Original Article

Assessment of Antimicrobial Efficacy of Bioceramic Sealer, Epiphany Self-etch Sealer, and AH-Plus Sealer against *Staphylococcus aureus and Candida albicans*: An *In vitro* Study

Ruchita Kishor Rathod[1], Pradeep Dilip Taide[2], Rohan Dattatraya Dudhale[3]

[1]Department of Conservative Dentistry and Endodontics, Khed, Ratnagiri, Maharashtra, India, [2]Department of Prosthodontics and Crown and Bridge and Implantology, Yogita Dental College and Hospital, Ratnagiri, Maharashtra, India, [3]Department of Bacteriology, Haffkine's Institute, Mumbai, Maharashtra, India

ABSTRACT

Aim: The aim and objective of this *in vitro* study was to evaluate the antimicrobial efficacy of root canal sealers (bioceramic [BC] sealer, Epiphany self-etch sealer, and AH-Plus sealer) on *Staphylococcus aureus* and *Candida albicans*. **Materials and Methods:** An agar well diffusion assay method was used to determine the efficacy of the root canal sealer against *S. aureus* (ATCC 6538) and *C. albicans* (ATCC 10231). Root canal sealers were divided into three groups: BC sealer, Epiphany self-etch sealer, and AH-Plus sealer, and the standard antibiotic disc of amoxiclav and fluconazole was kept as a control against *S. aureus* and *C. albicans*. The diameters of the growth inhibition zones against *S. aureus* and *C. albicans* for each group were recorded and compared at 24 h. The differences between groups were analyzed by one-way ANOVA and Tukey's *post hoc* tests for intergroup analysis. **Results:** AH-Plus sealer exhibited a larger zone of inhibition than the other two sealers against *S. aureus* and *C. albicans* at 24 h. The standard antibiotic disc of fluconazole, which was used as a control against *C. albicans*, exhibited a higher antimicrobial activity than the AH-Plus sealer at 24 h, whereas Epiphany self-etch sealer showed the least antimicrobial activity against S. *aureus* and C. a*lbicans*. **Conclusion:** The AH-plus root canal sealer exhibits a better antibacterial action against *S. aureu*s and *C. albican*s at 24 h.

KEYWORDS: *Agar diffusion test, brain-heart infusion broth, Candida albicans, inhibition zone, sealers, Staphylococcus aureus*

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INTRODUCTION

Microorganisms and their by-products have been responsible for dentinal, pulpal, and periapical pathologies, which were observed by Miller in 1890. The main aim of endodontic therapy is to eliminate microorganisms from the root canal.[1,2] Instrumentation, irrigation, and intracanal medicaments significantly reduce the population of microorganisms. However, it does not completely eliminate the microorganisms from the root canal due to the anatomical complexities such as dentinal tubules, ramification, deltas, and fins; hence, a good root canal filling material with antibacterial property would be beneficial in further reducing the number of residual microorganisms.[3] A variety of microbes ranging from anaerobes, aerobes, and fungi can cause root canal infection. Among these, *Enterococcus faecalis*, a Gram-positive anaerobic, facultative coccus, has been reported as one of the most frequently isolated microorganisms from the root canals at the time of retreatment ranging from 24 to 77%.[4,5] It is known to penetrate deeply into dentinal tubules (250–400 μm),[6] binding to collagen and hydroxyapatite,[4] which makes its complete elimination difficult. Due to these properties, *E. faecalis* has been adopted as a model test organism in many endodontic studies.[7] In the previous study by Hegde *et al*., the antimicrobial efficacy of root canal sealer was assessed against *E. faecalis*.

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Address for correspondence: Dr. Ruchita Kishor Rathod, 503, 56/A, Chintamani Chs Ltd, Near Pratikshanagar Bus Depot, Sion, Mumbai - 400 022, Maharashtra, India.
E-mail: ruchitarathod@yahoo.com

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Other than *E. faecalis, Candida albicans* and *Staphylococcus aureus* are other aerobic organisms associated with root canal infections.[8] Extraradicular microbial biofilm of *S. aureus* on tissue or biomaterial surface is related to the refractory periapical disease.[9] *C. albicans*, a part of normal microbiota, is associated with failed endodontic therapy and may be considered a dentinophilic microorganism.[10] *C. albicans* yeast is larger than bacteria and can colonize dentinal tubules to depths approximately 150 μm[11] and has the ability to form biofilms even in nutrient-deprived conditions such as those in clean and filled root canals.[12] It is the most frequently found fungal species in endodontically treated teeth with periradicular lesions.[13] Several factors and activities have been recognized to contribute to the pathogenic potential of this fungus. Among them, secretion of hydrolytic enzyme, molecule that causes adhesion and attack host cell, yeast-to-hypha mutation, biofilm formation, and phenotypic switching are considered the virulence factor of this fungus. Hence, we choose these organisms as our study parameters.

Bioceramic (BC) sealers are known to possess biological activity. It is highly radiopaque and hydrophilically forms hydroxyapatite on setting and chemically bonds to both dentin and gutta-percha points. It is antibacterial during setting attributable to its highly alkaline pH. It exhibits absolutely zero shrinkage. Hence, when these sealers are used along with obturating systems, it may greatly affect the survival of bacteria adversely. The use of sealers with antibacterial properties may be advantageous, especially in clinical situations of persistent or current infection. The endodontic sealers have been shown to give the greatest antimicrobial effects immediately after spatulation, following which there is a gradual loss of antimicrobial effects over time.[14]

Many studies have been performed to assess the antimicrobial efficacy of different root canal sealers. The agar diffusion test (ADT) was the most commonly used technique. Antibacterial activity of the endodontic sealers is tested based on measuring the effect of close contact between test bacteria and tested material on the kinetics of bacterial growth.[15]

Materials and Methods

In the current study, the root canal sealers tested were as follows: Group 1 – BC root canal sealer (Brasseler USA), Group 2 – self-etch Epiphany sealer (RealSeal, SybronEndo, Orange, CA, USA), and Group 3 – AH-Plus sealer (Dentsply, De-Trey, Konstanz, Germany). The standard antibiotic disc of amoxiclav and fluconazole disc were used as control groups against *S. aureus and C. albicans,* respectively.

Preparation of the medium for *Staphylococcus aureus*

The strains of *S. aureus* used for the study were standard strains of *S. aureus* ATCC 6538 and were subcultured in the blood agar plate and were incubated at 37°C for 24 h. A pure, single *S. aureus* colony was isolated from the same cultured plate and Gram's staining was performed to confirm its growth, which was checked under an oil immersion microscope and was then inoculated with a brain-heart infusion (BHI) broth. The BHI broth was incubated at 37°C for 24 h period and checked for bacterial growth by changes in turbidity. A drop of BHI broth containing *S. aureus* was placed into a saline solution and checked for correct bacterial concentration with a spectrophotometer. By analyzing the broth at a density associated with the barium sulfate standard of 0.5 McFarland units, which was equal in value to 1.5×10^8 CFU/ml, the density of the bacterial suspension is standardized. Mueller-Hinton agar was used to prepare Petri plates. The sterility of the plates was checked and the fresh inoculums of *S. aureus* of 0.5 McFarland standard suspensions were formulated.

Preparation of the medium for *Candida albicans*

The strains of *C. albicans* used for the study were standard strains of *C. albicans* ATCC 10231 and were subcultured in *candida* agar plate and were incubated at 37°C for 24 h. A pure, single *C. albicans* colony was isolated from the same cultured plate, and Gram's staining was performed to confirm its growth, which was checked under an oil immersion microscope and was then inoculated with a BHI broth. The BHI broth was incubated at 37°C for 24 h period and checked for bacterial growth by changes in turbidity. A drop of BHI broth containing *C. albicans* was placed into a saline solution and checked for correct bacterial concentration with a spectrophotometer. By analyzing the broth at a density associated with the barium sulfate standard of 0.5 McFarland units, which was equal in value to 1.5×10^8 CFU/ml, the density of the bacterial suspension is standardized. Sabouraud dextrose agar was used to prepare Petri plates. The sterility of the plates was checked and the fresh inoculums of *C. albicans* of 0.5 McFarland standard suspensions were formulated.

Antimicrobial activity by agar diffusion test

A sterile, nontoxic cotton swab was dipped on a wooden applicator into standardized inoculums and the soaked cotton swab was rotated firmly against the upper inside wall of the tube to express excess fluid. The plate was turned at a 60° angle between each streaking. The inoculums were allowed to dry for 5–15 min with lid in place. Four wells were created using an 8 mm sterile cork borer. A desired amount of the root canal sealers in paste form, which was treated by ADT, was

mixed and placed in the wells. 10–15 min was allowed for diffusion of the medicament in agar and then was incubated immediately at 35 ± 2°C for 24 h. The whole experiment was carried out under aseptic conditions and was repeated twelve times to ensure reproducibility.

Measurement of inhibition zones

Zones of bacterial growth inhibition were measured at the end of 24 h using a Vernier caliper.

Statistical analysis

After the results were collected, the results were entered into Microsoft Excel and analyzed using the SPSS software v20 (IBM Corp., Armonk, NY, USA). Descriptive statistics were shown as mean ± and standard deviation. To evaluate the differences between the antimicrobial efficacy of Endosequence BC sealer, Epiphany self-etch sealer, and AH-Plus sealer against *S. aureus* and *C. albicans*, one-way ANOVA test [Tables 1 and 2] was used, and for comparison within the groups, Tukey's multiple comparison test [Tables 3 and 4] was used. $P < 0.05$ was considered statistically significant.

Results

Antimicrobial activity of tested sealers against *Staphylococcus aureus*

The means of the diameters of the growth inhibition zones for each group of root canal sealers and control groups against *S. aureus* are presented in Figure 1 and Graph 1. The range of inhibitory values between experimental groups varied broadly. One-way ANOVA was used to calculate *P* value and showed significant differences ($P < 0.0001$) [Table 1]. Overall, AH-Plus sealer (Group 3) had larger zones of growth inhibition in the well diffusion assay [Figure 1 and Graph 1] than the standard antibiotic disc of amoxiclav which serves as a control against *S. aureus*. BC sealer and Epiphany self-etch sealer showed the least amount of inhibitory effect against *S. aureus*.

Antimicrobial activity of tested sealers against *Candida albicans*

The means of the diameters of the growth inhibition zones for each group of root canal sealers and control groups against *C. albicans* are presented in Figure 2 and Graph 2. The range of inhibitory values between experimental groups varied broadly. One-way ANOVA was used to calculate *P* value and showed significant differences ($P < 0.0001$) [Table 2]. The standard antibiotic disc of fluconazole which serves as a control against C. albicans had the highest zone of growth inhibition than AH-Plus (Group 3) sealer followed by BC sealer [Figure 2 and Graph 2]. Epiphany self-etch sealer showed the least amount of inhibitory effect against C. albicans.

Table 1: Statistical analysis ANOVA against *Staphylococcus aureus* at 24 h

Agents	Mean (zone of inhibition)	SD	*P* (Oneway ANOVA)
BC sealer	0.97	0.47	<0.001*
Epiphany self-etch	0.80	0.33	
AH-Plus	18.99	1.11	
Control (amoxiclav disc)	17.91	0.87	

*$P \leq 0.05$ is statistically significant. SD: Standard deviation, BC: Bioceramic

Table 2: Statistical analysis ANOVA against *Candida albicans* at 24 h

Agents	Mean (zone of inhibition)	SD	*P* (Oneway ANOVA)
BC sealer	14.76	1.02	<0.001*
Epiphany self-etch	0.80	0.33	
AH-Plus	21.97	1.27	
Control (fluconazole disc)	34.91	0.87	

*$P \leq 0.05$ is statistically significant. SD: Standard deviation, BC: Bioceramic

Table 3: Statistical analysis Tukey's *post hoc* multiple comparisons against *Staphylococcus aureus* at 24 h

Group	*P*	Conclusion
Control versus BC sealer	<0.001*	Significant
Control versus AH-Plus	0.006*	Significant
Control versus Epiphany	<0.001*	Significant
BC sealer versus AH-Plus	<0.001*	Significant
BC sealer versus Epiphany	0.950	Nonsignificant
AH-Pplus versus Epiphany	<0.001*	Significant

*BC: Bioceramic

Table 4: Statistical analysis Tukey's *post hoc* multiple comparisons against *Candida albicans* at 24 h

Group	*P*	Conclusion
Control versus BC sealer	<0.001*	Significant
Control versus AH-Plus	<0.001*	Significant
Control versus Epiphany	<0.001*	Significant
BC sealer versus AH-Plus	<0.001*	Significant
BC sealer versus Epiphany	<0.001*	Significant
AH-Plus versus Epiphany	<0.001*	Significant

*BC: Bioceramic

Discussion

In the present study, the standard antibiotic disc of amoxiclav was used as a control against *S. aureus* (*ATCC 6538*) strain, and the standard antibiotic

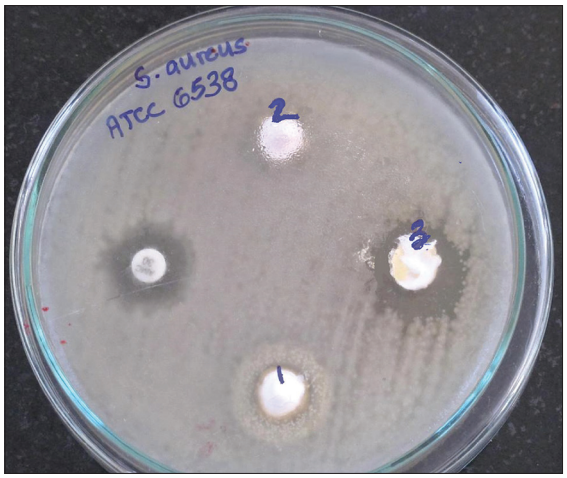


Figure 1: Zone of inhibition exhibited by the standard antibiotic disc of amoxiclav was higher than bioceramic sealer and Epiphany self-etch sealer, whereas AH-Plus sealer showed the highest zone of inhibition among all the groups against *Staphylococcus aureus*

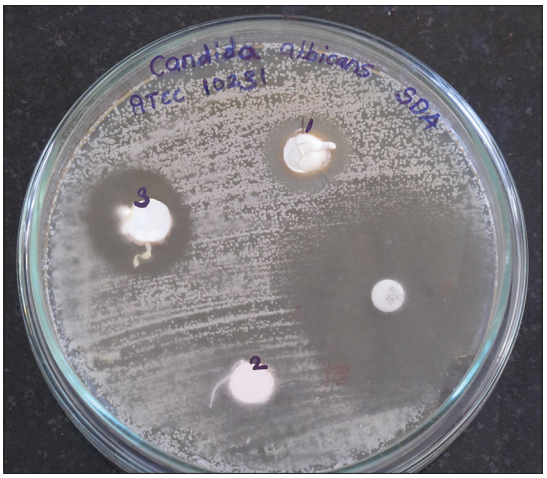
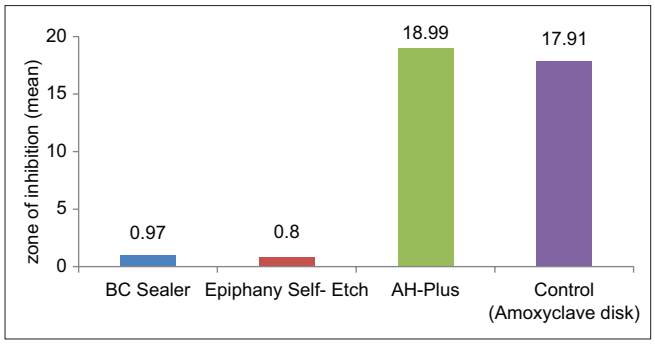
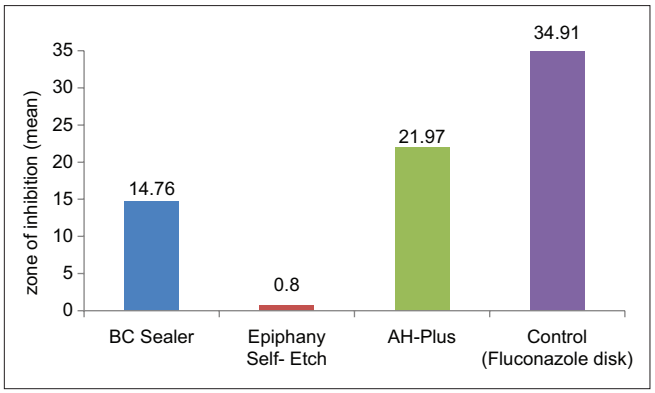


Figure 2: Zone of inhibition exhibited by AH-Plus sealer was higher than the other two sealers, whereas the standard antibiotic disc of fluconazole showed the highest zone of inhibition among all the groups against *Candida albicans*



Graph 1: Comparison of the zone of inhibition exhibited by all the groups against *Staphylococcus aureus* using agar diffusion test after 24 h



Graph 2: Comparison of the zone of inhibition exhibited by all the groups against *Candida albicans* using agar diffusion test after 24 h

disc of fluconazole was used as a control against *C. albicans* (ATCC 10231). The results demonstrated that AH-Plus sealer showed the highest zone of inhibition followed by the control group (standard antibiotic disc of amoxiclav) against *S. aureus*. This bactericidal action is due to the double inhibition of bacterial folic acid synthesis, whereas the control group (standard antibiotic disc of fluconazole) showed the highest zone of inhibition followed by AH-Plus and BC sealer against *C. albicans*.

BC sealer is a newly introduced endodontic sealer (Endosequence BC Sealer, Brasseler USA, Savannah, GA, USA). It has an alkaline pH, high calcium ions release, and suitable radiopacity and flow capacity. It also exhibits antibacterial activity and biocompatibility.[16] BC sealers are highly hydrophilic which allows them to spread easily over the root canal walls and fill the lateral microcanals too. During the setting, these sealers expand and form a chemical bond with the canal walls.[17]

The present study found the significant antimicrobial activity of BC sealer against *C. albicans*. The antibacterial effect of the BC sealer may be due to the combination of high pH and active calcium hydroxide diffusion.[3] AH-Plus sealer had a significantly greater ability to eliminate *S. aureus and C. albicans* than the Endosequence BC sealer. Studies by Candeiro *et al*. have similar findings as that of our study and they have shown that AH-Plus sealer had significantly greater cytotoxicity and genotoxicity than Endosequence BC sealer.

According to the Epiphany's manufacturer (Pentron Clinical Technologies, LLC Wallingford, Connecticut), when compared to epoxy resin or zinc oxide-eugenol-based sealers, this dual-cure, resin root canal sealer is nonmutagenic, noncytotoxic, biocompatible, and less irritating.[18] According to studies, Epiphany has a significant sealing capability to the root canal walls; however, this is supported by restricted data.[18,19] In the present study, Epiphany

sealer exhibited the least antimicrobial activity against *S. aureus* and *C. albicans*. This result partly agrees with the previous studies of Maekawa *et al*. that investigated the antibacterial efficacy of AH-Plus, EndoREZ, and Epiphany against *C. albicans, E. faecalis, Escherichia coli*, and *S. aureus* using the agar diffusion method. They found that Epiphany had no effect against the tested microorganisms, considering that no inhibition halo was observed.[20] Slutzky-Goldberg *et al.* reported that the least antimicrobial effect of Epiphany may result from its hydrophilic resin form.[21]

AH-Plus has the highest antimicrobial activity in the evaluation period 24 h on *C. albicans* and *S. aureus.* Miyagak *et al.* found that AH-Plus has antimicrobial activity on *C. albicans, S. aureus, and Escherichia coli*.[22] Besides, Yasuda showed that AH-plus has higher antimicrobial activity against all the tested microorganisms (*S. aureus*, *E. faecalis*, *C. albicans*, *Staphylococcus mutans*, and *Streptococcus saguinis*).[23] The action of AH-Plus is due to the presence of bisphenol A diglycidyl ether. Components of paste A (containing epoxy resin) and paste B (containing amines) are mixed together, whereby the sealer reduced the cell viability.[24] Furthermore, this sealer has a good flow, thereby diffusing into the dentinal tubules and creating microbial inhibition by means of entombment.[25] It has been also reported that material released formaldehyde in the polymerization process. This contributes sealers' antibacterial property.

In the present study, AH-Plus sealer exhibited the highest zone of inhibition compared to Endosequence BC sealer and Epiphany sealer against *S. aureus* and *C. albicans*, respectively. This result could imply that these sealers contain more potent antibacterial inhibitors than Endosequence BC sealer and Epiphany sealer. The study has shown that the antibacterial components of these sealers have better diffusion properties.

However, there are certain limitations associated with this study. The results of the agar diffusion method could be influenced by diffusion and affinity of the material to the culture medium since a material that diffuses easily usually results in a larger zone of inhibition of bacterial growth.

Furthermore, agar diffusion method is not completely reliable as it has its own limitations such as the intensity of agar, condition of plate storage, time of incubation, size and number of specimen or plate, quantity of culture medium, inability to distinguish between bacteriostatic and bacteriocidal properties, and can be tested only in water-soluble agents. There are contemporary and more reliable methods available to check antibacterial efficacy which can also be tried in future to test the same.

Conclusion

Within the experimental conditions of the present study, it can be concluded that

- BC sealer showed the least antimicrobial activity against *S. aureus*. BC sealer exhibited moderate antimicrobial activity against *C. albicans* though it was lesser than AH-Plus sealer
- Epiphany self-etch sealer showed least antimicrobial activity against *S. aureus and C. albicans*
- AH-Plus sealer exhibited a larger zone of inhibition than BC sealer and Epiphany self-etch sealer against *S. aureus and C. albicans*
- Antibiotic disc of amoxiclav, which was used as a control against *S. aureus*, exhibited moderate antimicrobial activity
- Antibiotic disc of fluconazole, which was used as a control against, *C. albicans* exhibited the highest antimicrobial activity.

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Conflicts of interest

There are no conflicts of interest.

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