ORIGINAL RESEARCH

Antimicrobial Activity of Five Calcium Silicate Based Root Canal Sealers against a Multispecies Engineered Biofilm: An *In Vitro* Study

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Abstract

Aim: The present study's objective was to compare the impact of CerasealR, total fill BC SealerR, Bio-C SealerR, AH Plus BioceramicR, and K-BiocerR on the elimination of a multispecies' endodontic biofilm at 3, 7 and 14 days.

Materials and methods: A total of 20 freshly extracted, caries-free premolars were prepared for the study to create dentinal disks. For the multispecies biofilm formation, *Enterococcus faecalis*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Candida albicans* were cultured and used to inoculate hydroxyapatite discs. After incubation, the biofilms were placed on blotting papers in petri dishes with an orthodontic bend. Different root canal sealers, including CeraSeal, total Fill BC Sealer, Bio-C Sealer, AH Plus Bioceramic, K-Biocer, and Sealite, were injected into the bend, facilitating contact with the biofilms. The samples were divided into seven groups, including a negative control. At specific intervals, 3, 7, and 14 days, 3 biofilm samples from each group were collected, diluted, and plated on Agar media for colony counting and analysis.

Results: In all tested groups, the total bacterial count significantly decreased between day 3 and 14 ($p < 0.05$) with no statistically significant differences among the different sealers' groups at all-time points for the total bacterial count, *E. faecalis* count, and *P. mirabilis* count. However, Sealite demonstrated the most consistent effectiveness in reducing bacterial counts across multiple categories. The sealite group was capable of decreasing the *C. albicans* count significantly between day 3 and day 14 ($p < 0.05$) in comparison with the bioceramic groups.

Conclusion: All sealers had antibacterial activity against the multispecies biofilm between day 3 and day 14. The ascending order of sealers in terms of their effectiveness in killing bacteria, based on the provided results, is as follows: Sealite, Bio-C Sealer, AH Plus, CeraSeal, TotalFill, and K-Biocer. However, there were no statistically significant differences in the bacterial counts among the different sealer groups at any time point.

Clinical significance: The role of sealers in combating biofilm-associated infections highlights their potential clinical utility in preserving root canal health. Understanding the antimicrobial properties of these sealers is vital for informed decision-making in selecting the most effective materials for improved treatment outcomes and long-term success in endodontic procedures.

Keywords: Biofilm, Bioceramics, calcium silicate based root canal sealers, Multispecies' biofilm.

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Introduction

Persistent apical periodontitis occurs when root canal treatment of initial apical periodontitis has not sufficiently eliminated the intraradicular infection.[1]

It is known that microorganisms present in root canals form biofilms, which makes them more resistant to antimicrobial agents than bacteria in the planktonic state.[2] The goal of endodontic treatment is to remove the microorganisms from the infected root canal in order to achieve clinical and radiological healing.[3] Although mechanical and chemical preparation significantly reduces the number of microorganisms in the infected root canal system, it is practically impossible to completely remove all microorganisms by irrigation and other methods.[4]

In the medical and dentistry disciplines, bioceramics are inorganic, nonmetallic, and biocompatible materials used in direct contact with living tissues.[5] Other bioceramic materials have been elaborated and applied successfully in endodontic treatments, including pulp capping, obturation, apical barrier construction, perforation repair, and root-end filling because they are chemically stable, non-corrosive, and interact well with organic tissues.[6] While some endodontic bioceramics are premixed materials that cure with moisture from the surrounding tissue, others are powder/

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liquid systems that require manual mixing.[7] The bioceramics can create a great seal with the tooth structure thanks to the curing procedure.[8]

By raising the pH and ion release from the material, the antibacterial and antibiofilm characteristics are applied during the setting process.[9] The outcome of endodontic therapy depends on both a high-quality seal and antibacterial characteristics.[10] By further physicochemical interactions (such as the biomineralization effect) with the surrounding dental hard tissues, antibiofilm qualities may continue to be present in a bioceramic-treated.[11]

These bioceramics possess unique compositions, and one key component responsible for their antimicrobial effects is calcium silicate.[3] Calcium silicate-based sealers, such as AH Plus Bioceramic, Bio-C Sealer, and K-Biocer, contain this component which exhibits inherent antimicrobial properties.[4] When these sealers come into contact with bacteria, calcium silicate releases calcium hydroxide ions that create an alkaline environment.[9] This alkalinity disrupts the cellular processes of microorganisms, inhibiting their growth and promoting antimicrobial effects.[11]

Comparative analysis enables direct comparisons of the different bioceramics' antimicrobial properties.[1–3] This information assists clinicians in selecting the most suitable bioceramic sealer for specific clinical scenarios, taking into account factors such as the spectrum of antimicrobial activity and compatibility with other endodontic materials and techniques.[4]

The aim of the present study was to compare the impact of 5 different calcium silicate-based root canal sealers on the bacterial reduction of a multispecies endodontic biofilm at 3, 7, and 14 days to aid clinicians in choose the most appropriate bioceramic sealer based on specific clinical circumstances.

Materials and Methods

Over a period of 2 years (2020–2022), the study aimed to assess the antimicrobial activity of five calcium silicate-based root canal sealers against a multispecies-engineered biofilm.

The study protocol was approved by the "Ethics Committee" of Saint Joseph University of Beirut, Lebanon (Study Reference: FMD200). The study was conducted in the Microbiological Laboratory at the same university.

Dentinal Disks Preparation

A total of 20 caries-free premolars were carefully selected for the study and extracted for orthodontic purposes. The teeth underwent a meticulous cleaning process to remove any soft tissue remnants using a 5% NaOCl solution, followed by rinsing with sterile water. Plastic molds were used to securely attach the teeth to a base using putty.

To create dentinal disks, a precise ratio mixture of EpoxyResin® base and hardener (3:1) was prepared. Thorough mixing ensured a homogeneous consistency and eliminated trapped air bubbles. The resin mixture was poured into the molds, ensuring complete coverage of the tooth, and left to set at room temperature for 24 hours. Afterward, the resin blocks were carefully removed from the molds.

Using an Isomet® 2000 precision saw, the resin blocks were cut into 2 mm thick wafers. To remove any residual resin layer, the wafers underwent polishing with TEXMET® and were thoroughly rinsed with water. An adhesive brush was then used to apply a 5% NaOCl solution and a 17% EDTA solution to remove the smear layer and surface contamination, each for 1 minute. Subsequently, the disks were rinsed under running water for five hours to ensure complete solvent removal. All samples underwent autoclaving at 120°C for 20 minutes to ensure sterility. The dentinal disks were stored in sterile water at 4°C until they were ready for use in the study.

Multispecies Biofilm Formation

E. faecalis derived from ATCC 29212 was obtained from the Microbiological laboratory and cultured aerobically on blood agar at 35°C for 48 hours according to the manufacturer's instructions. Colonies were later grown in brain heart infusion + 5% glucose (BHI) broth at 37°C for 24 hours in a shaker incubator followed by a 24 hours static incubation at 37°C. Inoculum was prepared in sterile BHI + 5% glucose broth and turbidity was set to 0.5 McFarland corresponding to approximately 1.5×10^8 colony forming units per milliliter (CFU/mL).

Ten µl of the inoculum were placed on 63 hydroxyapatite discs pretreated with collagen type I and were incubated for 16 days at 37°C.

P. mirabilis derived from ATCC 12453 and *P. aeruginosa* derived from ATCC 27853 were grown on plate count agar.

Plate Count Agar (PCA) at 37°C for 24 hours according to manufacturer's instructions, *C. albicans* derived from ATCC 10231 was grown on Yeast Glucose Chloramphenicole (YGC). Colonies were later grown in brain heart infusion + 5% glucose (BHI) broth at 37°C for 24 hours in a shaker incubator followed by a 24 hours static incubation at 37°C. Inoculum was prepared in sterile BHI + 5% glucose broth and turbidity was set to 0.5 McFarland corresponding to approximately 1.5×10^8 colony forming units per milliliter (CFU/mL).

P. aeruginosa was added to the pretreated hydroxyapatite discs on day 10, *C. albicans* on day 14, and *P. mirabilis* on day 16.

The multispecies biofilm was then incubated for 2 days at 37°C after the addition of all the microorganisms.

Sample Preparation

A total of 63 formed biofilms on the hydroxyapatite disks were carefully removed and placed on 10 mm round-shaped blotting papers. These biofilm-containing blotting papers were then positioned on a metallic net and placed in a 6 cm petri dish filled with sterile BHI broth. An orthodontic bend #2 was placed in the middle of the blotting paper. The bioceramics were injected in the orthodontic bend ensuring direct contact with the biofilms.

The sample was divided equally and randomly into seven groups (9 disks in each group): Group I: CeraSeal (Meta Biomed, Cheongju, Korea), Group II: Total Fill BC Sealer (TFBC; FKG Dentaire, La Chaux-des-Fonds, Switzerland), Group III: Bio-C Sealer (Angelus, PR, Brazil), Group IV: AH Plus Bioceramic (AHBC, Dentsply Sirona, York, PA, USA), Group V: K-Biocer (Rikitta, Lebanon), Group VI: Sealite (Pierre Rolland, Merignac, France), Group VII: negative group control with no intervention where the colonies were counted prior to the intervention.

At days 3, 7, and 14, 3 blotting papers underneath the orthodontic bend from each group were removed using forceps and placed in sterile BHI broth for 15 minutes. After vortex, the biofilm was dissected using the sterile needle technique for 15 minutes.

Table 1: Descriptive statistics of total bacterial count ($\times 10^7$) according to groups and time

	Time			
Groups	*Day 3*	*Day 7*	*Day 14*	*p-value*
Control ($n = 9$)				
Mean ± SD	4.62 ± 0.73^C	4.86 ± 0.72^B	5.11 ± 0.71^A	<0.001*
Median (Q1–Q3)	$4.6\ (3.95–5.30)^C$	$4.8\ (4.2–5.55)^B$	$5.1\ (4.45–5.75)^A$	
CeraSeal ($n = 9$)				
Mean ± SD	2.63 ± 0.23^A	2.36 ± 0.22^B	2.16 ± 0.17^C	<0.001*
Median (Q1–Q3)	$2.6\ (2.4–2.75)^A$	$2.3\ (2.2–2.5)^B$	$2.1\ (2.05–2.5)^C$	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
Total Fill ($n = 8$)				
Mean ± SD	2.56 ± 0.17^A	2.37 ± 0.18^B	2.19 ± 0.17^C	<0.001*
Median (Q1–Q3)	$2.5\ (2.42–2.67)^A$	$2.3\ (2.22–2.55)^B$	$2.1\ (2.1–2.35)^C$	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
Bio-C Sealer ($n = 9$)				
Mean ± SD	2.71 ± 0.23^A	2.50 ± 0.21^B	2.27 ± 0.19^C	<0.001*
Median (Q1–Q3)	$2.7\ (2.55–2.9)^A$	$2.5\ (2.35–2.7)^B$	$2.3\ (2.1–2.4)^C$	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
AH Plus (n = 9)				
Mean ± SD	2.73 ± 0.24^A	2.52 ± 0.26^B	2.31 ± 0.26^C	<0.001*
Median (Q1–Q3)	$2.8\ (2.45–2.95)^A$	$2.6\ (2.25–2.75)^B$	$2.3\ (2.05–2.55)^C$	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
K-Biocer ($n = 9$)				
Mean ± SD	2.76 ± 0.25^A	2.54 ± 0.25^B	2.33 ± 0.25^C	<0.001*
Median (Q1–Q3)	$2.8\ (2.5–2.95)^A$	$2.6\ (2.3–2.75)^B$	$2.4\ (2.1–2.55)^C$	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
Sealite ($n = 9$)				
Mean ± SD	2.70 ± 0.22^A	2.50 ± 0.22^B	2.28 ± 0.22^C	<0.001*
Median (Q1–Q3)	$2.7\ (2.5–2.9)^A$	$2.5\ (2.3–2.7)^B$	$2.3\ (2.1–2.5)^C$	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	

Different uppercase superscript letters indicate statistically significant differences between the timepoints within each group

About 50 µl of the liquid medium was serially diluted in sterile BHI broth and plated on different agars. Plate count agar for the determination of the total number of bacteria. Yeast Glucose Chloramphenicol for *C. albicans*, cetrimid agar (AC) for *P. aeruginosa*, Slantez and Bartley Agar (SBA) for *E. faecalis*, and uriselect agar for *P. mirabilis*.

Colonies were counted and confirmed by colony morphology observation on the agar of choice at 3, 7 and 14 days by 2 investigators.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, NY, USA). Descriptive statistics of the quantitative variables were summarized and presented as medians (1st and 3rd quartiles) and means ± standard deviations. The normality of the distribution of the quantitative variables was assessed using the Shapiro-Wilk test. The Friedman's test was used to compare values within each group between the three time points when data was not normally distributed, and repeated-measures ANOVA was used instead when the normality of distribution was assumed, both tests were followed by the Bonferroni *post-hoc* test for multiple comparisons. Mann-Whitney *U*-test (when data was not normally distributed) and student *t*-test (when data was normally distributed) were used to compare values within each time point between groups. All tests were two-tailed and the level of significance was set at 5%.

Results

The descriptive statistics of the total bacterial count for the seven different groups and three-time points, along with the comparisons between each sealer and the control group at each time point, are presented in Table 1. In the control group, there was a significant increase in the total bacterial count between day 3 and day 14 ($p < 0.05$). Conversely, in all other groups (CeraSeal, Total Fill, Bio-C Sealer, AH Plus, K-Biocer, and Sealite), there was a significant decrease in the total bacterial count between day 3 and day 14 ($p < 0.05$). The total bacterial count was significantly higher in the control group compared to all other groups at days 3, 7, and 14 ($p < 0.05$). However, there were no statistically significant differences in the total bacterial count among the different sealer groups at any time point ($p > 0.05$).

In the analysis of *C. albicans* count, an increase was observed in the control group between days 3 and 14, but this increase was not statistically significant ($p > 0.05$). However, a significant decrease in the *C. albicans* count was found in the sealite group between day 3 and day 14 ($p < 0.05$), as well as between day 7 and day 14. Among the other groups, although the *C. albicans* count decreased between days 3 and 14, the decrease was not

Table 2: Descriptive statistics of *C. albicans* count ($\times 10^6$) according to groups and time

	Time			
Groups	*Day 3*	*Day 7*	*Day 14*	*p-value*
Control ($n = 9$)				
Mean ± SD	12.78 ± 2.91	13.56 ± 2.96	14.22 ± 3.38	0.308
Median (Q1–Q3)	13 (10.5–15)	14 (11–15)	13 (11–17.5)	
CeraSeal ($n = 9$)				
Mean ± SD	6.89 ± 1.62	5.67 ± 1.50	5.78 ± 1.30	0.072
Median (Q1–Q3)	7 (5–8.5)	6 (4–7)	5 (5–7)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
TotalFill ($n = 8$)				
Mean ± SD	7.00 ± 1.60	7.12 ± 1.25	6.00 ± 1.07	0.252
Median (Q1–Q3)	7 (5.25–8.75)	7 (6.25–8)	6 (5.25–7)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
Bio-C Sealer ($n = 9$)				
Mean ± SD	7.22 ± 1.30	6.00 ± 1.50	6.00 ± 0.87	0.106
Median (Q1–Q3)	8 (6–8)	6 (4.5–7.5)	6 (5–7)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
AH Plus ($n = 9$)				
Mean ± SD	7.33 ± 1.00	6.22 ± 0.97	6.56 ± 1.33	0.070
Median (Q1–Q3)	7 (6.5–8)	6 (5.5–7)	7 (5.5–7.5)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
K-Biocer ($n = 9$)				
Mean ± SD	7.56 ± 1.24	6.56 ± 0.88	6.78 ± 1.20	0.148
Median (Q1–Q3)	7 (6.5–9)	7 (6–7)	7 (5.5–8)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
Sealite ($n = 9$)				
Mean ± SD	6.78 ± 1.56^A	6.44 ± 1.01^A	4.67 ± 0.87^B	0.001*
Median (Q1–Q3)	6 (5.5–8.5)^A	7 (5.5–7)^A	4 (4–5.5)^B	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	

Different uppercase superscript letters indicate statistically significant differences between the timepoints within the Sealite group

statistically significant ($p > 0.05$). The control group consistently had a significantly higher *C. albicans* count compared to all other groups at all-time points ($p < 0.05$). Furthermore, there were notable statistically significant differences observed at day 7 between CeraSeal and Total Fill ($p = 0.047$), at day 14 between Total Fill and Sealite ($p = 0.021$), at day 14 between Bio-C Sealer and Sealite ($p = 0.011$), at day 14 between AH Plus and Sealite ($p = 0.006$), and at day 14 between K-Biocer and Sealite ($p = 0.002$) regarding the *Candida Albicans* count (Table 2).

The *E. faecalis* count showed an increase over time in the control group, although this increase was not statistically significant ($p > 0.05$). In the K-Biocer group, a decrease in *E. faecalis* count was observed, but with borderline significance compared to the other groups ($p \approx 0.05$). On days 3, 7, and 14, the *E. faecalis* count was significantly higher in the control group compared to all other groups ($p < 0.05$). Notably, at day 7, a statistically significant difference was found in *E. faecalis* count between the Bio-C Sealer and Sealite groups ($p = 0.040$) (Table 3).

The analysis of *P. aeruginosa* count revealed a significant increase in the control group between days 3 and 14 ($p < 0.05$). In the other sealer groups, a decrease in *P. aeruginosa* count was observed between days 3 and 14, but this decrease was statistically significant only in the CeraSeal group ($p < 0.05$). At all-time points (days 3, 7, and 14), the control group exhibited a significantly higher *P. aeruginosa* count compared to all other groups ($p < 0.05$). No statistically significant differences were found among the different sealer groups for the *P. aeruginosa* count at any time point ($p > 0.05$) (Table 4).

The analysis of *P. mirabilis* count revealed a statistically significant decrease in the Bio-C Sealer group between day 7 and day 14 ($p < 0.05$). However, no statistically significant differences were observed in the *P. mirabilis* count between time points in the other groups ($p > 0.05$). At all-time points (days 3, 7, and 14), the control group exhibited a significantly higher *P. mirabilis* count compared to all other groups ($p < 0.05$). There were no statistically significant differences in the *P. mirabilis* count among the different sealer groups at any time point ($p > 0.05$) (Table 5).

In the control group, the total bacterial count increased significantly between day 3 and day 14. However, in all other groups, there was a significant decrease in the bacterial count during the same period. The *C. albicans* count increased in the control group but without statistical significance. The *E. faecalis* count increased in the control group, while in the K-Biocer group, there was a borderline significant decrease. The *P. aeruginosa* count increased significantly in the control group between day 3 and day 14. The *P. mirabilis* count showed no significant differences among the different groups and time points.

In all tested groups, there were no statistically significant differences among the different sealers' groups at all-time points for the total bacterial count, *E. faecalis* count, and *P. mirabilis* count.

Table 3: Descriptive statistics of Enterococcus count ($\times 10^6$) according to groups and time

	Time			
Groups	*Day 3*	*Day 7*	*Day 14*	*p-value*
Control ($n = 9$)				
Mean ± SD	12.00 ± 2.78	12.22 ± 2.44	12.89 ± 2.42	0.284
Median (Q1–Q3)	13 (9.5–15)	13 (10–13)	14 (10.5–15)	
CeraSeal ($n = 9$)				
Mean ± SD	5.89 ± 1.54	5.78 ± 1.64	5.56 ± 1.67	0.892
Median (Q1–Q3)	6 (4.5–7.5)	5 (4.5–7)	5 (4–6.5)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
TotalFill ($n = 8$)				
Mean ± SD	6.75 ± 1.03	5.87 ± 2.10	6.75 ± 1.28	0.261
Median (Q1–Q3)	7 (6–7.75)	5.5 (4–8.25)	7 (5.5–7)	
p-value (difference with the control group)	<0.001*	<0.001*	0.018*	
Bio-C Sealer ($n = 9$)				
Mean ± SD	7.00 ± 0.87	6.56 ± 1.01	5.67 ± 1.50	0.067
Median (Q1–Q3)	7 (6–8)	6 (6–7.5)	6 (4–7)	
p-value (difference with the control group)	0.003*	<0.001*	<0.001*	
AH Plus ($n = 9$)				
Mean ± SD	6.44 ± 1.59	6.44 ± 1.24	5.44 ± 1.67	0.233
Median (Q1–Q3)	6 (5–8)	6 (5.5–7)	5 (4–6.5)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
K-Biocer ($n = 9$)				
Mean ± SD	7.11 ± 0.93	6.56 ± 1.42	5.44 ± 1.01	0.053
Median (Q1–Q3)	7 (6.5–7.5)	7 (5.5–8)	5 (5–6.5)	
p-value (difference with the control group)	0.001*	<0.001*	<0.001*	
Sealite ($n = 9$)				
Mean ± SD	6.22 ± 1.56	5.56 ± 0.73	6.33 ± 0.71	0.217
Median (Q1–Q3)	7 (4.5–7.5)	5 (5–6)	6 (6–7)	
p-value (difference with the control group)	<0.001*	<0.001*	0.004*	

However, the total bacterial count significantly decreased between day 3 and day 14 ($p < 0.05$) in all groups. Notably, the Sealite group consistently demonstrated the most effective reduction in bacterial counts across multiple categories. Additionally, the Sealite group significantly decreased the *C. albicans* count between day 3 and day 14 ($p < 0.05$) compared to the bioceramic groups.

Discussion

One of the primary causes of pulp necrosis, periapical pathology, and unsuccessful root canal treatments is bacteria, their compounds, and their ability to form biofilms.[3] Hence, the primary goal of root canal therapy is to eliminate bacteria and stop them from spreading throughout the root canal system.[3,4] This highlights the need to maximizing the effectiveness of irrigants to improve disinfection,[12] before filling the root canal systems with sealing compounds and filling materials that have antibacterial effects, especially before setting.[13] The aim of the present study was to compare the impact of different bioceramics and sealite on multispecies endodontic biofilm elimination at 3, 7, and 14 days.

Most of the studies on antimicrobial activity use a monospecies biofilm which is far from the in vivo situation, in which infected canals host a polymicrobial infection where microorganisms create three-dimensionally structured communities with fluid channels for the transportation of food, waste, and signal molecules.[4,14] Further, several studies have shown that multispecies biofilms demonstrate increased resistance to antimicrobial treatment compared to monoculture biofilms.[3,13,15] For example, when the aerobic bacteria consume oxygen they provide anaerobic circumstances inside the deeper layers of the biofilm, for instance, anaerobic bacteria are able to survive aerobic conditions when grown in a mixed biofilm.[4,14,16]

Since only a specific set of microorganisms thrive in the environment of the necrotic root canal, 4 microorganisms were selected for the present study, *E. faecalis* is a pioneer bacteria in the formation of endodontic biofilm, resistant to all kind of irrigation techniques[17] which make it one of the main reasons of the persistence of a periapical lesion after endodontic treatment.[4,17,18] *P. aeruginosa*, *C. albicans,* and *P. mirabilis* are some of the main microorganisms in a necrotic pulpal infection.[4]

Bioceramic sealers have the advantage of extended antimicrobial activity in comparison with Sealite which can lose antimicrobial activity after setting.[19] Within all the bioceramic groups, the total bacterial count has significantly decreased between day 3 and day 14 ($p < 0.05$) this antibacterial activity is mostly related to their capacity to raise pH after releasing hydroxyl ions in comparison with traditional sealers.[19,20] The production of calcium silicate hydrogel and calcium hydroxide, which raise and maintain a high pH in the root canal environment is facilitated by moisture from dentin.[21] When silica is dissolved in a high pH environment, it can directly reduce the viability of bacteria.[9, 11, 21]

Total fill R bioceramic sealer, at day 14, killed more bacteria but no statistical difference was found in comparison with the

Table 4: Descriptive statistics of *P. aeruginosa* count ($\times 10^6$) according to groups and time

	Time			
Groups	*Day 3*	*Day 7*	*Day 14*	*p-value*
Control ($n = 9$)				
Mean ± SD	10.22 ± 1.92^{B}	10.44 ± 2.24^{B}	12.22 ± 2.82^{A}	0.012*
Median (Q1–Q3)	$9\ (9–11.5)^{B}$	$12\ (8–12)^{B}$	$12\ (10–14.5)^{A}$	
CeraSeal ($n = 9$)				
Mean ± SD	6.89 ± 1.69^{A}	6.44 ± 1.59^{AB}	5.22 ± 1.39^{B}	0.040*
Median (Q1–Q3)	$7\ (5.5–8.5)^{A}$	$6\ (5.5–8)^{AB}$	$5\ (4–7)^{B}$	
p-value (difference with the control group)	0.001*	<0.001*	<0.001*	
TotalFill ($n = 8$)				
Mean ± SD	6.12 ± 1.73	5.25 ± 1.03	4.87 ± 0.83	0.199
Median (Q1–Q3)	5.5 (5–7.75)	5 (4.25–6)	5 (4–5.75)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
Bio-C Sealer ($n = 9$)				
Mean ± SD	6.78 ± 1.39	6.22 ± 1.56	5.78 ± 1.39	0.356
Median (Q1–Q3)	7 (5.5–8)	7 (5–7)	6 (4.5–7)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
AH Plus ($n = 9$)				
Mean ± SD	6.89 ± 1.17	6.11 ± 1.62	5.56 ± 1.42	0.347
Median (Q1–Q3)	7 (6–7.5)	6 (5–7.5)	5 (4–7)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
K-Biocer ($n = 9$)				
Mean ± SD	6.22 ± 1.39	6.67 ± 1.22	5.89 ± 1.90	0.462
Median (Q1–Q3)	6 (5–7.5)	6 (6–7.5)	6 (4–8)	
p-value (difference with the control group)	0.001*	0.001*	0.001*	
Sealite ($n = 9$)				
Mean ± SD	7.11 ± 1.54	6.56 ± 1.74	6.22 ± 1.39	0.244
Median (Q1–Q3)	7 (5.5–8.5)	7 (5–8)	6 (5–8)	
p-value (difference with the control group)	0.002*	0.001*	0.005*	

Different uppercase superscript letters indicate statistically significant differences between the timepoints within the control and CeraSeal groups

other groups at all-time points. This result might be related to the capability of total fill to have a long-lasting antimicrobial ability for up to 30 days due to the biomineralization process induced by calcium silicates/phosphates from the sealer and from the dentin minerals.[22] In another, total fill BC demonstrated effective antibacterial activities against single-species and multi-species endodontic biofilm utilizing a direct contact test and confocal laser scanning microscopy. It also killed over 40% of *E. faecalis* biofilm in dentin.[23] Using a modified direct contact test, a recent study found that total fill BC had more antibacterial activity than AH-Plus sealer.[24]

In addition, the high solubility of AH Plus Bioceramic sealer and CeraSeal increases the production of calcium silicate facilitated by the moisture, this can positively impact the antibacterial effect.[25,26]

On the other hand, the present study compared the antibacterial properties of bioceramic sealers with zinc oxide eugenol cement, in the Sealite group, *C. albicans* count has significantly decreased between day 3 and day 14 ($p < 0.05$) in comparison with the other groups. In fact, a comparative study by Harni Priya et al. showed that zinc oxide eugenol had a maximum antifungal activity on *C. albicans,* this finding is also in concordance with the present study and with the comparative study of Saha et al.[27] where a zinc oxide eugenol-based sealer showed maximum zone of inhibition affirming the highest antimicrobial activity on *C. albicans*.[28] These results are probably due to the composition and biophysical characteristics of the sealer.[19,25–27]

The antimicrobial activity of bioceramic sealers plays a crucial role in endodontic therapy, as complete bacteria elimination during irrigation procedures can be challenging.[4,12] Bioceramic sealers, with their ability to raise pH levels and release hydroxyl ions, create an unfavorable environment for bacterial survival.[11] They exhibit extended antimicrobial activity, contributing to the reduction of bacterial counts over time.[5] The biomineralization process induced by calcium silicates/phosphates in bioceramics enhances their long-lasting antimicrobial ability.[11] These sealers offer a valuable solution to inhibit bacterial growth, minimize reinfection risks, and improve treatment outcomes in root canal therapy.[9] Further research is needed to optimize the antimicrobial properties of bioceramics for enhanced effectiveness and long-term success in endodontic treatments.

The findings of the present study are in agreement with Bukhari and Karabucak, demonstrating that EndoSequence BC Sealer exhibited significantly greater efficacy in eliminating *E. faecalis* biofilm on canal surfaces compared to AH-plus sealer and the control group at both time points.[20]

The findings of the present study are consistent with the results of Jerez-Olate et al. They reported that Biodentine and BioRoot RCS demonstrated higher antibacterial action, and Biodentine maintained its antibacterial activity even after prolonged aging *in vitro*. Similarly, in the present study EndoSequence BC Sealer exhibited significantly higher antibacterial activity against *E. faecalis* biofilm compared to AH Plus sealer and the control group.[29]

Table 5: Descriptive statistics of *P. mirabilis* count ($\times 10^6$) according to groups and time

	Time			
Groups	*Day 3*	*Day 7*	*Day 14*	*p-value*
Control ($n = 9$)				
Mean ± SD	11.22 ± 1.92	12.33 ± 1.93	11.78 ± 1.30	0.328
Median (Q1–Q3)	11 (10–13)	12 (10.5–14.5)	11 (11–13)	
CeraSeal ($n = 9$)				
Mean ± SD	6.67 ± 1.41	5.67 ± 1.58	5.22 ± 0.83	0.177
Median (Q1–Q3)	7 (5.5–8)	5 (4–7)	5 (4.5–6)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
TotalFill ($n = 8$)				
Mean ± SD	5.75 ± 2.05	5.62 ± 1.30	4.75 ± 0.89	0.341
Median (Q1–Q3)	5 (4–7.75)	5.5 (4.25–7)	4.5 (4–5.75)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
Bio-C Sealer ($n = 9$)				
Mean ± SD	6.11 ± 1.69^{AB}	6.22 ± 1.20^{A}	5.22 ± 1.39^{B}	0.040*
Median (Q1–Q3)	$6\ (4.5–7.5)^{AB}$	$6\ (5.5–7)^{A}$	$5\ (4–6)^{B}$	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
AH Plus ($n = 9$)				
Mean ± SD	6.67 ± 0.87	6.44 ± 1.67	5.56 ± 1.13	0.261
Median (Q1–Q3)	6 (6–7.5)	6 (5–8)	6 (4.5–6.5)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
K-Biocer ($n = 9$)				
Mean ± SD	6.67 ± 0.87	5.67 ± 1.58	5.33 ± 0.87	0.085
Median (Q1–Q3)	6 (6–7.5)	5 (4–7)	6 (4.5–6)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	
Sealite ($n = 9$)				
Mean ± SD	6.89 ± 1.05	6.44 ± 1.94	5.56 ± 1.01	0.234
Median (Q1–Q3)	7 (6–8)	6 (4.5–8.5)	5 (5–6.5)	
p-value (difference with the control group)	<0.001*	<0.001*	<0.001*	

Different uppercase superscript letters indicate statistically significant differences between the timepoints within the Bio-C Sealer group

The study findings should be interpreted in light of certain considerations. The focus on specific bioceramic sealers limits the generalizability of the results to other sealers within the same class. Furthermore, the *in vitro* experimental design may not fully capture the complexities and variations encountered in clinical settings. It is important to acknowledge that the sample size utilized in the present study may have impacted the statistical power and precision of the findings. Future research directions may involve investigations into the long-term antimicrobial efficacy of different sealers in clinical scenarios, comprehensive evaluations of their mechanical and physical properties, assessment of cytotoxicity and biocompatibility, as well as conducting in vivo studies to assess sealing ability and overall clinical performance. Additionally, exploring potential synergistic effects with other antimicrobial agents could provide valuable insights for improving treatment outcomes.

Conclusion

Even if the CSBS tested in the present study displayed antibacterial qualities comparable or even superior to those of traditional sealers, the clinician should place primary emphasis on the root canal disinfection procedure and effective irrigant activation techniques to ensure thorough removal of bacteria. While the antimicrobial properties of bioceramic sealers provide an additional layer of defense against bacterial colonization, they should not be solely relied upon as the primary means of achieving disinfection. Proper mechanical debridement, irrigation with antimicrobial solutions, and the use of activation methods such as ultrasonic or laser irrigation are essential for achieving optimal disinfection. Bioceramic sealers can serve as a valuable adjunct to these procedures, enhancing the overall effectiveness of root canal treatment and reducing the risk of persistent infection.

References

1. Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. Oral Surg Oral Med Oral Pathol 1983;55(3):307–312. DOI: 10.1016/0030-4220(83)90333-X.
2. Waltimo T, Trope M, Haapasalo M, et al. Clinical efficacy of treatment procedures in endodontic infection control and one year follow-up of periapical healing. J Endod 2005;31(12):863–866. DOI: 10.1097/01.don.0000164856.27920.85.
3. Duggan JM, Sedgley CM. Biofilm formation of oral and endodontic Enterococcus faecalis. J Endod 2007;33(7):815–818. DOI: 10.1016/j.joen.2007.02.016.
4. Swimberghe RCD, Coenye TRJ, De Moor G, et al. Biofilm model systems for root canal disinfection: A literature review. Int Endod J 2019;52(5):604–628. DOI: 10.1111/iej.13050.

5. Camilleri J. Will bioceramics be the future root canal filling materials? Curr Oral Health Rep 2017;4:228–238. DOI: 10.1007/s40496-017-0147-x.
6. Camps J, Jeanneau C, El Ayachi I, et al. Bioactivity of a calcium silicate–based endodontic cement (Bio Root RCS): Interactions with human periodontal ligament cells in vitro. J Endod 2015;41(9):1469–1473. DOI: 10.1016/j.joen.2015.04.011.
7. Silva Almeida LH, Moraes RR, Morgental RD, et al. Are premixed calcium silicate–based endodontic sealers comparable to conventional materials? A systematic review of in vitro studies. J Endod 2017;43(4):527–535. DOI: 10.1016/j.joen.2016.11.019.
8. Lim M, Jung C, Shin DH, et al. Calcium silicate-based root canal sealers: A literature review. Restor Dent Endod 2020;45(3):e35. DOI: 10.5395/rde.2020.45.e35.
9. Zordan-Bronzel CL, Tanomaru-Filho M, Rodrigues EM, et al. Cytocompatibility, bioactive potential and antimicrobial activity of an experimental calcium silicate-based endodontic sealer. Int Endod J 2019;52(7):979–986. DOI: 10.1111/iej.13086.
10. Urban K, Neuhaus J, Donnermeyer D, et al. Solubility and pH value of 3 different root canal sealers: A long-term investigation. J Endod 2018;44(11):1736–1740. DOI: 10.1016/j.joen.2018.07.026.
11. Candeiro GT, Correia FC, Duarte MA, et al. Evaluation of radiopacity, pH, release of calcium ions, and flow of a bioceramic root canal sealer. J Endod 2012;38(6):842–845. DOI: 10.1016/j.joen.2012.02.029.
12. Hage W, De Moor RJG, Hajj D, et al. Impact of different irrigant agitation methods on bacterial elimination from infected root canals. Dent J 2019;7:64. DOI: https://doi.org/10.3390/dj7030064.
13. Kapralos V, Koutroulis A, Ørstavik D, et al. Antibacterial activity of endodontic sealers against planktonic bacteria and bacteria in biofilms. J Endod 2018;44(11):149–154. DOI: 10.1016/j.joen.2017.08.023.
14. Siqueira JF Jr, Rôças IN. Present status and future directions: Microbiology of endodontic infections. Int Endod J 2021;55(Suppl 3):512–530. DOI: 10.1111/iej.13677.
15. Garg A, Mala K, Kamath PM. Biofilm models in endodontics – A narrative review. J Conserv Dent 2021;24(1):2–9. DOI: 10.4103/JCD.JCD_621_20.
16. Jhajharia K, Parolia A, Shetty KV, et al. Biofilm in endodontics: A review. J Int Soc Prev Community Dent 2015;5(1):1–2. DOI: 10.4103/2231-0762.151956.
17. Alghamdi F, Shakir M. The influence of Enterococcus faecalis as a dental root canal pathogen on endodontic treatment: A systematic review. Cureus 2020;12(3):e7257. DOI: 10.7759/cureus.7257.
18. Colaco AS. Extreme resistance of Enterococcus faecalis and its role in endodontic treatment failure. Prog Med Sci 2018;2(1):9–13. DOI: doi.org/10.47363/PMS/2018(2)109.
19. Sfeir G, Zogheib C, Patel S, et al. Calcium silicate-based root canal sealers: A narrative review and clinical perspectives. Materials (Basel) 2021;14(14):3965. DOI: 10.3390/ma14143965.
20. Bukhari S, Karabucak B. The antimicrobial effect of bioceramic sealer on an 8-week matured enterococcus faecalis biofilm attached to root canal dentinal surface. J Endod 2019;45(8):1047–1052. DOI: 10.1016/j.joen.2019.04.004.
21. Šimundić Munitić M, Poklepović Peričić T, Utrobičić A, et al. Antimicrobial efficacy of commercially available endodontic bioceramic root canal sealers: A systematic review. PLoS One 2019;14(10):e0223575. DOI: 10.1371/journal.pone.0223575.
22. Šimundić Munitić M, Budimir A, Jakovljević S, et al. Short-Term antibacterial efficacy of three bioceramic root canal sealers against *enterococcus faecalis* biofilms. Acta Stomatol Croat 2020;54(1):3–9. DOI: 10.15644/asc54/1/1.
23. Wang Z, Shen Y, Haapasalo M. Antimicrobial and antibiofilm properties of bioceramic materials in endodontics. Materials (Basel) 2021;14(24):7594. DOI: 10.3390/ma14247594.
24. Zhang H, Shen Y, Ruse ND. Antibacterial activity of endodontic sealers by modified direct contact test against Enterococcus faecalis. J Endod 2009;35(7):1051–1055. DOI: 10.1016/j.joen.2009.04.022.
25. de Souza LC, Neves GS, Kirkpatrick T, et al. Physicochemical and biological properties of AH Plus bioceramic. Journal of Endodontics 2023;49(1):69–76. DOI: 10.1016/j.joen.2022.10.009.
26. Kharouf N, Arntz Y, Eid A, et al. Physicochemical and antibacterial properties of novel, premixed calcium silicate-based sealer compared to powder–liquid bioceramic sealer. Journal of Clinical Medicine 2020;9(10):3096. DOI: 10.3390/jcm9103096.
27. Harini Priya M, Bhat SS, Sandeep Hegde K. Comparative evaluation of bactericidal potential of four root canal filling material against microflora of infected non-vital primary teeth. J Clin Pediatr Dent 2010;35(1):23–29. DOI: 10.17796/jcpd.35.1.u57p4500360g2752.
28. Saha S, Samadi F, Jaiswal JN, et al. Antimicrobial activity of different endodontic sealers: An *in vitro* evaluation. J Indian Soc Pedod Prev Dent 2010;28(4):251–257. DOI: 10.4103/0970-4388.76151.
29. Jerez Olate C, Araya N, Alcántara R, et al. In vitro antibacterial activity of endodontic bioceramic materials against dual and multispecies aerobic anaerobic biofilm models. Aust Endod J 2022;48(3):465–472. DOI: 10.1111/aej.12587.