An assessment of Antimicrobial Activity 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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Research article

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Abstract

For a successful endodontic treatment antimicrobial property of endodontic filling 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 (Group 1= AH Plus, 2= MTA Fillapex and 3= Endo Sequence BC) were tested against Enterococcus faecalis ATCC 29212, Candida albicans ATCC 10231 and Staphylococcus 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 in order to determine the colony count using digital colony counter. Readings were taken at 1 hr (fresh specimen), then at 24 hrs (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 hour and 24 hours).

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

Introduction

The exclusion of microorganisms from root canal is the priority of the 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 is because of the presence of Enterococcus faecalis (E. faecalis) [2]. Enterococci have the ability to grow in environment of low-nutrient which can also can survive as mono infection. According to Sundqvist et al., 38% of failed root canal systems were commonly associated with E.faecalis. Candida albicans (C. albicans), dentinophilic microorganism, is associated with failed treatment [3]. The refractory periapical disease is associated with biofilm of Staphylococcus aureus (S. aureus) on tissues. Hence these organisms were used as our study parameter. The application of sealers with antibacterial properties have 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 epoxy resins majorly. It also contains zirconium oxide, silica, iron oxide pigments and calcium tungstate. The Paste B contains tricyclodecane-diamine, dibenzyldiamine, aminoadamantane, calcium tungstate, silica, zirconium oxide, and silicone oil [4]. Mineral trioxide aggregate (MTA) has been used as sealer after modifications. MTA- Fillapex has good biocompatibility and capacity in formation of mineralized tissues. MTA Fillapex can also be used as perforation repair

material in root canal. It is also used as 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 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 sealer 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 diffusibility and solubility of the bio materials 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 by using direct contact test. 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.

Methods

Grouping of Sample

This study was divided into 3 groups based on the following sealers.

1 = AH Plus, 2 = MTA Fillapex and 3 = Endo Sequence BC

Depending on the microorganisms to be tested, these three groups were further subdivided into 3 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 analysed at 1 hour and the remaining nine samples were analysed after 24 hours. For this study, the bacterial growth was measured by microplate spectrophotometer. For DCT, 50mg 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 x 10^8 CFU/ ml) of microorganisms were spread over the sealers. McFarland standards was 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 were in direct contact for 1 hour and 24 hours. The test tubes will be incubated at 37°C, following which the test tubes were inspected for evaporation of suspension.

Ethics

Ethical clearance was taken from the ethical committee institute IIEC (I. T.S Ethical Committee).

Statistics

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

Result

The DCT showed a significant difference in microbial count among the groups (ANOVA p=0.0001) at 1hour and 24 hours. In paired t test, Group 3 (Endosequence BC) showed 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 the time intervals i.e 1hr and 24 hrs and it was significant [table 2]. Group 2 (MTA Fillapex) showed 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 1]. 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 i.e 1hr and 24 hrs and it was significant [table 3]

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			P value
					Lower	Upper		
Pair 1	E.faecalis1hr. - E.faecalis24hr.	6.322	3.279	1.093	5.854	6.813	5.050	.016
Pair 2	C.albicans1hr - C.albicans24hr	7.222	2.949	.983	7.489	4.956	6.348	<.001**
Pair 3	S.aureus 1hr – S.aureus 24hr	5.444	3.167	1.056	5.179	1.010	5.263	.011

Table 1. Comparison of means of microbial count between two intervals in MTA Fillapex by paired T test

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			P value
					Lower	Upper		
Pair 1	E.faecalis1hr. - E.faecalis24hr.	8.980	2.345	.782	7.803	4.197	7.675	<.001**
Pair 2	C.albicans1hr- C.albicans24hr	7.889	2.977	.992	11.177	6.601	8.958	<.001**
Pair 3	S. aureus 1hr S.aureus 24hr	6.540	2.345	.782	7.803	4.197	7.675	<.001**

Table 2. Comparison of means of microbial count between two intervals in Endosequence BC by paired T test

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			P value
					Lower	Upper		
Pair 1	E.faecalis1hr. - E.faecalis24hr.	5.444	5.503	1.834	3.674	5.215	-5.149	.001
Pair 3	S.aureus 1hr – S.aureus 24hr	5.114	5.503	1.834	3.674	5.215	-5.149	.001

Table 3. Comparison of means of Microbial count between two intervals in AH Plus by paired T test

* - Significant, **- Highly Significant, NS- Not Significant

Discussion

The predominant cause of failure of endodontic treatment is because of resistant micro organisms such as E. faecalis, C. albicans and S. aureus [11]. Bioceramic (Endosequence BC) sealers are known for its antimicrobial property during setting and exhibits no shrinkage. During setting, there if formation of hydroxyapatite which chemically bonds to dentin and gutta percha [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 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 hr and 24hrs). It showed maximum antimicrobial efficacy against E.faecalis, followed by C.albicans and least against S.aureus. These outcome resembled with 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 was in accordance in the studies in which ADT, DCT, and modified DCT were implemented [17, 18, 19, 20]. Endosequence BC showed 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 Enterococcus faecalis followed by Candida albicans and Staphylococcus aureus using direct contact test. 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 exclusion of microorganisms like E.faecalis which cease to survive at high pH, near to 11.5 or more. Also active calcium hydroxide diffusion over the period of time can be the reason for the antimicrobial efficacy [21]. On 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 Enterococcus faecalis. Such discrepancies can be due to the methodology used in the study which was agar diffusion test [22].

MTA Fillapex showed second most statistically significant microbial count reduction for both the time intervals, i.e 1 hour and 24 hours against C.albicans, E.faecalis and S.aureus. This result was in accordance to Rahman H 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 [23]. Another study by Stowe et al (2013) verified the antimicrobial properties of MTA which inhibited the growth of both E. faecalis and Streptococcus sanguis [24]. 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 gives the high pH

which could be related to its antimicrobial property to MTA Fillapex. [25] On contrary to this study, Ustun et al. (2013) in his study showed that MTA based sealer has least antibacterial effect at 20 mins, whereas bioceramic sealer and epoxy resin sealer had maximum antibacterial properties [16]. AH Plus, resin based sealer, exhibited least antimicrobial efficacy against. Aravind et al (2006) evaluated the antimicrobial property five root canal sealers. The results showed that AH Plus has no antimicrobial action against Candida albicans and Enterococci [26]. The result of Andre Mickel et al (2003) was in accordance with our study, who also verified AH Plus to be show minimum efficiency against E.faecalis [27]. The ineffective property of AH Plus is because of the elimination of formaldehyde. The presence bisphenol A diglycidyl ether in resin based sealers induces its antimicrobial properties [23].

Our study showed a significant difference in antimicrobial properties observed in MTA Fillapex, AH Plus and Endosequence BC at one hour. The antimicrobial activity of tested sealers decreased over the time. This shows that resin based and bioceramic root canal sealers are more efficient in freshly mixed state and their antimicrobial properties decreases with time. However, the lowest long-time efficacy of AH Plus may be due to the paraformaldehyde released by this material only during setting period. Similar studies were also reported by Hyeder et al (2013) discussing the antimicrobial properties for AH Plus owing formaldehyde, which is released in small quantities during the setting reaction [23]. According to the manufacturers, the processing time of AH Plus is 4 hrs and setting time at 37 ∘C for another 8hr. Pizzo G et al (2006) suggested that the 24-h samples of AH Plus is ineffective in irradicating all E. faecalis in direct contact [28].

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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Figures

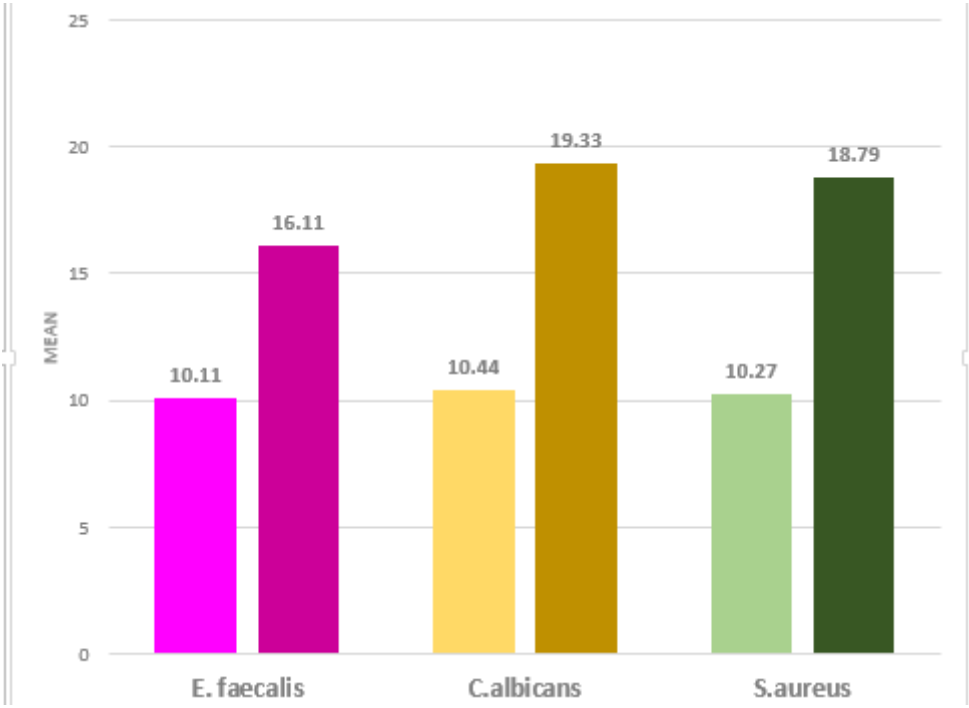

Figure 1

Mean microbial count of Endosequence BC at 1 hr and 24 hours

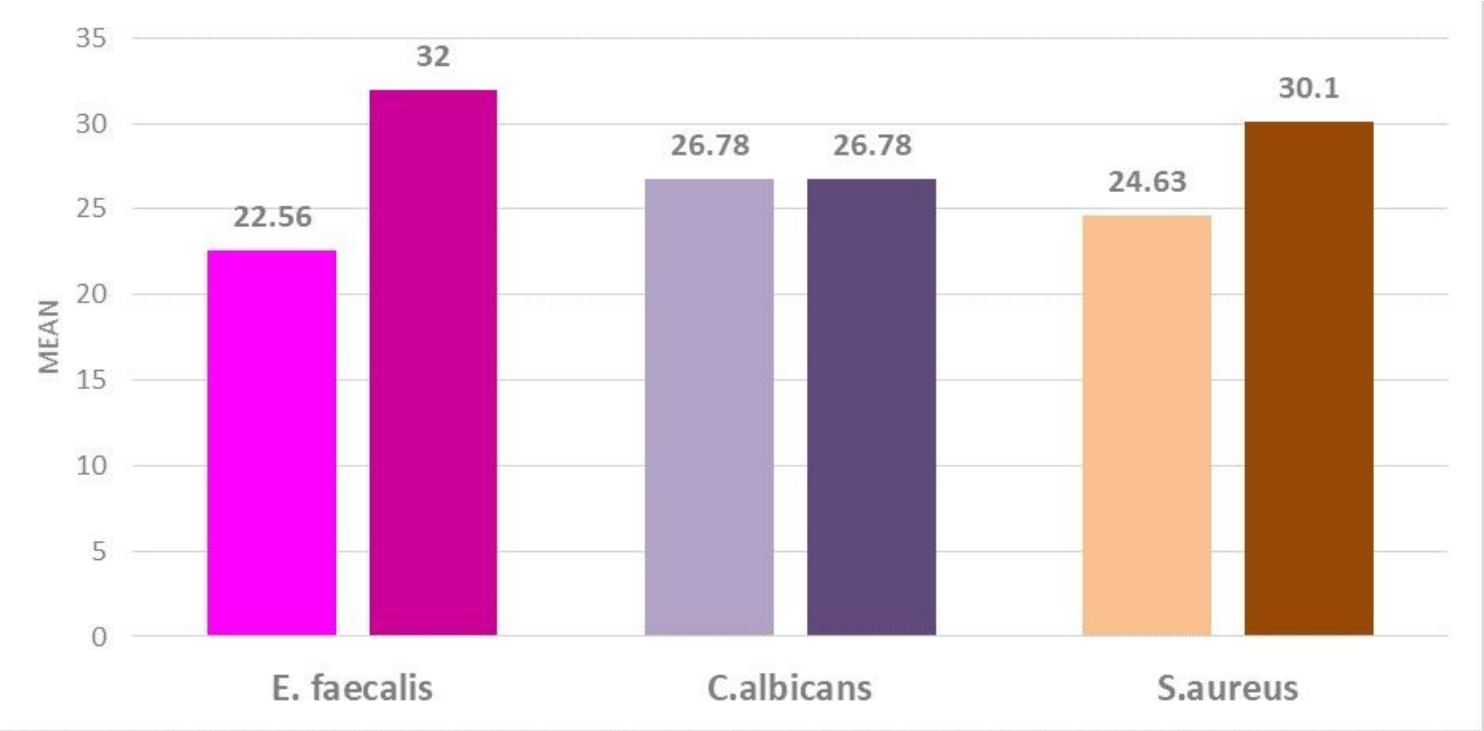

Figure 2

Mean microbial count of AH Plus at 1 hr and 24 hours

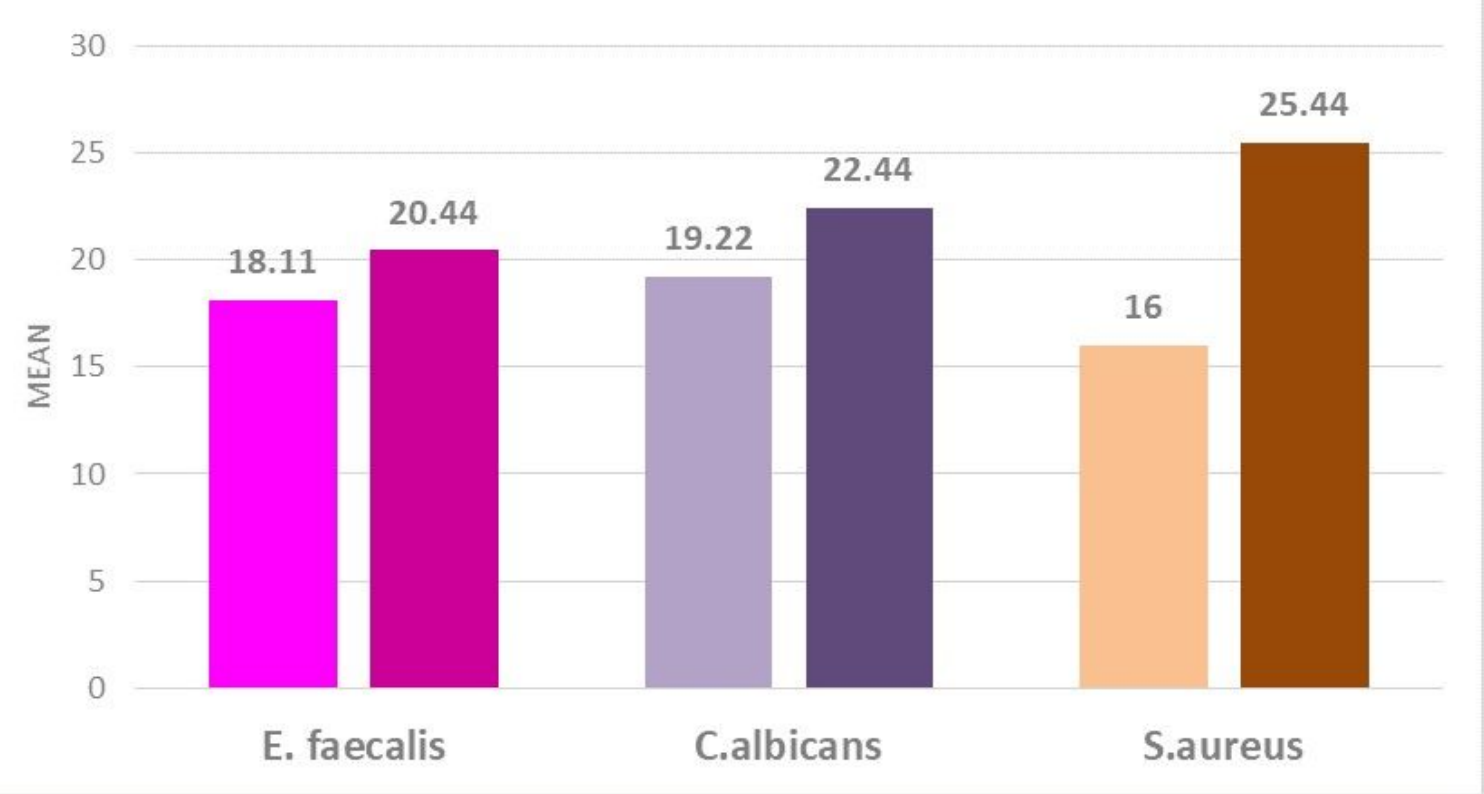

Figure 3

Mean microbial count of MTA Fillapex at 1 hr and 24 hours

Figure 4

AH Plus, MTA Fillapex, and Endoseq BC

Supplementary Files

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