little research have been taken about antimicrobial properties of these sealers. Increasing the types of these bioceramic sealers and the more tendency to use them due to the biocompatibility properties [12] instigate us to evaluate the antibacterial properties of three bioceramic sealers against two bacterial species E. faecalis and S. aureus that are commonly found in failure of root canal treatment.

Materials & Method

Endoseal-MTA from Maruchi products (MARUCHI Products, South Korea), MTA-fillapex fron Angelus Lordina (Angelus, Londrina, PR, Brazil) and SureSeal from Sure-endo (Sure-endo, South Korea) were purchased.

The Mueller Hinton Agar (MHA- Merck; Germany) medium was made according to the manufacturer in 10 cm plates. Using normal saline and based on half-McFarland turbidity, a suspension of E. faecalis (ATCC 29212) and S. aureus (ATCC 25923) contained 1.5×108 CFU/milliliter was prepared. The bacterial suspension was cultured on the plate using a sterile swab. Then, certain wells were made up in each plate by a sterile punch at a distance of 2 cm from each other. Sealers were prepared according to the manufacturer's instructions in sterile conditions and 100 µl of each sealers was transferred to the wells. The prepared plates were incubated at 37 °C for 72 hours and the inhibition zones around each well were measured by a proper ruler at 24, 48, 72 hours in 16 replicates for each sealer against each bacterial species. The antibacterial effects of 100 µl of serial dilutions of 0.5, 0.25, 0.125, 0.0625, 0.0312 of phenol in the MHA was also evaluated as standard against both bacterial species.

Statistical analysis

Data were analyzed using SPSS version 24. One way analysis of variance (ANOVA) and TuKey as Posthoc test

were used to compare the antibacterial effects of different sealers and standard. $p<0.05$ was considered as significant difference.

Results

The mean and SD of diameters of inhibition zone for each sealers in each bacterial species are presented in Table1. As shown, all sealers showed antibacterial effects against S. aureus and among them, the Endoseal-MTA was more portent due to higher diameter of inhibition zone. However, only MTA-Fillapex sealer showed antibacterial effect against E. faecalis and two other sealers had no antibacterial effect.

	Mean diameter of inhibition zone (mm) ± SD	
Sealer	S. aureus	E. faecalis
MTA-Fillapex	11.62±0.5	13.65±0.47
Sureseal	11.56±0.51	0
Endoseal MTA	13.68±0.47	0
p-value	0.00	0.00

Table 1: Mean±SD of diameters of inhibition zone in each groups

The inhibition zones of different concentration of phenol are shown in Table 2. As seen, the sealers showed lower antibacterial activities in comparison of almost all concentration of phenols.

	Diameter of inhibition zone (mm)	
Phenol concentrations	S. aureus	E. faecalis
0.5	30	24
0.25	26	19
0.125	15	15
0.0625	11	11
0.0312	10	10

Table 2: Antibacterial effect of different concentration of phenol on each bacteria.

Discussion

Bacteria and their products are known as one of the main etiologies of pulp necrosis and root canal treatment failure.[4,5,13] Therefore, the main purpose of root canal treatment is the removal of microorganisms and preventing them from spreading in the root canal system.[2,4,5,8,9,14,15] To reach that, filling materials and sealers must have antimicrobial properties, especially before setting.[15,16] In this study, the effect of three types of bioceramic bases sealers against E. faecalis and S. aureus was investigated. We found that these sealers were more potent against S. aureus and just MTA-Fillapex sealer had antibacterial effect against E. faecalis. Moreover, the largest inhibition zones (12 mm) against S. aureus and E. faecalis obtained by Endoseal-MTA and MTA-Fillapex, respectively.

S. aureus is a Gram-positive bacterium, which causes secondary endodontic infections, which occur in the root canal system after the beginning of tooth treatment, and is found in endodontic failed treatment.[14,16] In addition, this bacterium is commonly used as the standard organism in antimicrobial tests.[17] The agar diffusion test is routine technique for evaluation of antimicrobial properties. Although this test does not consider factors such as tooth anatomy and biofilm formation by microorganisms, but it provides the ability to comprise antimicrobial properties of sealers against tested microorganisms and shows which sealer have the potential to eliminate the bacteria in local microenvironment of root canal.[17,18] Also, this test is an accurate method for evaluation of newly mixed sealers and non-set materials.[15,16,19] The disadvantages of this method are that the results of the study not only depend on the effect of the toxicity of the material on a specific microorganism, but also largely influenced by the diffusion of the material in the agar medium.[15,18]

Bioceramic sealers are biocompatible agents with osteogenic constituents such as calcium silicate and calcium phosphate and present suitable antibacterial activity and flow properly.[12,20-23] It has been reported that the antimicrobial properties of root canal sealers are related with alkaline structure and calcium ion release.[5] Indeed, use of alkaline materials improves the mineralization of hard tissue and increases antibacterial activity.[24] On the best of our knowledge, there are no previous studies on the antibacterial properties of Endoseal-MTA and Sureseal sealers. In line with our study, Morgental *et al*. evaluated the antibacterial activity of MTA-Fillapex against E. faecalis and found that MTA-Fillapex exhibited the largest inhibition zone compared to two White MTA and Endofill.[15] Also, Kuga *et al*. reported that MTA-Fillapex, AHplus, and Sealapex had antibacterial properties against E. faecalis and S. aureus. They found that all sealers had approximately same effects against E. faecalis but in the Sealapex showed higher antibacterial effect against S. aureus.[18] In our study, all sealers showed antibacterial properties against S. aureus and Endoseal-MTA had the highest antibacterial effect; while Endoseal MTA and Sureseal had no antibacterial activity against E. faecalis unlike MTA-Fillapex. This can be considered as the presence of resins in the MTA-Fillapex compositions and its effect on E. faecalis.[15]

On the other hand, we compared the antibacterial properties of these sealers with phenol as a standard antibacterial substance. Phenol (C_6H_5OH) is one of the oldest organic disinfectants compound which is bacteriostatic and antifungal at concentration of 1-2%.[25] We found that MTA-Fillapex and SureSeal sealers had antibacterial effect against S. aureus as similar as to 6% phenol. While antibacterial activity of Endoseal-MTA sealer is equivalent to 6-12% phenol. About such equivalency against E. faecalis, the antibacterial property of the MTA-Fillapex sealer is equivalent to a concentration of 6 to 12% phenol. However, SureSeal and Endoseal-MTA sealers did not show any antibacterial effect against E. faecalis.

The results of the present study showed that sealers with bioceramic bases had restrictive effects on S. aureus, while

only MTA-Fillapex sealer exhibited an inhibitory effect on E. faecalis, as a resistant bacterium of root canal infections. The other two sealers did not show any limiting activity of bacterial growth.

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