Original Article

Antibacterial efficacy of bioceramic root canal sealers against planktonic *Enterococcus faecalis* after different contact and setting time: An *in vitro* study

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Abstract

Introduction: Bioceramic root canal sealers are the newest generation of root canal sealers. There are contradictory results in the literature about their antimicrobial activity. The aim of the study was to evaluate the antimicrobial efficacy of four root canal sealers against *Enterococcus faecalis*.

Materials and Methods: Four root canal sealers were used in order to examine antibacterial efficacy: TotalFill, BioRoot Root Canal Sealer, mineral trioxide aggregate (MTA) Fillapex, and AH Plus. The bacterial suspension was placed on a freshly mixed sealer, one or 3 days set sealer in vertically held microtiter plates. After incubation of 2, 5, 20, and 60 min in 100% humidity at 37 C, Trypticase Soy Broth was added to each well and mixed. Then, bacterial suspension from each well was transferred, serially diluted, and placed on *Mitis salivarius* agar plates. After incubation, colony-forming units were counted. All experiments were performed in triplicate. The outcomes of antimicrobial properties of tested materials were analyzed by one-way analysis of variance.

Results: All bioceramic root canal sealers showed significantly better efficacy than the control group and epoxy resin sealer ($P < 0.05$). TotalFill presented the highest efficacy comparable with BioRoot RCS ($P > 0.05$), followed with MTA Fillapex ($P < 0.05$). Also, freshly mixed sealers showed comparable efficacy as sealers set for 3 days ($P > 0.05$), but better than sealers set for 1 day ($P < 0.05$). All sealers exhibited the highest efficacy after 20 min of contact time, independently of materials that were freshly mixed or set for 1 or 3 days.

Conclusion: Bioceramic sealers have greater antimicrobial activity than commercially used epoxy resin sealer. These sealers exhibited the strongest efficacy at 20 min of contact time, independently of their setting condition.

Keywords: Antibacterial action, bioceramic, *Enterococcus faecalis*, planktonic, sealer

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Submission: 16-01-21 **Revision:** 18-01-21 **Acceptance:** 25-03-21 **Web publication:** 08-01-22

Access this article online	
Quick Response Code:	Website: www.saudiendodj.com
	DOI: 10.4103/sej.sej_12_21

How to cite this article: Munitic MS, Marijan S, Kero D, Bago I. Antibacterial efficacy of bioceramic root canal sealers against planktonic *Enterococcus faecalis* after different contact and setting time: An *in vitro* study. Saudi Endod J 2022;12:56-60.

INTRODUCTION

Primary ethiological factors in pulpal and periapical diseases are microorganisms and their products. Root canal treatment aims to eliminate microorganisms from the infected root canal system and prevent its reinfection.[1] Although conventional chemomechanical intracanal protocols reduce significantly the number of intracanal microorganisms, their complete eradication is unattainable due to the anatomical irregularities of endodontic space (extensions, lateral and accessory canals, and ramifications).[2] Consequently, these residual microorganisms and their endotoxins impair periapical immune response and prevent the healing of the periapical lesion.[3]

It has already been proven that complete cleaning of infected dentin walls is impossible, regardless of the instrumentation techniques used.[4] Furthermore, conventional irrigation protocols have limited ability in cleaning intracanal irregularities, especially the apical part of the canal, which has been considered as the critical area in debridement and disinfection.[5] Therefore, attention has been given to improving the antimicrobial efficacy of the root canal sealers.

The aim of a filling material is to entomb the residual microorganisms in the root canal system and to prevent their further growth and re-infection.[2] All commercially available sealers show a certain degree of antimicrobial activity,[6] which changes over time.[2] Epoxy resin sealers, which have been considered as a gold standard in endodontics, showed efficacy against *Enterococcus faecalis* within a zone of 300 μm around the root canal.[7] Their antimicrobial action has been claimed to be the result of the activity of unpolymerized residues (i.e. epoxide and amide) which are released during the setting process, but this activity decreases over the time.[8]

Bioceramic root canal sealers are the latest generation of endodontic sealers.[9] They are based on tricalcium phosphate, mineral trioxide aggregate (MTA), and tricalcium silicate. They have promising biological and physicochemical properties as alkaline pH, high calcium ions release, and suitable radiopacity and flow capacity.[10,11] Their antimicrobial activity has been reported in earlier *in vitro* studies against planktonic microbial cells or bacterial biofilms. The results of the studies showed contradictory results regarding the duration of their antimicrobial activity.[2,12,13] Observing the long-term antimicrobial activity could be useful in considering the maximal effect of sealers.

The aim of this *in vitro* study was to evaluate the antibacterial activity of three bioceramics and one epoxy-based endodontic sealer after different setting times (20 min, 24 h, and 72 h after mixing) and different contact times (2, 5, 20, and 60 min).

MATERIALS AND METHODS

Three different bioceramic materials and an epoxy resin-based sealer were tested in this study:

1. Total Fill Bioceramic Sealer (TotalFill BC Sealer, FKG, Switzerland).
2. BioRoot Root Canal Sealer (BioRoot RCS, Septodont, Saint Maur Des Fosses, France).
3. MTA Fillapex (Angelus, Londrina, PR, Brazil).
4. Epoxy resin-based sealer, AH Plus (Dentsply, Konstanz, Germany).

Antibacterial efficacy of the bioceramic root canal sealers was evaluated using modified direct contact test (DCT) described by Zhang *et al*.[2] with some modifications.

The tested materials were prepared according to the manufacturers' instructions.

Approximately 20 μL of each freshly prepared sealer, measured by a spoon excavator (Premium instruments, New York, USA) of the same volume, was used for modified DCT.

Preparation of bacterial suspension

The antibacterial efficacy of the sealers was tested against *E. faecalis* (ATCC 29212). The bacteria was grown overnight in Tryptic Soy Broth (TSB; Becton, Spark, MD) at 37°C. Bacteria were prepared in sterile water and optical density was adjusted to a density of 3×10^8 colony-forming units (CFU)/ml using a spectrophotometer (PrimLight SECOMAM, France) at 405nm.

The modified DCT was performed in 96-microtiter plates (Sarstedt Inc, Newton, NC). An equal amount of each sealer was placed, using a cavity liner applicator, on the sidewall of the microtiter plate which was held vertically. Microtiter plates which were purposed for 1 or 3-day material setting were incubated in a 100% humid atmosphere at 37°C before application of the bacterial suspension.

A 10 μl of bacterial suspension was placed on the surface of each sealer. For the control group, wells where the bacterial suspension was placed on the wall without sealer were used. Antimicrobial efficacy was tested for freshly set sealers, sealers set for 1 day, and for the sealers set for 3 days. Sealers purposed to set 1 or 3 days before application

of the bacterial suspension were placed in 100% humidity at 37°C. After the bacterial suspension was placed, microtiter plates were incubated for 2, 5, 20, and 60 min in 100% humidity at 37°C. Three wells were used for each time period for each material (in triplicate). Also for the control group, the same number of wells were used.

Microbiological evaluation

After incubation, 190 µl of TSB was added to each well and mixed with a pipette for 1 min. Then, bacterial suspension from each well was transferred and serially diluted in TSB. After 12-fold dilution, 10 µl of suspension was placed on *Mitis salivarius* agar plates. After incubation for 24 h at 37°C, CFU was counted and the CFU/ml was calculated. One examiner counted the colonies on the each field of the microtiter plate.

Statistical analysis

The outcomes of antimicrobial properties of tested materials were analyzed by one-way analysis of variance. The level of confidence was set at 95%. Statistical analysis was performed with the statistical software SPSS v. 11.0 (SPSS for Windows; SPSS Inc., Chicago, IL, USA) and Microsoft Office Excel 2016 (Microsoft Corp., Redmond, WA, USA).

RESULTS

All tested sealers showed significant antibacterial efficacy when compared to the control group ($P < 0.05$). The TotalFill BC Sealer showed the highest, while AH Plus showed the lowest efficacy in all tested setting times and contact times. There was no statistically significant difference between TotalFill and BioRoot RCS in all time periods ($P > 0.05$). Furthermore, MTA Fillapex and BioRoot RCS presented statistically better antibacterial efficacy compared to AH-Plus ($P < 0.05$) in all settings and contact times.

Considering the setting time of the sealers, all freshly mixed sealers had significantly greater antibacterial efficacy compared to the sealers set for 1 day ($P < 0.05$). There was no statistically significant difference between freshly mixed sealers and sealers set for 3 days ($P > 0.05$). Furthermore, the sealers, that were set for 3 days, were statistically more efficient than the sealers, that were set for 1 day ($P < 0.05$).

All tested bioceramic sealers (TotalFill BC Sealer, BioRoot RCS, and MTA Fillapex) exhibited the highest antibacterial efficacy after 20 min of contact time, independently of the setting time ($P < 0.05$). In contact time periods of 2, 5, and 60 min, the bioceramic sealers did not show statistically different results ($P > 0.05$). Antibacterial activity of the sealers at different settings and contact times are presented in Figures 1-3.

DISSCUSION

The results of this study showed that the bioceramic sealers had better antibacterial activity compared to the epoxy resin-based sealer. The calcium silicate and phosphate-based sealer (TotalFill BC Sealer) was the most efficient against *E. faecalis*, followed by the pure tri-calcium silicate sealer (BioRoot RCS), whereas the MTA-based salicylate resin sealer (MTA Fillapex) was the least efficient, but still

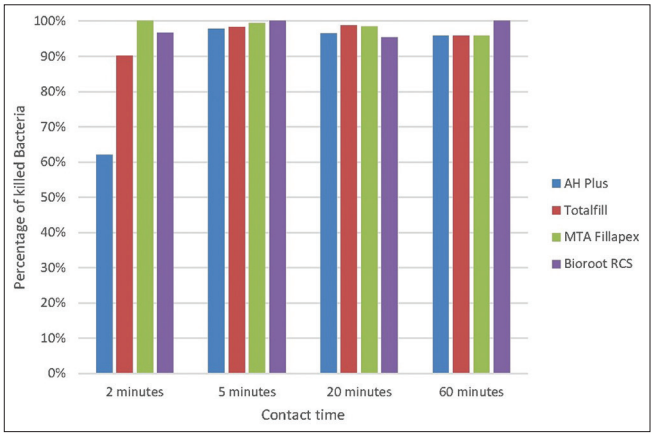

Figure 1: Antibacterial efficacy of freshly prepared materials

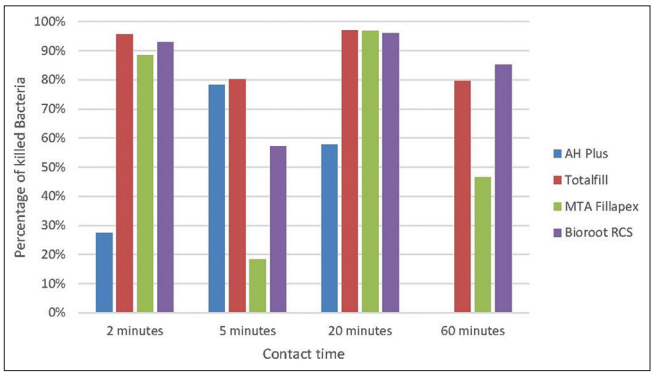

Figure 2: Antibacterial efficacy of materials after one setting day

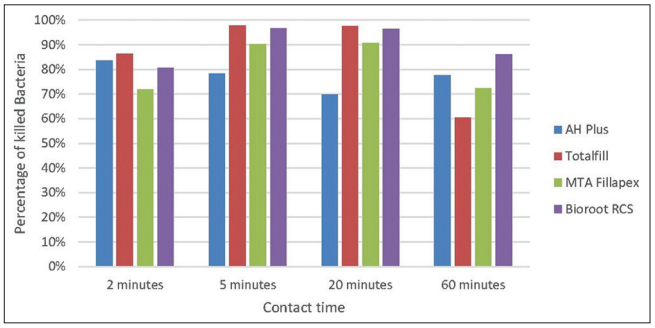

Figure 3: Antibacterial efficacy of materials after three setting days

better than the AH Plus. A modified direct contact technique described by Zhang *et al*.[2] with some modifications was used in the study. Modifications were made due to the insufficient growth of bacterial colonies on agar plates.

The microorganism used in this study was *E. faecalis*. It has a significant role in the etiology of persistent periradicular lesions because of its virulence factors and ability to survive the conventional chemo-mechanical root canal procedures.[2] Also, it has the ability to penetrate deep into dentin tubules, form biofilms, survive nutrition deprivation, and resist commonly used disinfection agents.[14,15]

The significant antibacterial efficacy of the bioceramic sealers is result of their high pH, hydrophilicity, and active calcium hydroxide, which is released during the setting process.[2,10,16] According to the best knowledge, there has been only one study[16] published so far comparing the antibacterial efficacy of few bioceramic sealers. In the study of Poggio *et al*.,[17] the TotalFill BC eradicated all bacteria evaluated by the DCT. The results of this study are in accordance with the previous results of Bronzel *et al*.[13] and Bukhari *et al*.[18] who also found significant and better antibacterial efficacy of calcium silicate and phosphate sealers (TotalFill BC and Endosequence BC) against *E. faecalis* compared to the epoxy resin sealer (AH Plus).

However, there have still been some controversies in the literature regarding the superior antibacterial efficacy of the calcium silicate and phosphate-based sealers. In the studies of Shin *et al*.[19] and Candeiro *et al*.,[12] the EndoSequence BC Sealer did not present any significant antibacterial efficacy over the epoxy-resin-based sealer. The differences among the studies could be explained by the different microbiological methodology used. So far in the literature, the antibacterial efficacy of the root canal sealers has been evaluated mostly using an agar diffusion test (ADT).[20,21] ADT is the most common used test in this kind of study. The results of the test are recorded after analyzing of the inhibition zones of bacteria growth around testing materials. If the inhibition zone is larger, the material had better antibacterial efficacy. However, this test has certain limitations such as influence of diffusion characteristics of agar and solubility of materials on test results. Therefore, this test is not appropriate for testing endodontic materials.[22] DCT is another method for testing the antibacterial efficacy of materials based on contact between the tested sealer and microbial cells. It is a more reliable method, since it evaluates the bactericidal efficacy of the sealer, which is clinically more relevant. Furthermore, the DCT has the possibility to test the efficacy of sealers in different setting times.[2]

According to the best knowledge, there have been only three studies[6,14,23] published so far on the antibacterial efficacy of BioRoot RCS. In the study of Poggio *et al*.,[17] BioRoot RCS had similar efficacy as MTA Fillapex, but lower than the TotalFill BC. The authors used DCT for the evaluation of antibacterial efficacy of the sealers as done in this study. In another study by Alsubait *et al*.,[6] the BioRoot RCS and the TotalFill BCS showed similar antibacterial efficacy using confocal laser scanning microscopy. Ariaz-Moliz *et al*.[23] reported superiority of BioRoot RCS over MTA Fillapex and AH Plus. From the above-presented studies and the results of this study, it could be concluded that BioRoot RCS, in *in vitro* conditions, had significant antibacterial efficacy superior to AH Plus and MTA Fillapex.

The most commonly investigated bioceramic material in the literature is MTA Fillapex.[24] However, the results of its antibacterial efficacy are conflicting. In this study, MTA Fillapex had the lowest antibacterial efficacy when compared to other tested bioceramic sealers, but better than the epoxy resin-based sealer. Similar results are reported in other previous studies.[23,25,26] Contrary, there have been few studies showing its lower efficacy compared to the AH Plus.[27,28] This difference could be attributed to the use of ADT, whose limitations have been mentioned previously.[22] The lowest antibacterial efficacy of MTA Fillapex when compared with other bioceramic sealers might be due to lower pH because of the lower percentage of available calcium hydroxide.[29]

In this study, there were no differences in antibacterial activity between the freshly mixed sealers and the sealers set for 3 days. This topic is quite controversial in the literature showing different conclusions. Most past studies reported decrease of the antimicrobial activity of root canal sealers during setting.[2,19,30] However, Kapralos *et al*.[31] showed that the efficacy of set sealers might be valuable in longer time periods. In their study TotalFill, BC Sealer had similar antibacterial activity when freshly mixed or when set 24 h or 7 days. The long-term efficacy of bioceramic sealers could be explained by their long-term releasing of calcium hydroxide ions even after the initial setting reaction.[32]

The limitation of the study was the usage of the planktonic cells for the investigation of the antibacterial activity of root canal sealers. In the clinical situation, after conventional chemo-mechanical root canal preparation, there are remaining bacterial biofilms as well as planktonic bacterial cells left on intracanal walls and in intracanal anatomical irregularities.[33] However, since the aim of this study was to compare three well-known bioceramic sealers in well

standardized *in vitro* conditions, the next studies should be a focus on their individual efficacy against one-species or multispecies biofilms of different maturation stage.

CONCLUSION

Under the limitations of this study, the TotalFill BC Sealer showed the best antibacterial activity compared to other bioceramic sealers. Its efficacy was most evident at freshly mixed sealer and after 20 min of contact.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Sundqvist G. Ecology of the root canal flora. J Endod 1992;18:427-30.
2. Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against *Enterococcus faecalis*. J Endod 2009;35:1051-5.
3. Siqueira JF Jr., Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod 2008;34:1291-301.e3.
4. Zuolo ML, Zaia AA, Belladonna FG, Silva EJ, Souza EM, Versiani MA, *et al*. Micro-CT assessment of the shaping ability of four root canal instrumentation systems in oval-shaped canals. Int Endod J 2018;51:564-71.
5. Adcock JM, Sidow SJ, Looney SW, Liu Y, McNally K, Lindsey K, *et al*. Histologic evaluation of canal and isthmus debridement efficacies of two different irrigant delivery techniques in a closed system. J Endod 2011;37:544-8.
6. Alsubait S, Albader S, Alajlan N, Alkhunaini N, Niazy A, Almahdy A. Comparison of the antibacterial activity of calcium silicate- and epoxy resin-based endodontic sealers against *Enterococcus faecalis* biofilms: A confocal laser-scanning microscopy analysis. Odontology 2019;107:513-20.
7. Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D. Survival of *Enterococcus faecalis* in infected dentinal tubules after root canal filling with different root canal sealers *in vitro*. Int Endod J 2004;37:193-8.
8. Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M. Combined antibacterial effect of sodium hypochlorite and root canal sealers against *Enterococcus faecalis* biofilms in dentin canals. J Endod 2015;41:1294-8.
9. Antunovic M, Vukmanovic LJ, Budimir A, Kabil E, Anic I, Bago I. Evaluation of sealing ability of four bioceramic root canal sealers and an epoxy resin-based sealer: An *in vitro* study. Saudi Endod J 2021;11:66-72.
10. Candeiro GT, Correia FC, Duarte MA, Ribeiro-Siqueira DC, Gavini G. Evaluation of radiopacity, pH, release of calcium ions, and flow of a bioceramic root canal sealer. J Endod 2012;38:842-5.
11. Borges RP, Sousa-Neto MD, Versiani MA, Rached-Júnior FA, De-Deus G, Miranda CE, *et al*. Changes in the surface of four calcium silicate-containing endodontic materials and an epoxy resin-based sealer after a solubility test. Int Endod J 2012;45:419-28.
12. Candeiro GT, Moura-Netto C, D'Almeida-Couto RS, Azambuja-Júnior N, Marques MM, Cai S, *et al*. Cytotoxicity, genotoxicity and antibacterial effectiveness of a bioceramic endodontic sealer. Int Endod J 2016;49:858-64.
13. Wang Z, Shen Y, Haapasalo M. Dentin extends the antibacterial effect of endodontic sealers against *Enterococcus faecalis* biofilms. J Endod 2014;40:505-8.
14. Kayaoglu G, Ørstavik D. Virulence factors of *Enterococcus faecalis*: Relationship to endodontic disease. Crit Rev Oral Biol Med 2004;15:308-20.
15. Alabdulmohsen ZA, Saad AY. Antibacterial effect of silver nanoparticles against *Enterococcus faecalis*. Saudi Endod J 2017;7:29-35.
16. Zordan-Bronzel CL, Tanomaru-Filho M, Rodrigues EM, Chávez-Andrade GM, Faria G, Guerreiro-Tanomaru JM. Cytocompatibility, bioactive potential and antimicrobial activity of an experimental calcium silicate-based endodontic sealer. Int Endod J 2019;52:979-86.
17. Poggio C, Trovati F, Ceci M, Colombo M, Pietrocola G. Antibacterial activity of different root canal sealers against *Enterococcus faecalis*. J Clin Exp Dent 2017;9:e743-8.
18. 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;45:1047-52.
19. Shin JH, Lee DY, Lee SH. Comparison of antimicrobial activity of traditional and new developed root sealers against pathogens related root canal. J Dent Sci 2018;13:54-9.
20. Singh G, Gupta I, Elshamy FM, Boreak N, Homeida HE. *In vitro* comparison of antibacterial properties of bioceramic-based sealer, resin-based sealer and zinc oxide eugenol based sealer and two mineral trioxide aggregates. Eur J Dent 2016;10:366-9.
21. Tanomaru JM, Tanomaru-Filho M, Hotta J, Watanabe E, Ito IY. Antimicrobial activity of endodontic sealers based on calcium hydroxide and MTA. Acta Odontol Latinoam 2008;21:147-51.
22. Cobankara FK, Altinöz HC, Ergani O, Kav K, Belli S. *In vitro* antibacterial activities of root-canal sealers by using two different methods. J Endod 2004;30:57-60.
23. Arias-Moliz MT, Camilleri J. The effect of the final irrigant on the antimicrobial activity of root canal sealers. J Dent 2016;52:30-6.
24. Šimundić Munitić M, Poklepović Peričić T, Utrobičić A, Bago I, Puljak L. Antimicrobial efficacy of commercially available endodontic bioceramic root canal sealers: A systematic review. PLoS One 2019;14:e0223575.
25. Omidi S, Hoshyari N, Mirzadeh A, Ebrahimzadeh H, Ahajan M, Charati J. Comparison of antibacterial activity of three endodontic sealers against Enterococcus faecalis. J Res Med Dent Sci 2018;6:2347-67.
26. Faria-Júnior NB, Tanomaru-Filho M, Berbert FL, Guerreiro-Tanomaru JM. Antibiofilm activity, pH and solubility of endodontic sealers. Int Endod J 2013;46:755-62.
27. Gurel M, Demiryurek O, Ozyurek T, Gulhan T. Antimicrobial activities of different bioceramic root canal sealers on various bacterial species. Int J Appl Dent Sci 2016;2:19-22.
28. Colombo M, Poggio C, Dagna A, Meravini MV, Riva P, Trovati F, *et al*. Biological and physico-chemical properties of new root canal sealers. J Clin Exp Dent 2018;10:e120-6.
29. Kuga CM, Farial G, Weckwerthl H, Hungaro Duaertel MA, de Campos EA, Reis Só MV. Evaluation of the pH, calcium release and antibacterial activity of MTA Fillapex. Rev Odontol UNESP 2013;42:330-5.
30. Dalmia S, Gaikwad A, Samuel R, Aher G, Gulve M, Kolhe S. Antimicrobial efficacy of different endodontic sealers against *Enterococcus faecalis*: An *in vitro* study. J Int Soc Prev Community Dent 2018;8:104-9.
31. Kapralos V, Koutroulis A, Ørstavik D, Sunde PT, Rukke HV. Antibacterial activity of endodontic sealers against planktonic bacteria and bacteria in biofilms. J Endod 2018;44:149-54.
32. Vitti RP, Prati C, Silva EJ, Sinhoreti MA, Zanchi CH, de Souza e Silva MG, *et al*. Physical properties of MTA Fillapex sealer. J Endod 2013;39:915-8.
33. Nair PN. On the causes of persistent apical periodontitis: A review. Int Endod J 2006;39:249-81.