Print ISSN: 2321-6379
Online ISSN: 2321-595X

Original Article

An Antimicrobial Activity Assessment of Three Endodontic Sealers on *Enterococcus faecalis*, *Candida albicans*, and Staphylococcus aureus by a Direct Contact Test: An *In Vitro* Study

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Abstract

Introduction: For a successful endodontic treatment, antimicrobial property of endodontic filing is mandatory. Hence, the aim of this *in vitro* study was to assess the antibacterial efficacy of three endodontic sealers on *Enterococcus faecalis*, *Candida albicans*, and *Staphylococcus aureus* by direct contact test (DCT).

Materials and Methods: The antimicrobial efficacy of three different sealers, AH Plus, mineral trioxide aggregate (MTA) Fillapex, and Endosequence BC was tested against *E. faecalis* ATCC 29212, *C. albicans* ATCC 10231 and *S. aureus* ATCC 25923 by DCT. Freshly mixed sealers were placed in flat bottom test tubes incubated at 37°C. Thereafter, 10 μL of suspension was drawn and spread over cultural plates to determine the colony count using a digital colony counter. Readings were taken at 1 h (fresh specimen), then at 24 h (after setting). The results were tabulated and statistical analysis was done using one way ANOVA and Tukey HSD test.

Results: DCT showed a significant decrease in microbial count in AH Plus, MTA Fillapex, and Endoseq BC at both the time intervals. Group 3 (Endosequence BC) showed minimum microbial count followed by Group 2 (MTA Fillapex) and maximum for Group 1 (AH Plus) for both the time intervals (1 h and 24 h).

Conclusion: Endosequence BC showed maximum antimicrobial efficacy against all the tested microorganisms for both the time intervals, followed by MTA Fillapex and AH Plus.

Key words: *Candida albicans*, Direct contact test, Endodontic sealers, *Enterococcus faecalis*, *Staphylococcus aureus*

INTRODUCTION

The exclusion of microorganisms from root canal is the priority of endodontic treatment. This is implemented by biomechanical preparation, irrigation with irrigants, and satisfactory filling of the three dimensional root canal.[1] The failure in the treatment is dominated by facultative and resistant microbial species. The persistent periradicular lesions after root canal treatment are because of the presence of *Enterococcus faecalis*.[2] Enterococci have the ability to grow in an environment of low-nutrient which can also survive as mono-infection. According to Sundqvist *et al*., 38% of failed root canal systems were commonly associated with *E. faecalis*. *Candida albicans*, dentinophilic microorganism, is associated with failed treatment.[3] The refractory periapical disease is associated with the biofilm of *Staphylococcus aureus* on tissues. Hence, these organisms were used as our study parameter. The application of sealers with antibacterial properties has further lowered the remaining microorganisms. One of the most commonly epoxy resin-based sealer is AH Plus (Dentsply International Inc., York, PA), which is eugenol-free, biocompatible, and radio-opaque. The Paste A of AH Plus has bisphenol-A

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www.ijss-sn.com

Month of Submission : 06-2020
Month of Peer Review : 06-2020
Month of Acceptance : 07-2020
Month of Publishing : 07-2020

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epoxy resins majorly. It also contains zirconium oxide, silica, iron oxide pigments, and calcium tungstate. Paste B contains tricyclodecane-diamine, dibenzyldiamine, aminoadamantane, calcium tungstate, silica, zirconium oxide, and silicone oil.[4] Mineral trioxide aggregate (MTA) has been used as a sealer after modifications. MTA Fillapex has good biocompatibility and capacity in the formation of mineralized tissues. MTA Fillapex can also be used as perforation repair material in the root canal. It is also used as a retrograde filling material and used in cases of apexification.[5,6] In current times, bioceramic sealer is used for root repair material and also as a sealer because of its biocompatibility, alkaline pH (<12). It has other advantages like easily introducible in canal, non-shrinkable, and non-resorbable. The studies have shown bioceramic sealer to strengthen the root canal following obturation. One of the newer bioceramic endodontic sealers is Endosequence BC Sealer (Brasseler, Savannah, GA, USA), which majorly comprises of zirconium oxide and calcium phosphate. It also consists of calcium silicates and calcium hydroxide.[7,8] Agar diffusion test (ADT), a semi-quantitative technique, is the most frequent method used to study the *in vitro* antimicrobial activities. ADT cannot differentiate between bacteriostatic and bactericidal effects of materials. The outcome is influenced by the diffusibility and solubility of the biomaterials through the agar. Hence, it is not used for water-insoluble materials.[9] Therefore, the methodology adopted was direct contact test (DCT). DCT by Weiss *et al.* measures the effect between the tested microorganism and the material when they are in contact, on the basis of microbial viability. It measures the antimicrobial property of the biomaterial irrespective of the solubility and diffusibility of the tested materials. DCT is a quantitative test and reproducible assay which can also be used to study the insoluble biomaterials and can be used for standardized settings.[10] Therefore, the aim of this study was to compare the *in vitro* antimicrobial efficacy of three endodontic sealers against *E. faecalis*, *C. albicans*, and *S. aureus* using DCT. The null hypothesis tested was that there were no differences in the antimicrobial efficacy of Endosequence BC Sealer, MTA Fillapex, and AH Plus against *E. faecalis, S. aureus*, and *C. albicans*.

MATERIALS AND METHODS

Grouping of Sample

This study was divided into three groups based on the following sealers.

1 = AH Plus, 2 = MTA Fillapex and 3 = Endosequence BC.

Depending on the microorganisms to be tested, these three groups were further subdivided into three groups of 18 each:

Sub Group A: *E. faecalis* (n = 18), sub Group B: *C. albicans* (n = 18), and sub Group C: *S. aureus* (n = 18).

Nine samples from each subgroup were analyzed at 1 h and the remaining nine samples were analyzed after 24 h. For this study, bacterial growth was measured by a microplate spectrophotometer. For DCT, 50 mg of sealer was mixed and settled in nine flat bottom tubes. The tubes for each sealer were prepared in triplicate. Following which 50 μL of 0.5 ml McFarland standard suspension (1.5×10^8 CFU/ml) of microorganisms was spread over the sealers. McFarland standards were used as a reference to evaluate the number of bacteria within a given range to standardize the microbial testing. It is based on the turbidity of bacterial suspensions. The samples were then incubated at 37°C to ensure the direct contact between bacteria and test sealers. The suspension of microorganisms and test sealers was in direct contact for 1 h and 24 h. The test tubes were incubated at 37°C, following which the test tubes were inspected for evaporation of suspension.

Ethics

Ethical clearance was taken from the I.T.S Institutional Ethics Committee (IIEC).

Statistics

The data were analyzed using SPSS 16.0. The intergroup comparison for normal data was tested by one-way ANOVA and Tukey HSD test. The intragroup comparison was tested by paired *t*-test (parametric test). The level of significance and confidence interval was 5% and 95 %, respectively.

RESULTS

The DCT showed a significant difference in microbial count among the groups (ANOVA $P = 0.0001$) at 1 h and 24 h. In paired *t*-test, Group 3 (Endosequence BC) showed a minimum microbial count of *E. faecalis* with a mean difference of 8.980, *C. albicans* with a mean difference of 7.889, and *S. aureus* with a mean difference of 6.540 for both time intervals, that is, 1 h and 24 h and it was significant [Table 1]. Group 2 (MTA Fillapex) showed the second-highest microbial count with a mean difference of 6.322 for *E. faecalis*, 7.222 for *C. albicans,* and 5.444 for *S. aureus*, for both the time intervals [Table 2]. In this study, Group 3 showed the highest microbial count of *E. faecalis* with a mean difference of 5.444 for *E. faecalis*, and 5.114 for *S. aureus* for both the time intervals, that is, 1 h and 24 h and it was significant [Table 3].

DISCUSSION

The predominant cause of failure of endodontic treatment is because of resistant microorganisms such as *E. faecalis*,

Table 1: Comparison of means of microbial count between two intervals in Endosequence BC by paired t-test

Parameter/Variable	Mean	Std. deviation	Std. error mean	95% confidence interval of the difference		*t*-test value	*P*-value
				Lower	Upper		
Enterococcus faecalis 1–24 h	8.980	2.345	0.782	7.803	4.197	7.675	<0.001**
Candida albicans 1–24 h	7.889	2.977	0.992	11.177	6.601	8.958	<0.001**
Staphylococcus aureus 1 h–24 h	6.540	2.345	0.782	7.803	4.197	7.675	<0.001**

*: Significant, **: Highly significant

Table 2: Comparison of means of microbial count between two intervals in mineral trioxide aggregate Fillapex by paired t-test

Parameter/Variable	Mean	Std. deviation	Std. Error mean	95% confidence interval of the difference		*t*-test value	*P*-value
				Lower	Upper		
Enterococcus faecalis 1–24 h	6.322	3.279	1.093	5.854	6.813	5.050	0.016*
Candida albicans 1–24 h	7.222	2.949	0.983	7.489	4.956	6.348	<0.001**
Staphylococcus aureus 1 h–24 h	5.444	3.167	1.056	5.179	1.010	5.263	0.011*

*: Significant, **: Highly significant

Table 3: Comparison of means of microbial count between two intervals in AH Plus by paired t-test

Parameter/Variable	Mean	Std. deviation	Std. error mean	95% confidence interval of the difference		*t*-test value	*P*-value
				Lower	Upper		
Enterococcus faecalis 1–24 h	5.444	5.503	1.834	3.674	5.215	□5.149	0.001**
Staphylococcus aureus 1 h–24 h	5.114	5.503	1.834	3.674	5.215	□5.149	0.001**

*: Significant, **: Highly significant

C. albicans, and *S. aureus*.[11] Bioceramic (Endosequence BC) sealers are known for its antimicrobial property during the setting and exhibit no shrinkage. The hydrophilic nature helps to form hydroxyapatite om setting and chemically bonds to dentin and gutta percha points.[12] MTA-based sealers (MTA Fillapex, Angelus, Brazil) are known for its properties such as remarkable biocompatibility, stimulating mineralization, and exhibiting higher push-out strengths than zinc oxide eugenol cements.[13] MTA consists of calcium oxide which has a similar mode of action to calcium hydroxide.[14] Epoxy resin-based sealers (AH Plus) have good antimicrobial, physical, and chemical properties.[15] Hence, these sealers are diminishes the survival of microorganisms during obturation. The result of the present study showed significant microbial count reduction with Endosequence BC than MTA Fillapex and AH Plus for both time intervals (1 h and 24 h). It showed maximum antimicrobial efficacy against *E. faecalis*, followed by *C. albicans* and least against *S. aureus* (Graph 1). This outcome resembled other studies where fresh Endoseq BC, MTA Fillapex, and AH Plus had antibacterial action against *E. faecalis* when tested by time-kill assay.[16] The results also were in accordance in the studies in which ADT, DCT, and modified DCT were implemented.[15,17,18,19] Endosequence BC showed the least microbial count against *E. faecalis* followed by *C. albicans* and least in *S. aureus*. The literature search does not have studies to support our result in relation to the highest reduction in *E. faecalis* followed by *C. albicans* and *S. aureus* using DCT. MTA Fillapex showed maximum antimicrobial efficacy against *C. albicans* followed by *E. faecalis* and least against *S. aureus*. AH Plus showed maximum antimicrobial efficacy against *E. faecalis* followed by *S. aureus* and showed no antimicrobial effect against *C. albicans*.

The antimicrobial property of the BC sealer is contributed by its alkaline pH that aids in the exclusion of microorganisms like *E. faecalis* which cease to survive at high pH, near to 11.5 or more. Furthermore, active calcium hydroxide diffusion over the period of time can be the reason for the antimicrobial efficacy.[20] On the contrary to our study, Hegde and Rathod (2017) stated that AH Plus sealer had better results than Bioceramic sealer against *E. faecalis*, in their study on *E. faecalis*. Such discrepancies can be due to the methodology used in the study which was ADT.[21]

MTA Fillapex showed second most statistically significant microbial count reduction for both the time intervals, that is, 1 h and 24 h against *C. albicans*, *E. faecalis*, and *S. aureus*. (Graph 2). This result was in accordance with Rahman *et al.* (2017), who found that MTA Fillapex and Real Seal SE both showed antifungal activity whereas only MTA Fillapex was effective against *E. faecalis*, rest of the materials did not depict any antimicrobial activity.[22] Another study by

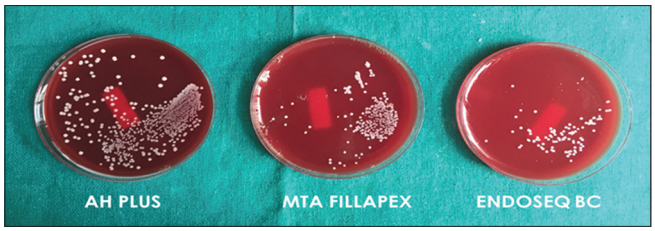
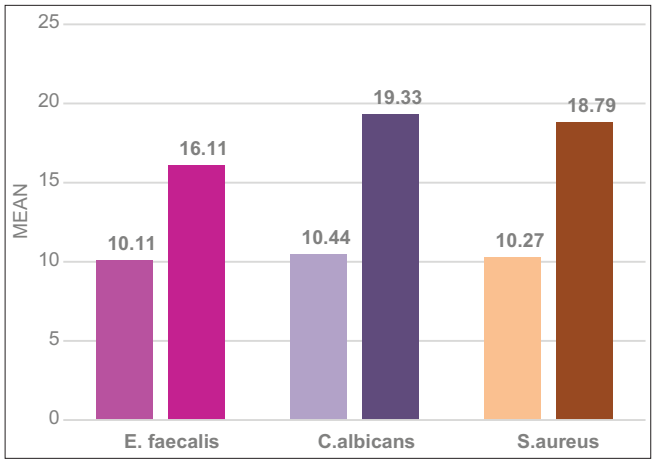
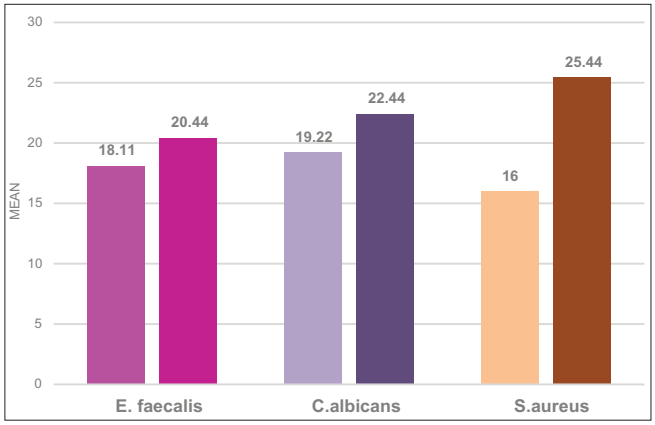


Figure 1: Microbial count for sealers for *Candida albicans* for 1 h

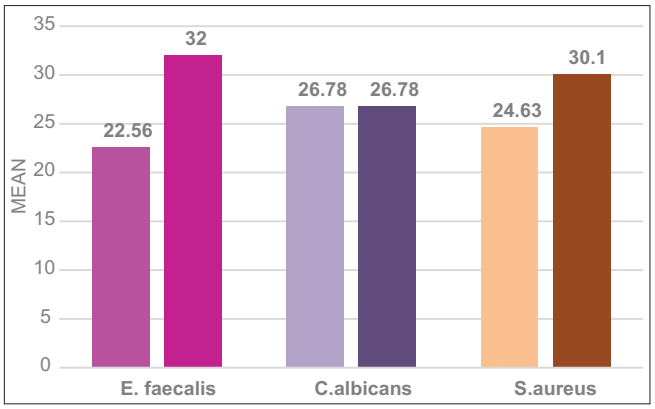


Graph 1: Comparison of mean microbial count for Endosequence BC in different time intervals



Graph 2: Comparison of mean microbial count for MTA Fillapex in different time intervals

Stowe *et al.* (2013) verified the antimicrobial properties of MTA which inhibited the growth of both *E. faecalis* and *Streptococcus sanguis*.[23] MTA Fillapex which contains calcium silicate which on contact with the moisture from dentin, begins the hydration of calcium silicates. The calcium silicate hydrogel and calcium hydroxide give a high pH which could be related to its antimicrobial property to MTA Fillapex.[24] On the contrary to this study, Ustun *et al.* (2013) in his study showed that MTA-based sealer has a least antibacterial effect at 20 min, whereas bioceramic sealer and epoxy resin sealer had maximum antibacterial



Graph 3: Comparison of mean microbial count for AH Plus in different time intervals

properties.[16] AH Plus, resin-based sealer, exhibited least antimicrobial efficacy against (Graph 3). Aravind *et al.* (2006) evaluated the antimicrobial property of five root canal sealers. The results showed that AH Plus has no antimicrobial action against *C. albicans* and Enterococci.[25] The result of Mickel *et al.* (2003) was in accordance with our study, who also verified AH Plus to be show minimum efficiency against *E. faecalis*.[26] The ineffective property of AH Plus is because of the elimination of formaldehyde. The presence of Bisphenol A diglycidyl ether in resin-based sealers induces its antimicrobial properties.[22]

Our study showed a significant difference in antimicrobial properties observed in MTA Fillapex, AH Plus, and Endosequence BC at 1 h (Figure 1). The antimicrobial activity of tested sealers decreased over time. This shows that resin-based and bioceramic root canal sealers are more efficient in a freshly mixed state and their antimicrobial properties decrease with time. However, the lowest long-time efficacy of AH Plus may be due to the paraformaldehyde released by this material only during the setting period. Similar studies were also reported by Heyder *et al.* (2013) discussing the antimicrobial properties for AH Plus owing formaldehyde, which is released in small quantities during the setting reaction.[22] According to the manufacturers, the processing time of AH Plus is 4 h and setting time at 37°C for another 8 h. Pizzo *et al.* (2006) suggested that the 24-h samples of AH Plus are ineffective in irradicating all *E. faecalis* in direct contact.[27]

CONCLUSION

Within the limitations of the study, it was concluded that:

Endosequence BC had the maximum antimicrobial efficacy against all the tested microorganisms for both the time intervals, followed by MTA Fillapex. The minimum efficacy was seen in AH Plus.

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How to cite this article: Chakraborty T, Taneja S, Malik S. An Antimicrobial Activity Assessment of Three Endodontic Sealers on *Enterococcus faecalis*, *Candida albicans*, and *Staphylococcus aureus* by a Direct Contact Test: An *In Vitro* Study. Int J Sci Stud 2020;8(4):35-39.

Source of Support: Nil, **Conflicts of Interest:** None declared.