Antibacterial Effects of Bioceramic and Mineral Trioxide Aggregate Sealers Against Enterococcus Faecalis Clinical Isolates

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Abstract

To analyze the antibacterial effect of Bioceramic and mineral trioxide aggregate (MTA) sealers against Enterococcus faecalis clinical isolates at 2 minutes (fresh), 4 hours (initial setting), 1 day, and 7 days after the sealers were mixed. The antibacterial effects of Bioceramic and MTA sealers were assessed by direct contact tests. Each sealer directly contacted clinical isolates of E. faecalis at 2 minutes, 4 hours, 1 day, and 7 days after the sealers were mixed. The suspension was swabbed in an agar medium and incubated for 24 hours to determine the number of bacterial colonies that grew. The colonies in the agar plates were counted with colony-forming units (CFUs).

There were significant differences between Bioceramic and MTA at 7 days, between Bioceramic at 2 minutes (fresh) and 4 hours (initial setting), and between Bioceramic at 4 hours (initial setting) and 7 days. Both Bioceramic and MTA sealers showed good antibacterial effects against E. faecalisat fresh, initial setting, and 1 day after the sealers were mixed. At 7 days after mixing, the MTA sealer was better compared with Bioceramics. Moreover, MTA sealer had a constant antibacterial effect for up to 7 days.

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Introduction

Microorganisms and their products are the main etiology of pulpal and periapical diseases. The main purpose of a root canal treatment is to eliminate a number of microorganisms as well as other factors that can lead to a reinfection. The main challenge in eliminating the infection in a root canal is to not only eliminate microorganisms within the root canal lumen, but also microorganisms in the dentinal tubules and area of ramification[1–3]. Enterococcus faecalis is a Gram-positive bacteria, group D streptococci, and facultative anaerobe, which singly, or in pairs or short chains, can survive in less than favorable conditions. *E. faecalis* may cause periapical disease or a failure in the root canal treatment, and its actions depend on its virulence factor. The virulence factor may enable *E. faecalis* to survive in high temperature environments and at high pH levels[3–5].

E. faecalis bacteria from clinical isolates is a species of bacteria derived from the root canal of a natural tooth with a periapical lesion.[6] *E. faecalis* is considered more persistent than the American Type Culture Collection (ATCC) bacteria because it has the ability to survive and has a high virulence. The ATCC bacteria is one that has been cultured in an environment and has received suitable nutrition for bacterial viability[7]. To ensure the success of a root canal treatment, especially in eliminating E. faecalis, a good seal is needed for the root canal filling after root canal cleaning is done by chemomechanical methods. A sealer is one of the root canal fillers, and ideally it should have certain properties, such as biocompatibility, an ability to penetrate the dentinal tubules, and be bactericidal[8].

A recently developed Bioceramic-based sealer may be an alternative for sealers that still have many deficiencies, especially with regard to their antibacterial properties. The advantages of Bioceramic sealer are that it can produce calcium hydroxide as a result of the setting reaction and it is bactericidal with a very high pH of 12.8. Studies have shown that Bioceramic sealer can

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kill *E. faecalis* bacteria within 2 minutes (fresh) following contact, and this antibacterial action is based on a combination of a high pH levels, hydrophilic conditions, and an active diffusion of calcium hydroxide[8]. Sealers that are based on bioactive materials, such as mineral trioxide aggregate (MTA), also have a very high pH because they contain hydroxyl ions that can kill *E. faecalis*[1,9]. No previous studies have compared Bioceramic sealer and MTA, especially against *E. faecalis* clinical isolates at various times. Therefore, the aim of this study was to compare and analyze the antibacterial effects of Bioceramic sealer and MTA against clinical isolates of *E. faecalis* bacteria at 2 minutes (fresh), 4 hours (initial setting), 1 day, and 7 days.

Materials and methods

The samples used in this study were Bioceramicsealer (IRoot® SP, Innovative Bioceramix Inc., Vancouver, BC, Canada) and MTA Fillapex®(Angelus, Londria, Brazil). The bacteria used were clinical isolates of *E. faecalis* bacteria taken by a previous researcher from non-vital teeth with periapical disease[7]. The Bioceramic sealer and MTA were placed on a paper pad and weighed (0.03 grams), then applied to a 96-well plate using ballpointed instrument up to 5 mm thick, or equal to 100 µl of bacteria on brain-heart infusion (BHI) broth.

The sealer was applied to the plate and then incubated at 37°C at predetermined times of 2 minutes (fresh), 4 hours (initial setting), 1 day, and 7 days after the sealers were placed. After the sealer was incubated, it was placed in contact with as much as 100 ml of the *E. faecalis* clinical isolate, and then it was placed back in incubation at 37 °C for 3 hours. Each sample was then placed in microtube 15 ml and diluted with 990 µl of phosphate buffered saline (PBS), placed on a vortex until it was homogeneous, and then diluted up to four times dilution (10^{-6}). Then, 10 µl of the sample was placed on BHI-agar medium and incubated for 24 hours at 37 °C to determine the number of colony-forming units (CFUs).

Results

Means and standard deviations for the Bioceramic sealer and MTA groups are shown in Table 1.

Group	N	Mean ± SD	95% CI	
			Lower limit	Upper limit
Bioceramic				
2 minutes (fresh)	3	5.00 ± 2.000	0.03	9.97
4 hours (initial setting)	3	0.33 ± 0.577	-1.10	1.77
1 day	3	10.33 ± 14.572	-25.86	46.53
7 days	3	3.67 ± 0.577	2.23	5.10
MTA				
2 minutes (fresh)	3	3.00 ± 1.000	0.52	5.48
4 hours (initial setting)	3	0.67 ± 1.155	-2.20	3.54
1 day	3	3.33 ± 1.528	-0.46	7.13
7 days	3	1.00 ± 1.000	-1.48	3.48
Positive Control		26.00 ± 8.888		
2 minutes (fresh)	3	47.67 ± 3.786	3.92	48.08
4 hours (initial setting)	3	72.33 ± 56.977	38.26	57.07
1 day	3	16.00 ± 12.124	-69.20	213.87
7 days	3		-14.12	46.12

Table 1. Mean and standard deviation (SD) of CFUs formed after direct contact with *E. faecalis* between Bioceramic sealer and MTA at 2 minutes (fresh), 4 hours (initial setting), 1 day, and 7 days after the sealers were mixed.

Table 1 shows that the fewest number of colonies found was 4 hours after mixing the Bioceramicsealer and MTA. This means that both sealers had a better antibacterial effect against *E. faecalis* clinical isolates compared with the test groups at 2 minutes (fresh), 1 day, and 7 days.

To determine which hypothesis test was to be selected, the data normality test was done first using the Shapiro-Wilk test. Data analyses were performed using non-parametric statistical Kruskal-Wallis tests with ap value = 0.003 ($p<0.05$), which suggests there was a difference between the Bioceramic sealer and the MTA group. To test the significance of these differences, Mann-Whitney tests were done and the results are shown in Table 2.

Test Group 1	Test Group 1	p-value
Bioceramic 2 minutes (fresh)	Bioceramic 4 hours (initial setting)	0.046*
	Bioceramic 1 day	0.827
	Bioceramic 7 days	0.369
Bioceramic 4 hours (initial setting)	Bioceramic 1 day	0.246
	Bioceramic 7 days	0.043*
Bioceramic 1 day	Bioceramic 7 days	0.817
*Mann-Whitney test, significance value p< 0.05		

Table 2. Significance values on the number of *E. faecalis* colonies after contact with Bioceramic sealer at 2 minutes (fresh), 4 hours (initial setting), 1 day, and 7 days after the sealers were mixed.

Table 2 shows that there were significant differences between the colony numbers of *E. faecalis* clinical isolates in the Bioceramic sealer

group at 2 minutes (fresh) and 4 hours (initial setting) after the sealers were mixed, with p = 0.046. The descriptive data showed that the average value for the Bioceramic sealer at 2 minutes (fresh) was greater (5.00 ± 2.000) compared to 4 hours (initial setting) (0.33 ± 0.577). This indicates that the antibacterial effect on the initial setting in the Bioceramic sealer was better than the freshly mixed sealer.

A significant difference was also seen in the Bioceramic sealer group at 4 hours (initial setting)compared with7 days after mixing, with p = 0.043 (p<0.05). From the descriptive data shown, the average value of the Bioceramic sealer at 4 hours (initial setting) was less (0.33 ± 0.577) than the Bioceramic sealer at 7 days (3.67 ± 0.577). This shows that the antibacterial effect against *E. faecalis* for the Bioceramic sealer decreased after 7 days compared to the initial levels.

Test Group II	Test Group II	p-value
MTA 2 minutes (fresh)	MTA 4 hours (initial setting)	0.072
	MTA 1 day	0.822
	MTA 7 days	0.077
MTA 4 hours (initial setting)	MTA 1 day	0.072
	MTA 7 days	0.637
MTA 1 day	MTA 7 days	0.077

Table 3. Significance values on the number of E. faecaliscolonies after contact with MTA sealer at 2 minutes (fresh), 4 hours (initial setting), 1 day, and 7 days after the sealers were mixed.

Table 3 shows that there were no significant differences between the MTA sealer at 2 minutes (fresh), 4 hours (initial setting), 1 day, and 7 days after the sealers were mixed. The descriptive data show that the average number of colonies after contact with the MTA sealer at 4 hours (initial setting) was less (0.67 ± 1.155) than the MTA at 2 minutes (fresh), 1 day, and 7 days, but the significance test using the Mann-Whitney test found this difference to be not significant. This indicates that the antibacterial effectof MTA sealer is constant up to 7 days after mixing.

From the analyses using Mann-Whitney tests, there was a significant difference between the number of colonies ofE. faecalisclinical isolates in the Bioceramic sealer group andthe MTA group at 7 days after mixing, witha value of p = 0.046 (p<0.05). According to the descriptive data,the mean MTA sealer value was less (1.00 ± 1.000) thanwith the Bioceramic sealer (3.67 ± 0.577). This indicates that the MTA sealer hada better antibacterial effect against E. faecalisclinical isolates than the Bioceramic sealer at 7 days after mixing

Discussion

This study was conducted to analyze the antibacterial effects of Bioceramic sealer and MTA against *E. faecalis* clinical isolates. *E. faecalis* was chosen because it is found in about 29–77% of teeth that have had root canal treatments and filled but still have persistent periapical disease. *E. faecalis* has a number of virulence factors that help it survive in less favorable conditions, it is alsoresistant to medicaments, such as calcium hydroxide[5,6,10,11].

This study used a standardized sample of clinical isolate bacteria taken by a previous researcher from a non-vital tooth with periapical disease in a patient at the Conservative dentistry Clinic Teaching Dental Hospital Faculty of Dentistry Universitas Indonesia and has been standardized[7]. According to Elsner *et al*, virulence factors, such as aggregation substance (AS), are found in many *E. faecalis* clinical isolates[12]. AS is used to mediate an efficient contact of the donor and recipient and facilitates the exchange of plasmids, making it possible for E. faecalis to attach to host cells and secrete certain proteins that can make bacterial cells more attached. Kanemitsu et al (2001) have suggested that a high production of virulence factors in clinical isolates, such as gelatinase, is greater than in the usual ATCC strain. Gelatinase assists in the attachment of E. faecalis clinical isolates to dentinal walls[13]. In the case of an endodontic infection with a periodontal disease, the level of gelatinase is detected more in the gingival fluid. Cytolysin is also a virulence factor of *E. faecalis* clinical isolate that is often found. The role of cytolysinis to lysis erythrocytes, Peripheral Nervous System PNS, and macrophages, and decrease phagocytosis[12,13].

Bioceramic sealer was chosen for this study because it has a high alkalinity with a pH of 12.8, which can improve the mineralization process and bactericidalaction. This sealer is also hydrophilic, and root canal hydration helps in the formation of calcium phosphate. This sealer also has smaller-sized particles so it can fill the entire walls, including lateral canals[14,15].

MTA sealer is a root canal sealer which base are mineral trioxide aggregate and salicylate resin that is often used and is known

forits good antibacterial properties, high flow rate, and low firm thickness. It is composed of 40% MTA and salicylate resin, which is intended for its antibacterial properties and biocompatibility. MTA sealer has alkaline properties with a pH = 10.2, which can eliminate bacteria, especially *E. faecalis*[16]. While setting, MTA sealer can produce calcium silicate hydrate and calcium hydroxide. According to Al Haddad (2016), the antibacterial effect of MTA sealer is also caused by the resin content, but to be able to maintain the pH value for a long time, it must release hydroxyl ions during setting[17].

Bioceramic sealer and MTA equally release hydroxyl ions as a result of the setting reaction, which leads to high pH levels. Hydroxyl ions are highly oxidativent free radicals that can react with biomolecules. Hydroxyl ions can destroy the bacterial cytoplasmic membrane that serves as a defense for rom E. faecalis. The bacterial cytoplasmic membrane is selectively permeable and transports liquids and electrons, excretes hydrolytic exoenzymes, linking enzymes, and molecules that synthesize DNA, cell wall polymers, the lipid membrane, and connecting receptors that act aschemotactic proteins. Hydroxyl ions induce lipid peroxidation that can destroy phospholipids and structural components of cell membranes. Hydroxyl ions eliminate hydrogen atoms from unsaturated fatty acids and produce lipid free radicals. These lipid free radicals then react with oxygen to produce lipidic peroxide radicals that can eliminate more of the hydrogen atoms of the fatty acid as well as produce more lipid ic peroxide. The formed peroxide acts as a free radical that initiates an autocatalytic chain reaction that causes unsaturated fatty acids to collapse and cause extensive damage to the bacterial cytoplasm[18].

Cellular metabolism of *E. faecalis* depends on enzymatic activity. The enzyme has an optimum activity and stability at limited pH levels. The alkalinity produced by calcium hydroxide induces the outbreak of ionic bonds that maintain the protein structure. Consequently, the enzyme retains acovalent structure but the polypeptide chain of the protein becomes irregular. This change leads to a loss of biological activity of the enzymes and disrupts cellular metabolism. Protein structures can be destroyed by hydroxyl ions. Hydroxyl ions can also react with bacterial DNA and induce the separation of the DNA chains, so that DNA replication and cellular activity is inhibited. Free radicals can also induce mutations in bacteria[18].

The contact time between the sealer and the *E. faecalis* bacteria in this study was 2 minutes, 4 hours, 1 day, and 7 days after the sealers were mixed. These contact times were selected to determine whether there were differences in antibacterial effects between the Bioceramicsealer and MTA and also to investigate whether the antibacterial effects of both sealers increased or decreased over time.

This study used the direct contact test (DCT). According to Shwaimi (2011), the DCT can measure antibacterial effects without considering the solubility and the ability to diffuse of the antibacterial component to diffuse in sealer. This method can be used on materials that are less soluble in water, so it is good to measure the antibacterial effect of sealers[19].

In this study, the fewest number of bacterial colonies were those in the Bioceramic sealer and MTA groups at 4 hours after the sealers were mixed. This indicates that both sealers had a similar antibacterial effect at the time of the initial setting, which may be due to the release of hydroxyl ions at the time of setting the reaction[20].

A significant difference was observed in the Bioceramic sealer group at 2 minutes (fresh) compared to 4 hours (initial setting) after the sealers were mixed, with the antibacterial effectsat 4 hours (initial setting)better than at 2 minutes (fresh). This might have been caused by the greater amount of calcium hydroxide produced at the initial setting rather than in the freshly mixed sealer[20]. A significant difference was also found between the Bioceramic sealer group at 4 hours (initial setting) and 7 days after the sealers were mixed, with the antibacterial effect at the initial setting being better than at7 days. These results are in accordance with research by Ustun *et al.* (2013), which states that the antibacterial effect of a Bioceramic sealer will be reduced along with the setting of the sealer[21].This is probably due to the low solubility of the Bioceramic sealer after setting, so there will be less production of hydroxyl ions. This is also supported by a statement by Zeid *et al.* (2015) that states that the solubility of a sealer is directly proportional to the pH level; if the solubility is low, and then the production of hydroxyl ions is also low, prompting a decrease in pH levels[22]. Further research on the antibacterial effect of Bioceramic sealers with

contact times of more than 7 days after the sealers are mixed is needed.

There was no significant difference in the MTA sealer group at 2 minutes (fresh), 4 hours (initial setting), 1 day, and 7 days after the sealers were mixed (Table 3). This is not in accordance with research by Morgental *et al.* (2001), which states that the antibacterial effect of MTA sealer decreases after setting. This difference in findings may be due to differences in research methodologies. Morgental *et al* used the agar diffusion test to measure the antibacterial effect. This test depends on the diffusion and solubility of the component in a medium before producing an antibacterial effect[23]. The results of this study might be due to the MTA sealer component that has resin and MTA, which areable to eliminate *E. faecalis* clinical isolates.

Test Group 1	Test Group 2	p-value
Bioceramic 2 minutes (fresh)	MTA 2 minutes (fresh)	0.184
Bioceramic 4 hours (initial setting)	MTA 4 hours (initial setting)	0.796
Bioceramic 1 day	MTA 1 day	0.827
Bioceramic 7 days	MTA 7 days	0.046*
*Mann-Whitney test, significance value p< 0.05		

Table 4. Significance values on the number of E. faecaliscolonies after contact with Bioceramic sealer and MTA at 2 minutes (fresh), 4 hours (initial setting), 1 day, and 7 days after the sealers were mixed.

The significance of our test results shows that the MTA sealer has a better antibacterial effect than the Bioceramic at 7 days after the sealers were mixed (Table 4). These results are in accordance with the research by Ustun *et al* (2013), which states that MTA sealer has the best antibacterial effect at 7 days. This effect also extends up to 30 days compared to the Bioceramic sealer, and the antibacterial effect of the Fillapex® MTA sealer is based on its component calcium silicate in MTA and resin[21]. The assumption that can be inferred from previous studies are, that in addition to having the silicate component, MTA sealer also has a resin component that is cytotoxic to cells, so it can kill *E. faecalis* clinical isolates. The significance tests also showed there were no significant differences between the Bioceramic sealer and the MTA at 2 minutes, 4 hours, and 1 day. This indicates that both sealers had the same antibacterial effects at the immediate time (fresh), initial settings, and 1 day after the sealers were mixed. These actions were based on the components of the hydroxyl ions being released equally by both sealers as a result of the setting reaction.

Conclusions

The antibacterial effect against *E. faecalis* clinical isolates was best shown in the Bioceramic sealer at the time of the initial setting. The antibacterial effect of the MTA sealer was constant up to 7 days after the sealers were mixed. Overall, it can be concluded that both the Bioceramic sealer and the MTA have good antibacterial effects against *E. faecalis* at fresh, initial setting, and 1 day after the sealers were mixed. However, at 7 days, the antibacterial effect of the MTA was better than the Bioceramic sealer. Further research is needed with regard to the antibacterial effect of the Bioceramic sealer against *E. faecalis* clinical isolates with contact times more than 7 days after the sealers are mixed.

Declaration of Interest

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References

1. Tyagi S, Tyagi P, Mishra P. Evolution of root canal sealers: An insight story. *Eur J Gen Dent*. 2013;2(3):199.
2. Kothari A, Langalia A. Comparative Evaluation Of The Antimicrobial Activity Of Different Endodontic Sealers On Enterococcus Faecalis - An Invitro Study. *Natl J Integr Res Med*. 2013;4(3):121-127.
3. Vanapatla A, Vemisetty H, Punna R, et al. Comparative evaluation of antimicrobial effect of three endodontic sealers with and without antibiotics – An in-vitro study. *J Clin Diagnostic Res*. 2016;10(4):ZC69-ZC72.
4. Arora R, Rawat P, Bhayya DP. " A Comparative Evaluation of Antimicrobial Efficacy of Three Endodontic Sealers : Endoflas FS , AH Plus and sealapex against Enterococcus faecalis - an in vitro study ." Abstract : *IOSR J Dent Med Sci*. 2014;13(3):90-93.
5. Mallick R, Mohanty S, Behera S, Sarangi P, Nanda S, Satapathy SK. Enterococcus faecalis: A resistant microbe in endodontics. *Int J Contemp Dent Med Rev*. 2014;2014.
6. Artiningsih DANP, Bachtiar BM, Akbar SMS, Victor AA. Quantitative Real-Time PCR of cps Type 1, 2 and 5 of Enterococcus faecalis and Candida albican Isolated from Infected Root Canal of Subject Requiring Endodontic Treatment. *J Int Dent Med Res*. 2016;9(3):157-163.
7. Artiningsih DANP. Efek Laser Terapi Foto Dinamik dan Larutan Kalsium Hidroksida 50% Terhadap Karakter Fenotip Pada Genotip Cps E. Faecalis Isolat Infeksi Intraradikular Persisten. 2016.
8. Nasseh A. The rise of bioceramics. *Endod Prac*. 2009;(August):21-25.

9. Singh H, Markan S, Kaur M, et al. Endodontic Sealers : Current Concepts and Comparative Analysis. *Dent Open J*. 2015;2(1):32-37.
10. Peciuliene V, Maneliene R, Balcikonyte E, Drukteinis S, Rutkunas V. Microorganisms in root canal infections: a review. *Stomatologija*. 2008;10(1):4-9.
11. Djauharie N, Kemala N. Antibacterial Efficacy of 5% Ethanolic Extract of Propolis (EEP) Solution against Enterococcus faecalis (Laboratory Experiment). *J Int Dent Med Res*. 2017;10(1):19-23.
12. Elsner H a, Sobottka I, Mack D, Claussen M, Laufs R, Wirth R. Virulence factors of Enterococcus faecalis and Enterococcus faecium blood culture isolates. *Eur J Clin Microbiol Infect Dis*. 2000;19(1):39-42.
13. Kanemitsu K, Nishino T, Kunishima H, et al. Quantitative determination of gelatinase activity among enterococci. *J Microbiol Methods*. 2001;47(1):11-16.
14. Malhotra, Sakshi; Hegde, Mithra N; Shetty C. Bioceramic Technology in Endodontics - ProQuest. *Br J Med Med Res*. 2014;4(12):2446-2454.
15. Koch K, Brave D, Nasseh AA. A review of bioceramic technology in endodontics. *CE Artic*. April. 2012:6-12.
16. Chang S-W. Chemical characteristics of mineral trioxide aggregate and its hydration reaction. *Restor Dent Endod*. 2012;37(4):188-193.
17. Al-Haddad A, Aziz ZACA. Bioceramic-Based Root Canal Sealers : A Review. *Int J Biomater*. 2016;2016:1-10.
18. Jr JFS, Lopes HP, Siqueira JF. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. *Int Endod J*. 1999;32(5):361-369.
19. Al-Shwaimi E. Evaluation of Antimicrobial Effect of Root Canal Sealers. *Pakistan Oral Dent J*. 2011;31(2):432-435.
20. Vitti RP, Prati C, Silva EJNL, et al. Physical properties of MTA fillapex sealer. *J Endod*. 2013;39(7):915-918.
21. Ustun Y, Sagsen B, Durmaz S, Percin D. In vitro antimicrobial efficiency of different root canal sealers against Enterecoccus faecalis. *Eur J Gen Dent* |. 2013;2(2):134-138.
22. Zeid A, Saleh M, Treat JT, et al. Trauma & Treatment Solubility , pH Changes and Releasing Elements of Different Bioceramic and Mineral Trioxide Aggregate Root Canal Sealers Comparative Study. *J Trauma Treat*. 2015;4(2):2-5.
23. Morgental RD, Vier-Pelisser F V., Oliveira SD, Antunes FC, Cogo DM, Kopper PMP. Antibacterial activity of two MTA-based root canal sealers. *Int Endod J*. 2011;44(12):1128-1133.