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ANTIBACTERIAL EFFECT OF DIFFERENT BIOCERAMIC ROOT CANAL SEALERS

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ABSTRACT

Aim: "The aim of this in vitro study was to compare the antibacterial efficacy of different bioceramic root sealers (Well Root ST, CeraSeal, NeoSEALER Flo) in combination with AH plus against Enterococcus faecalis (ATCC 19433) and Streptococcus mutans (ATCC 25175).

Materials and methods: The samples were categorized according to the sealer type that was utilized. Group 1: Well Root ST, group 2: CeraSeal, group 3: NeoSEALER Flo, group 4: AH plus. Manufacturer's instructions were followed when preparing the sealers and placed in sterile plastic cylinders with a diameter and depth of 5 mm. Following that, specimens were incubated at 37°C and 100% humidity for 7 days. The bacterial suspension was made with a standardized density of 0.5 on the MacFarland (1.5x108/ml). The suspensions were diluted 10,000 times after mixing for six, fifteen, and sixty minutes, and then a triplicate amount of 0.01 from each diluted suspension was placed onto the pre-prepared BHI agar plates. (Difco Lab., Detroit, MI, USA). The number of colonies forming unit for each sealer was calculated in different time throughout the experiment.

Results: For the effect on Enterococcus faecalis at 6 minutes, NeoSEALER Flo showed lowest bacterial count, at 15 minutes AH plus showed lowest bacterial count and 60 minutes CeraSeal showed lowest bacterial count. For the streptococcus mutans at 6 minutes, NeoSEALER flo showed lowest bacterial count, at 15 min AH plus and Well Root ST showed lowest bacterial count and at 60 min Well Root ST showed lowest bacterial count.

Conclusion: All sealers showed antibacterial effect, where increasing the time resulted in decreasing Log10 CFU.

KEYWORDS: CeraSeal, NeoSEALER Flo, antibacterial, AH plus, Enterococcus Faecalis Streptococcus Mutans, Well Root ST.

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INTRODUCTION

The main objective of a successful root canal treatment, apart from achieving complete sealing, is to eliminate any infection present in the area. The combined actions of solid core materials and root canal sealers enable this outcome. [1]

The primary causative factor for pulp necrosis and consequent periapical diseases is microorganisms and their by products. There are a multitude of factors that can account for failures of endodontic treatment. The primary causes of unsuccessful endodontic treatment are persistent intraradicular or secondary infections.[2] Even if endodontic treatment aims to completely eradicate micro-organisms from the root canal space or at least to reduce them to levels compatible with the health of the periradicular tissue; numerous studies have shown that bacteria can remain in the cementum and dentinal tubules even after treatment. [3] Microorganisms can persist when there is insufficient intracanal irrigation, inadequate mechanical preparation that leaves a significant portion of the root canal surfaces untouched and inadequate chemo-mechanical preparation due to anatomical constraints. [4] Research has shown that the microorganisms present in teeth with unsuccessful root canal therapy substantially differ from those typically observed in untreated teeth. [5] Facultative Gram-positive cocci, specifically Enterococcus faecalis, are found in more than one-third of the root canals of teeth having persistent periapical lesions. [6]

As it is not always possible to entirely eliminate microorganisms from the endodontic area, the antimicrobial properties of root canal sealers can aid in eradicating any remaining microorganisms that were not impacted by the chemo-mechanical preparation of the root canal space. [7] Endodontic sealers with strong antibacterial properties might therefore inhibit or stop the growth of microorganisms and speed up the healing of the apical and periapical tissues. [8]

So, it is crucial to assess root canal sealers based on their antimicrobial characteristics. [9] Eugenol, thymol, and paraformaldehyde are a few of the specific ingredients found in sealers, and these compounds give them their antibacterial properties. Although these compounds have some antibacterial properties, their toxicity may endanger the periapical tissues. [10] Comparing newly developed calcium silicate-based bioceramic sealers to other sealants, they have shown greater biocompatibility and decreased cytotoxicity. [11]

Therefore, in this research, we assessed the antibacterial effectiveness of different bioceramic root canal sealers and AH plus sealer on Enterococcus Faecalis and Streptococcus Mutans.

MATERIAL AND METHODS

Four endodontic sealers were studied:

Three bioceramic sealers; CeraSeal (Meta Biomed Co., Ltd., Republic of Korea), Well Root ST (Vericom, Chuncheon-si, Gangwon-Do, Korea) and NeoSEALER Flo (NuSmile, Texas, USA) compared with epoxy resin based sealer; AH plus (Dentsply/Maillefer, Konstanz, Germany).

Direct contact test (DCT)

Counting the colonies of bacteria on agar plates after plating, the DCT was utilised to assess the root canal sealers' antibacterial properties. According to manufacturer's directions, all sealers were combined and put into sterile cylinder-shaped plastic blocks with a diameter and depth of 5 mm diameter. For 7 days, the specimens were kept in an incubator set to 37 °C with 100% humidity. Using a ceramic mixer, we ground and powdered the sealer blocks we obtained. (Coors Tek, Goled Co, USA). With the use of ethylene oxide gas, the powder was sterilized after being packed in special sterile packs. A precise balance was used to weigh 50 mg of each sealers powder. (Mettler-Toledo, model AE1633, Novate Milanese, Italy, metering accuracy 0.01 mg) and us-

ing sterile pipettes, each powdered sealer was mixed with 1 ml of sterilised saline suspension to produce a 50 mg/ml-density suspension. A standard density of 0.5 McFarland (1,5 x 108/ml) of bacterial suspension were prepared. Using a Bench Mixer Vortexer (Sigma, St. Louis, MO, USA), equal volumes of the bacterial suspension and the sealer suspension (1 ml) were blended. The sealer-free saline suspension was designated as the positive control. At time intervals of six, fifteen, and sixty minutes after mixing, the suspensions were diluted ten thousand times, subsequently, triplicate plating of 0.01 ml of the diluted suspension was carried out on the BHI agar plates provided earlier (Difco Lab., Detroit, MI, USA). Following a 24-hour incubation period at 37°C, the colonies that had developed on the agar plates were enumerated. Subsequently, the CFU (colony-forming units) count was determined for each sealer at various time intervals during the experiment. These experiments were repeated three times.

Sample size was calculated based data extracted from previously published paper (Markus et al 2013). For comparison between AH group and other sealer group the true difference is 2.2 for Log CFU and the effect size it large at f=1.1. The minimum sample size is 9 in each group will be sufficient to detect an 80% power. Sample size was calculated using G*Power 3.1.9.7

Statistical analysis:

Data presented as mean and standard deviation (SD). Data explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. One-way ANOVA used to compare between tested groups within each time point and to compare between time points within each group followed by for pairwise comparison with Tukey's HSD.

The significance level was set at $P \leq 0.05$.

Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.

RESULTS

A. Enterococcus Faecalis

After 6 min, NeoSEALER Flo showed the lowest significant Log10 CFU compared to all other groups. Insignificant results between AH plus, CeraSeal and Well-Root ST resulted after 6 min. After 15 min, NeoSEALER Flo showed the lowest significant Log10 CFU compared to all groups followed by AH plus and CeraSeal. The highest significant Log10 CFU resulted for Well-Root ST. After 60 min, NeoSEALER Flo showed the lowest significant Log10 CFU values followed by Well-Root ST followed by CeraSeal and followed by the highest Log10 CFU values for AH plus, all groups were significant with each other's.

TABLE (1): Mean and SD for Log10/CFU of Enterococcus faecalis for different tested group.

		6 min	15 min	60 min	p-value
Enterococcus faecalis	**AH plus**	8.59^{bA}±0.17	7.43^{bB}±0.11	5.75^{dC}±0.08	<0.001
	CeraSeal	8.7^{bA}±0.05	7.53^{bB}±0.04	5.27^{cC}±0.07	<0.001
	NeoSEALER Flo	8.21^{aA}±0.08	6.71^{aB}±0.12	4.4^{aC}±0.09	<0.001
	Well-Root ST	8.81^{bA}±0.07	7.91^{cB}±0.04	4.88^{bC}±0.03	<0.001
p-value		0.001	<0.001	<0.001	

Different lowercase letters within each column indicate significant difference.
Different uppercase letters within each row indicate significant difference.
NS= Non-significant, *= significant

For all cement used, increasing the time resulted in decreasing Log10 CFU after 15 min and followed by a further decrease after 60 min.

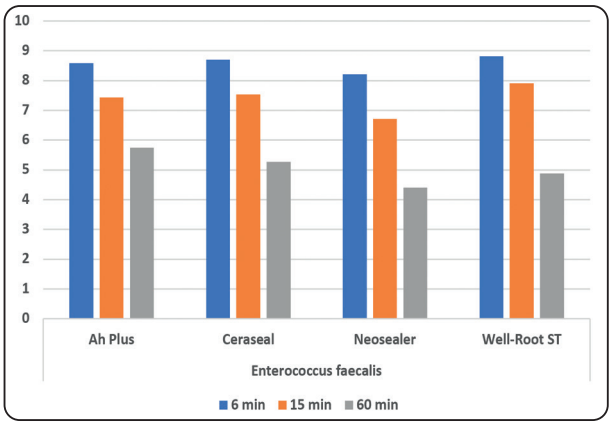

Fig. (1) Bar chart showing the mean for Log10/CFU of Enterococcus faecalis for different tested.

B. Streptococcus mutans

After 6 min, NeoSEALER Flo showed the lowest significant Log10 CFU compared to CeraSeal and Well-Root ST. Insignificant results between AH plus, CeraSeal and NeoSEALER Flo resulted after 6 min. After 15 min, NeoSEALER Flo showed the lowest significant Log10 CFU compared to all groups followed by AH plus and Well-Root ST. The highest significant Log10 CFU resulted for CeraSeal. After 60 min, NeoSEALER Flo showed the lowest significant Log10 CFU values followed by Well-Root ST followed by AH plus and followed by the highest Log10 CFU values for CeraSeal, all groups were significant with each other's.

For all cement used, increasing the time resulted in decreasing Log10 CFU after 15 min and followed by a further decrease after 60 min.

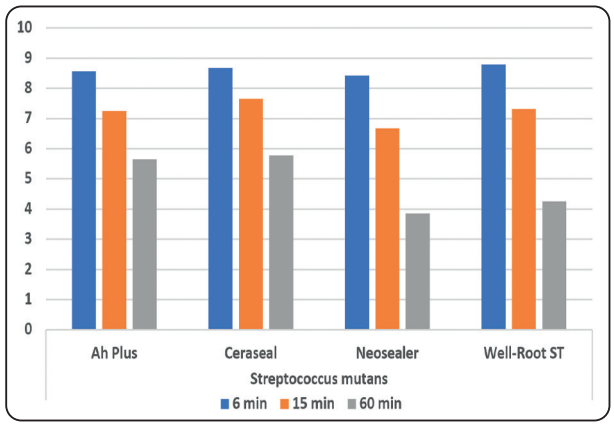

Fig. (2) Bar chart showing the mean for Log10/CFU of Streptococcus mutans for different tested.

TABLE (2) Mean and SD for Log10/CFU of Streptococcus mutans for different tested group.

		6 min	15 min	60 min	p-value
Streptococcus mutans	**AH plus**	$8.56^{abA}\pm0.14$	$7.25^{bB}\pm0.16$	$5.65^{cC}\pm0.06$	<0.001
	CeraSeal	$8.67^{bcA}\pm0.04$	$7.65^{cB}\pm0.05$	$5.78^{dC}\pm0.06$	<0.001
	NeoSEALER Flo	$8.41^{aA}\pm0.08$	$6.67^{aB}\pm0.05$	$3.85^{aC}\pm0.03$	<0.001
	Well-Root ST	$8.79^{cA}\pm0.04$	$7.31^{bB}\pm0.08$	$4.26^{bC}\pm0.03$	<0.001
p-value		0.003	<0.001	<0.001	

Different lowercase letters within each column indicates significant difference.
Different uppercase letters within each row indicate significant difference.
NS= Non-significant, *= significant

DISCUSSION

Ideally, for optimal performance, endodontic sealers should possess dimensional stability and non-toxicity. Additionally, they should possess the ability to establish a robust bond with the root canal dentin, ensuring effective sealing and preventing the occurrence of microleakage. [12] Furthermore, it is advantageous if the endodontic sealers demonstrate potent and durable antimicrobial properties as well as therapeutic effects. [13]

The utilization of an endodontic sealer possessing antibacterial characteristics can offer benefits, especially in cases involving pulpal or periapical infections. The antibacterial properties of root canal sealers can assist in eradicating any residual microorganisms that may remain unaffected by the chemo-mechanical preparation of the root canal system. [7]

Enterococcus faecalis, the predominant intraradicular microorganism found in periapical periodontitis [14,15] is frequently employed as a standard bacterium for assessing the antimicrobial efficacy of root canal sealer. E. faecalis, a Gram-positive facultative anaerobic microorganism, is commonly found in cases of unsuccessful root canal treatments. It possesses the capability to survive within the root canal, either in isolation or alongside other microorganisms. [16] The ability of E. faecalis to enter the dentinal tubules and stick to dentinal collagen makes it difficult to remove from the root canal. [17]

Streptococcus mutans was chosen as our focus due to its prevalence as the most found bacterium in persistent endodontic infections and cases of unsuccessful root canal treatments. [18] These microorganisms exhibit resistance to intracanal medicaments like calcium hydroxide and have the ability to enter secondary accessory canals and isthmuses.[19] The direct contact test is widely recognized as the preferred technique for evaluating the antibacterial properties of endodontic sealers.[20] This test is quantitative and reproducible, effectively simulating the interaction between microorganisms and endodontic sealers within the canal space. It offers valuable insights into the bactericidal effects of the sealers [21], and as a result, it yields reliable and pertinent results. [22]

To assess the antibacterial activity through DCT, the recommended duration was from 5 to 60 minutes was proposed to allow sufficient time for the sealers to exert their effects on resistant bacteria such as E. faecalis. [23]

NeoSEALER flo showed the highest antibacterial effect on enterococcus faecalis on all time interval as the antimicrobial efficacy of the bioceramic sealer is attributed to a combination of factors, including a high pH, hydrophilicity, and the active diffusion of calcium hydroxide. [24] This discovery confirms the antibacterial properties of the bioceramic sealer, which can be attributed to the rapid exchange of ions, releasing calcium and hydroxyl ions. [25] This reaction creates a highly alkaline environment surrounding the bioceramics, enabling their antibacterial effect. These findings are consistent with the research conducted by Singh et al. [26]

After 60 min, AH plus showed the lowest antibacterial effect which may be attributed to the paraformaldehyde produced by this material during setting period which was in accordance with (Mohammadi Z,2012). [27] The significant reduction of antibacterial effectiveness of the set AH plus could be attributed to the polymerization process which leads to a depletion of the epoxy resin and amines. This was in accordance with (Castillo-Villagomez P 2022). [28]

For the antibacterial effect on streptococcus mutans, also NeoSEALER Flo showed the highest antibacterial effect on all time interval. The results of this study corroborate the previously reported antibacterial properties of bioceramic root canal sealers. [29] The alkaline pH of these sealers improves their osteogenic potential and biocompatibility in addition making them more resistant to streptococci

strain development. According to the findings of this study, bioceramic root canal sealers demonstrate satisfactory antibacterial efficacy in inhibiting the spread of Streptococci strains. After15 min and 60 min, CeraSeal showed the lowest antibacterial effect which is consistent with (Dagna A 2022). [30]

AH plus possesses antibacterial efficacy due to the emission of bisphenol-A-diglycidyl ether during polymerization. [31]

AH plus has strong flow, which diffuses into the dentinal tubules and inhibits microbial growth by means of entombment. [32] However, according to Kayaoglu et al [33], fresh AH-Plus sealer had antibacterial activity against E. faecalis that decreased in samples that were aged for 24 and 7 days. This might be explained by how easily the antibacterial component diffused into the environment prior to the material setting. [34]

Bioceramic sealants, in contrast, have hydrophilic characteristics. Calcium silicates undergo a hydration process upon contact with dentinal moisture, resulting in the creation of calcium hydroxide and calcium silicate hydrogel. [35] Calcium hydroxide and calcium phosphate partially react to generate hydroxyapatite and water. The water that is created, in turn, restarts the cycle, producing additional calcium hydroxide and calcium silicate hydrogel and raising pH levels to above 12.5. When the sealer sets, the pH also drops to roughly 9.14, which lessens its antibacterial effectiveness. It was noted that seven days after combining, their antibacterial properties significantly decreased. [36]

CONCLUSION

"Within the limitations of this study, the demonstrated antibacterial activity of bioceramic root canal sealers is effective for endodontic applications. These findings suggest the promising potential of bioceramic root canal sealers for further study and investigation of their use."

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