ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)
IJDSIR : Dental Publication Service
Available Online at:www.ijdsir.com
Volume – 8, Issue – 4, July – 2025, Page No. : 76 - 83

The In Vitro Antimicrobial Efficacy of Two Different Bioceramic Sealers against Enterococcus Faecalis

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Citation of this Article: Anisha M Fernandes, B S Keshava Prasad, "The In Vitro Antimicrobial Efficacy of Two Different Bioceramic Sealers against Enterococcus Faecalis", IJDSIR- July – 2025, Volume – 8, Issue – 4, P. No. 76 – 83.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: The purpose of this study was to evaluate the antimicrobial effect of two bioceramic sealers (Ceraseal-B and Bio-C) against Enterococcus faecalis.

Methods: The antimicrobial effect of the two bioceramic sealers were assessed by the agar diffusion test (ADT) method using AH Plus sealer as a control. The results were reported in terms of the diameter of the growth inhibition zones in mm. The zone of inhibition for each sealer was measured at 24h, 48h and 72 hrs.

Results: The results showed that amongst the bioceramic sealers tested, Bio-C had a greater zone of inhibition compared to Ceraseal-B. This showed that Bio-C had a greater antimicrobial activity compared to Ceraseal-B. However, both of the freshly mixed bioceramic sealers had a lesser antimicrobial activity against the control group (AH Plus).

Conclusion: The invitro antimicrobial efficacy of Bio-C sealer was greater than that of Ceraseal-B sealer.

Keywords: Bioceramic sealers, Ceraseal-B, Bio-C, AH Plus, E.faecalis, agar diffusion test, zone of inhibition

Introduction

Bacteria and their byproducts are the main contributors to pulpal and periapical diseases. Eradicating bacteria from the root canal involves both chemical disinfection and mechanical preparation of the canal system. However, despite the range of chemical irrigants and mechanical techniques available, it is not always possible to completely remove all microbes from the canal. Consequently, using root canal filling materials with antibacterial properties is regarded as advantageous. The root canal is classically filled using gutta-percha in combination with a root canal sealer. [1]

The success of root canal therapy primarily relies on the elimination of infecting microorganisms. Hence, root

canal sealers with effective sealing properties and antimicrobial activity are essential to entomb and eradicate any remaining microbes. Microorganisms within the root canal dentin may either adhere superficially to the dentinal walls or penetrate deeper into the dentinal tubules (Ando & Hoshino 1990, Peters et al. 2001). Bacteria on the surface are generally easier to eliminate, whereas those embedded within the dentinal tubules are more protected. However, microorganisms within the tubules can still be affected by antimicrobial agents released from the sealer. Therefore, testing the antimicrobial nature of sealers should account for both direct contact with bacteria and the effects of antimicrobial leaching. [2]

Root canal filling is one of the most critical stages of endodontic treatment. Root canal sealers are used to `ill spaces, seal the voids between the root canal walls and the obturating material, obturate the accessory canals and inhibit bacterial infiltration into the root canal space. Over the years, root canal sealers has seen significant developmental advancements. [3] The latest innovation in this field is the introduction of bioceramic-based root canal sealers, known for their biocompatibility, the ability to induce and promote healing. Bioceramics are ceramic materials specifically developed for medical and dental applications which include alumina, zirconia, calcium silicates, bioactive glass, glass ceramics, hydroxyapatite, and calcium phosphates. [6] They are the latest generation of root canal sealers based on tricalcium phosphate, tricalcium silicate and mineral trioxide aggregate (MTA). [4]

E. faecalis is an organism that is frequently recovered from root canals with signs of apical periodontitis.

Being challenging to eliminate this bacteria from the root canals through root canal medication, it might be helpful if the sealer exerts some kind of antimicrobial activity. [1] It's ability to survive in the root canal as a sole organism and to resist nutrient starvation for long periods of time makes E. faecalis a resilient organism in the root canals. [2]

The aim of this invitro study aims at comparing the antimicrobial efficacy of two calcium silicate-based bioceramic sealers [Bio-C sealer (Angelus) and Ceraseal-B sealer (MAARC)] against Enterococcus faecalis using AH Plus sealer as a control using Agar Diffusion Test.

Materials and Methodology

Three different types of sealers were used in this study- Bio-C sealer (Angelus), Ceraseal-B sealer (MAARC) and AH Plus sealer (Dentsply). AH Plus was used as a control.

The three sealers were divided into three groups:

Group I- Bio-C sealer

Group II- Ceraseal-B sealer

Group III- AH Plus sealer

The test bacterial strains:

Enterococcus faecalis (MTCC 439) were obtained from Microbial Type Culture Collection and Gen Bank (IMTECH, Chandigarh, India).

For evaluating Zone of Inhibition (ZOI), 9 plates of Enterococcus faecalis agar base media were used. Inoculum of Enterococcus faecalis strains was prepared by harvesting colonies with a circular loop. The bacteria were cultivated in brain heart infusion (BHI) agar medium and suspension culture was prepared. Aliquots of the suspension was evenly spread over Muller-Hilton Agar contained in petri dish (n=9). The Petri dishes containing media were left to solidify for 15min at 37oC. Each of the 9 petri dishes were then evenly divided into four sections. A sterile stainless-steel cylinder was used to create wells of 4mm depth and 5mm diameter in each of the sections for all the 9 plates.

Agar Diffusion Test

Complying with the manufacturers' instructions, each of the sealers were mixed, and it was utilized within the given time of mixing using a digital timer. Four samples of each of the sealers were placed into the wells in each of the four sections of the pre-labelled media-containing petri dishes. For the evaluation of ZOI, three petri dishes per sealer type were used, amounting to 12 samples each. Incubation was carried out for1 week at 37°C under anaerobic conditions, during which measurements of the inhibition zones of the bacteria were recorded at 24 hrs, 48 hrs and 72 hrs.

Statistical Analysis of Data

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses.

Descriptive Statistics

Descriptive analysis includes expression of Zone of Inhibition in terms of Mean & SD in each group.

Inferential Statistics

One-way ANOVA Test followed by Tukey's post hoc Test was used to compare the mean zone of inhibition between 3 groups at different time intervals. Repeated measures of the ANOVA test followed by Bonferroni's post hoc test was used to compare the mean zone of inhibition between different time intervals in each group. The level of significance was set at $p<0.05$.

Results

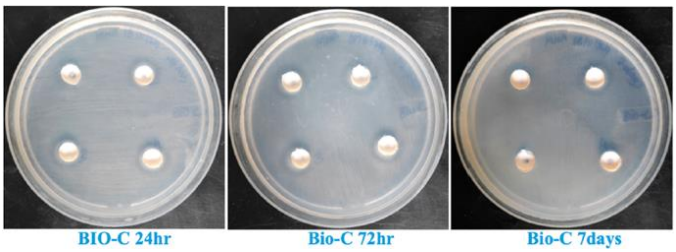


Figure 1: Zones of Inhibition of Bio-C against E. faecalis

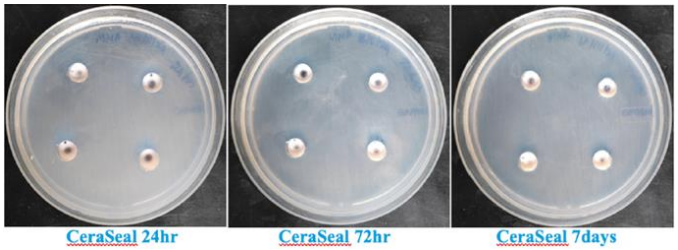


Figure 2: Zones of Inhibition of Ceraseal -B against E. faecalis

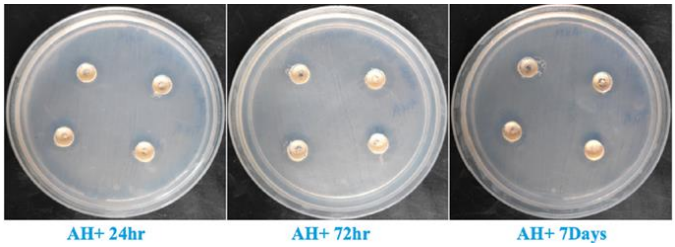
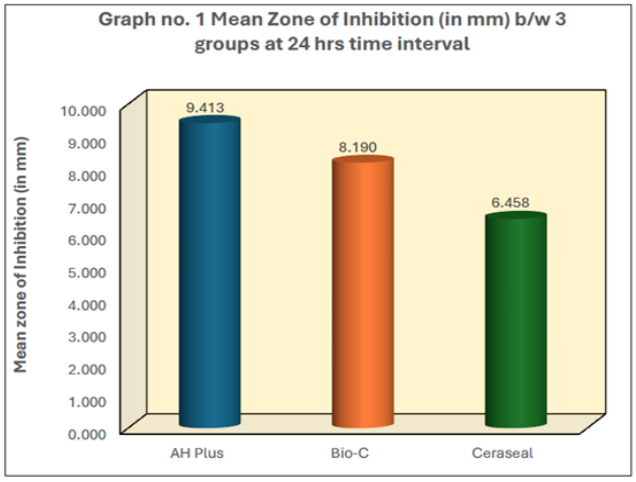


Figure 3: Zones of Inhibition of AH Plus against E. faecalis

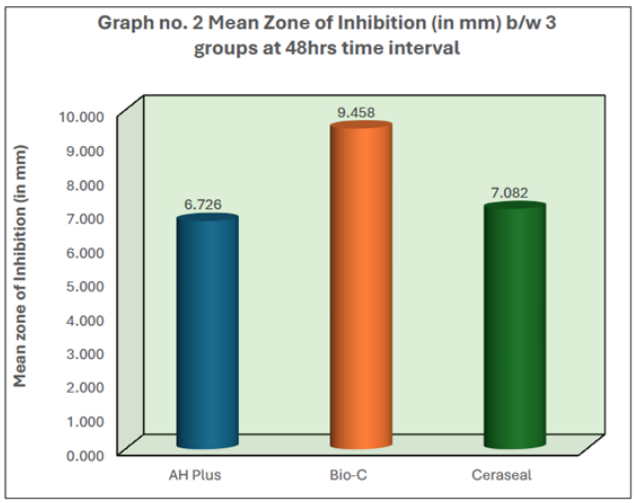
Comparison of mean Zone of Inhibition (in mm) b/w diff. time intervals in each group using Repeated measures of ANOVA Test followed by Bonferroni's post hoc Test

Groups	Time	N	Mean	SD	Min	Max	p-value [a]	Sig. Diff	p-value [b]
AH Plus	24 hrs	12	9.413	0.131	9.15	9.62	<0.001*	A vs B	<0.001*
	48 hrs	12	6.726	0.011	6.71	6.74		A vs C	<0.001*
	72 hrs	12	2.504	0.034	2.43	2.55		B vs C	<0.001*
Bio-C	24 hrs	12	8.190	0.057	8.09	8.25	<0.001*	A vs B	<0.001*
	48 hrs	12	9.458	0.029	9.39	9.48		A vs C	<0.001*
	72 hrs	12	4.337	0.014	4.31	4.36		B vs C	<0.001*
Ceraseal	24 hrs	12	6.458	0.019	6.44	6.49	<0.001*	A vs B	<0.001*
	48 hrs	12	7.082	0.022	7.05	7.12		A vs C	<0.001*
	72 hrs	12	3.464	0.017	3.44	3.48		B vs C	<0.001*

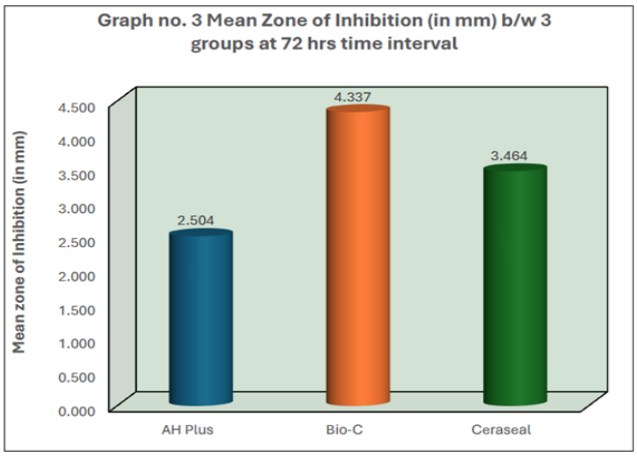
Table 1: Comparison of mean Zone of Inhibition (in mm) b/w diff. time intervals in each group using Repeated measures of ANOVA Test followed by Bonferroni's post hoc Test



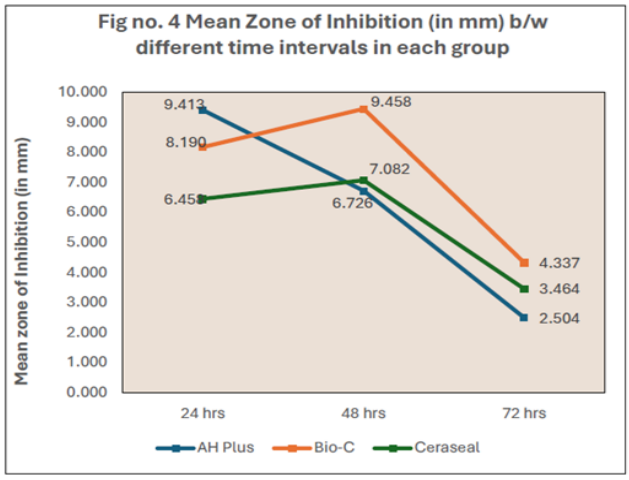
Graph 1: Mean Zone of Inhibition (In Mm) B/W 3 Groups At 24 Hrs Time Interval



Graph 2: Mean Zone of Inhibition (In Mm) B/W 3 Groups At 48 Hrs Time Interval



Graph 3: Mean Zone of Inhibition (In Mm) B/W 3 Groups At 72 Hrs Time Interval



Graph 4: Mean Zone of Inhibition (In Mm) B/W Different Time Intervals In Each

Group

Fig 1, Fig 2 and Fig 3 show the zones of inhibition of Bio-C sealer, Ceraseal-B sealer and AH Plus sealer against E.faecalis respectively. Freshly mixed AH Plus showed the highest ZOI against E. faecalis after 24 hours. This was followed by Bio-C and Ceraseal-B. After 72 hours, the ZOI of AH Plus decreased significantly whereas that of the bioceramic sealers increased. Bio-C had a larger ZOI in comparison to Ceraseal-B. ZOI after 7 days was still the highest for Bio-C as compared to Ceraseal-B and AH Plus. With respect to the bioceramic sealers, the ZOI was higher for Bio-C sealer. The ZOI for all the three sealers reduced over time.

In summary, all three groups exhibit significant changes in antimicrobial activity over time, with AH Plus showing a continuous decline, Bio-C peaking at 48 hours before decreasing at 72 hrs, and Ceraseal showing a moderate increase at 48 hours followed by a decrease at 72 hours. The statistically significant p-values underscore these significant differences across the time intervals. Graph 1, Graph 2 and Graph 3 show the mean zone of inhibition between the different sealers at 24hrs,

48hrs and 72hrs. Graph 4 shows the mean zone of inhibition between different time intervals in each group.

Discussion

In this study, 3 sealers were used namely, Bio-C sealer, Ceraseal-B sealer and AH Plus sealer (control group).The primary aim of this study was to evaluate the antimicrobial effect of Bio-C and Ceraseal-B against Enterococcus faecalis (E.faecalis). E. faecalis is a gram positive facultative anaerobic bacteria that is frequently recovered from root canals with signs of endodontic failure.[1] E.faecalis is a gram positive anaerobic bacteria that is present in 4 to 40% of primary endodontic infections and in 24 to 77% of persistent or secondary endodontic infections.[2] Due to its aggressive invasiveness, it can invade dentinal tubules and attach to collagen, complicating its eradication. This creates a serious obstacle in root canal treatment.[5] Calcium silicate-based root canal sealers have become increasingly favored in endodontics because of their superior biocompatibility and physicochemical characteristics.[2]

The antibacterial activity was evaluated using the agar diffusion test (ADT). This method involves creating an inhibition zone, often referred to as a halo, around the test material on an agar plate. The diameter of this zone shows the antibacterial effectiveness of the sealer. The ADT offers several benefits, including its simplicity, cost-effectiveness, and ease of use, making it suitable for testing a wide range of microorganisms and antimicrobial agents. Additionally, the results are easy to interpret, contributing to its widespread application in antimicrobial studies. [6]

Spangberg et al. firstly reported that AH Plus presents high antibacterial effectiveness due to its release of formaldehyde or bisphenol A ether during polymerisation[7] AH Plus exhibited limited effectiveness against Enterococcus faecalis. These results align with earlier studies, which demonstrated that only freshly manipulated AH Plus displayed antibacterial activity, while samples aged 48 hours and 7 days showed reduced antibacterial effects against E. faecalis[8,9]. The antimicrobial activity of fresh AH Plus may be explained by the toxic properties of amines and epoxy resin present in its composition. Additionally, this activity could result from the minimal release of formaldehyde during the polymerization process.[10]

The results of this study align with earlier research by Bronzel et al.[11] and Bukhari et al [12], who also reported that calcium silicate and phosphate-based sealers (TotalFill BC and Endosequence BC) demonstrated significantly greater antibacterial effectiveness against Enterococcus faecalis compared to the epoxy-based resin sealer (AH Plus).

However, there remains some debate in endodontic literature about the superior antimicrobial efficacy of calcium silicate and calcium phosphate-based sealers. For instance, in the studies by Candeiro et al [13], the EndoSequence BC Sealer failed to show significant antibacterial effectiveness compared to the epoxy-resin based sealer. These discrepancies among studies may be attributed to differences in the microbiological methodologies used. To date, the antibacterial efficacy of root canal sealers has primarily been evaluated using the agar diffusion test (ADT) [14,15] which is the most commonly employed method in such research. The ADT measures the inhibition zones of bacterial growth around the tested materials, with larger inhibition zones indicating greater antibacterial efficacy.

The literature shows that in addition to be able to induce biomineralization after implantation into connective tissue [16], Bio-C Sealer has the capability to alkalinise and reach a pH of 10, up to 21 days [17] It is known that

pH higher than 9 can inactivate cell membrane enzymes of microorganisms, causing loss of biological activity or integrity of the plasma membrane. Hence, it is necessary to maintain high pH levels, as several species remain stable at pH 9 or higher.[18] Despite the sealer's short setting time (≤ 240 minutes) and high solubility, the considerable release of calcium hydroxide may further contribute to the antibacterial properties of fresh Bio-C Sealer. [19]

The alkaline pH of calcium silicate-based root canal sealers is considered one of their primary advantages.

This alkalinity facilitates the creation of deposits resembling apatite on the surface of the sealer when it comes into contact with body fluids, enhancing bioactivity and establishing a strong chemical bond[20, 21].

Additionally, the alkaline environment promotes healing of the apical region and mineralization of the tissues [22], while also providing bacteriostatic effects that inhibit bacterial growth [23].

Another critical feature is the release of calcium ions from these sealers. This release plays a significant role in promoting a strong chemical bond and boosting bioactivity by encouraging the precipitation of an apatite-like layer along the dentin walls. This process further strengthens the seal and integration of the sealer with the neighbouring tissues. [24,25]

It has been noted from the present study that the antimicrobial activity of both the bioceramic sealers eventually decreases with time. It was the maximum at 24 h and the minimum at 7 days. Based on the results of this study, Bio-C Sealer emerges as a promising choice for managing root canal infections that are persistent. However, it is important to acknowledge that this study was conducted in vitro, and its findings may not fully reflect real-world clinical conditions. The presence of dentin can significantly influence the antimicrobial performance of sealers, potentially altering their efficacy. Therefore, further in vivo clinical studies are necessary to confirm the effectiveness of Bio-C Sealer and validate its application in endodontic practice.

Conclusion

Within the limitations of this study, it can be concluded that Bio-C sealer has a greater antimicrobial efficacy against Enterococcus faecalis in comparison to Ceraseal-B at varying incubation periods.

Abbreviations

ADT: Agar Diffusion Test

E. faecalis: Enterococcus faecalis

HRS: hours

MM: millimeter

ZOI: Zone of Inhibition

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