e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15 (12); 778-783

Original Research Article

Comparative Evaluation of Antimicrobial Activity of Different Endodontic Sealers against Three Root Canal Pathogens-An in-Vitro Study

Manjula.C.G[1], Rosamma George[2], Gayathree.L[3]

[1]Assistant Professor, Department of Dentistry, Hassan Institute of Medical Sciences, Hassan -573201
[2]Associate Professor, Department of Dentistry, Hassan Institute of Medical Sciences, Hassan -573201
[3]Assistant Professor, Department of Microbiology, Hassan Institute of Medical Sciences, Hassan -573201

Received: 25-09-2023 / Revised: 28-10-2023 / Accepted: 30-11-2023
Corresponding author: Dr. Manjula.C.G
Conflict of interest: Nil

Abstract:

Introduction: Main objective of endodontic treatment is removal of micro-organisms from root canal and preventing them from re-infecting. Endodontic sealers exhibit antimicrobial activity thereby eliminate micro-organisms from root canals and facilitate success of endodontic treatment.

AIM: To evaluate and compare antimicrobial activity of different endodontic sealers against three root canal pathogens.

Material and Methods: Microbial suspension of Enterococcus faecalis, Escherichia coli, and Candida albicans was prepared. Petri-dish containing Mueller Hilton agar medium were streaked with sterile cotton swabs dipped in microbial suspensions. Four wells of equal dimension were prepared, and were immediately filled with equal volumes of freshly mixed selected four sealers i.e., Zinc Oxide Eugenol based, Epoxy Resin based, Mineral Trioxide Aggregate based, Bio Ceramic based sealers. After pre-diffusion plates were incubated at 37^0C and microbial growth inhibition zones were measured using digital callipers, after 24 hours, 48 hours and 72 hours. All the assays were conducted in triplicate under aseptic precautions and data was recorded in terms of average diameter of inhibition zone. Data was statistically analysed using Epi Info and Statistical Package for Social Sciences (SPSS) software.

Results: Four sealers exhibited microbial growth inhibition. Epoxy Resin sealer showed the greatest microbial growth inhibition of 13.89±2.96 millimetres followed by MTA sealer (9.67 ±3.84 millimetres) and Bioceramics sealer (9.22±1.75 millimetres). ZOE sealer comparatively exhibited mild antimicrobial activity (8.89±1.91 millimetres).

Conclusion: All sealers exhibited antimicrobial activity. Epoxy Resin sealer showed highest antimicrobial activity followed by MTA, Bioceramics sealer and ZOE sealer against tested organisms.

Keywords: Agar Diffusion Method, Endodontic Sealers, Root Canal Pathogens.

Introduction

Microbes and their products are main causative factors in development and progression of pulpal and periapical pathology. Main objective of Endodontic treatment is to promote root canal disinfection, preventing microorganisms from impairing periapical healing or even contributing to the development of apical pathalogy. [1,2]

Absence of irritating agents like bacterial metabolic products and chemicals released from sealing materials will act as a stimulus for the peri apical healing process. [2] Extinction of microorganism from root canal, or at least their reduction to compatible levels is main objectives of endodontic treatment. [3] Root canal treatment (RCT) is achieved by proper diagnosis, instrumentation, biomechanical preparation, intracanal disinfection by irrigation and intracanal dressings, and three-dimensional obturation under aseptic conditions, are essential to avoid reinfection of root canal space and increase success rate. [2-7] Root canal filling materials plays an important role in control of reinfection by entombing residual organisms through antimicrobial activity. [4,5]

Certain facultative aerobic and anaerobic microorganisms like Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus anginosus, Bacteroides fragilis, Actinomyces, Fusobacterium nucleatum, and Candida albicans are usually associated with root canal infections. [2,6,7] In spite of utmost care, persistence of residual microbes and their growth in dentinal tubules, lateral canals, bifurcations, apical ramification leads to flare-ups, and associated endodontic

failures. Therefore, complete eradication of the infection is necessary for the success of endodontic treatment. [2,6,7]

Endodontic sealers play an important role in controlling infection by entombing residual bacteria and prevent leakage and reinfection. [4-6] multiple commercial endodontic sealers are available. Sealers with good sealing ability, biocompatibility, long antimicrobial activity, stability, low solubility in oral fluids and disintegration, able to penetrate into dentinal tubules, induce repair, mineralization are desirable. [8-12] Several classes of endodontic sealers based on calcium hydroxide, Zinc Oxide Eugenol (ZOE), Epoxy resin, Mineral Tri-oxide Aggregate (MTA), Methacrylate, Silicon, Calcium silicate, Iodoform, Bioceramics etc are available. [10-14] Many studies have compared antimicrobial activity of different endodontic sealers against different root canal pathogens. [8,13] Agar diffusion method, Direct contact test, are widely used to evaluate the antimicrobial activity of root canal sealers. [14-18]

The objective of the present study was to evaluate and compare antimicrobial activity of different endodontic sealers against root canal pathogens by measuring the diameter of inhibition zones using digital callipers on the surface of agar plates.

Aim: To evaluate and compare antimicrobial activity of different endodontic sealers against three root canal pathogens.

Material and Methods: **[1-18]**

This study was conducted in, Hassan Institute of Medical Sciences, Hassan, Karnataka. Study was conducted after approval from Institutional Ethical Committee (IEC/HIMS/ RR 462/05-09-2023). Microbial suspension of selected microorganisms i.e., Enterococcus faecalis (ATCC 29212), Escherichia coli (ATCC 25922), and Candida albicans (ATCC 10231) were prepared. Microorganisms were diluted to obtain a suspension of approximately 5×10^8 colony-forming units/ml in sterile Brain heart infusion (BHI) broth. Double base layer of Mueller Hilton agar was prepared on 90 millimetres diameter petri dishes.

Petri-dish containing Mueller Hilton agar medium were streaked with sterile cotton swabs dipped in microbial suspensions in all direction evenly. Four wells of 3 millimetres diameter and 3 millimetres deep were prepared using glass puncher. The sealers were mixed according to manufacturer instructions. The wells were immediately filled with equal volumes of freshly mixed selected four sealers i.e., Zinc Oxide Eugenol based, Epoxy Resin based, Mineral Trioxide Aggregate based, Bio Ceramic sealers. For pre-diffusion the petri dishes were kept for 2 hours at room temperature. The plates were incubated at 37^0C and microbial growth inhibition zones were measured using digital calliper, after 24 hours, 48 hours and 72 hours. All the assays were conducted in triplicate under aseptic precautions and the data was recorded in terms of the average diameter of inhibition zone. Data was statistically analysed. [1-18]

Statistical Analysis: Data was collected and statistically analysed using Epi Info and Statistical Package for Social Sciences (SPSS) software. Quantitative data were expressed as the mean and standard deviation. Kruskal Wallis H test equivalent to Chi-square test was carried out to compare the differences among different groups and four sealers. The $P<0.05$ level is considered statistically significant. Conclusion were drawn on statistical treatment outcome.

Results

Table 1,2,3 shows the antimicrobial activity of ZOE, Epoxy Resin, MTA, Bioceramics based sealers against three microbial strains like E. Coli, Candida Albicans and E. Faecalis. Microbial growth inhibition zones were measured after 24,48 and 72 hours using digital calliper. All sealers were significantly effective against all tested microorganisms. Epoxy Resin sealer showed the highest antimicrobial activity with an inhibition zone of 13.88±2.96 millimetres among all the sealers after 24 hours and was statistically significant ($p<0.05$). The antimicrobial action of Epoxy Resin sealer was superior to that of MTA sealer (9.66±3.84 millimetres). Bioceramics sealer and ZOE sealer showing inhibition zone of 9.22±1.75 and 8.88±1.91 millimeter respectively. Epoxy Resin and MTA based sealer, showed greater inhibition zones for Candida albicans followed by E. faecalis, and E. coli ($P<0.05$ statistically significant). Bioceramics sealer showed greater inhibition zones for E. faecalis, followed by Candida albicans and E. coli. ZOE sealer showed greater inhibition zones for E. coli and Candida albicans then E. faecalis. Effectiveness of Epoxy based and Zinc Oxide Eugenol based sealers declined gradually with time showing inhibition zone of 12.55±2.43 and 7.88±1.65 millimeter after 48 hours, 11.55±2.41 and 6.66±1.48 millimeter after 72 hours respectively and was statistically significant ($p<0.05$). MTA and Bioceramics sealer did not show much change, with inhibition zone of 9.55±3.64 and 8.88±1.69 millimeter after 48 hours, 9.22±3.65 and 8.33±2.05 millimeter after 72 hours respectively and was statistically significant ($p<0.05$). Size of inhibition zones does not necessarily reflect the exact strength of sealers used.

Table 1: Inhibition zones of sealers in triplicates after 24 hours (in millimetres)

Microorganisms	Test	SEALERS			
		ZOE	Epoxy Resin	MTA	Bioceramic
E. Coli	1	9.2	10.1	6.2	6.2
	2	10.8	11.9	5.8	7.0
	3	10.0	12.0	6.0	7.8
C. Albicans	1	11.4	17.8	14.6	9.8
	2	10.1	18.0	16.1	10.2
	3	8.5	17.2	12.3	10.0
E. Faecalis	1	7.7	12.0	9.8	10.4
	2	6.3	12.8	8.2	10.5
	3	6.0	13.2	8.0	11.1
Mean± Standard Deviation		8.88±1.91	13.88±2.96	9.66±3.84	9.22±1.75
p-value	p<0.0042 Statistically Significant				

Table 2: Inhibition zones of sealers in triplicates after 48 hours (in millimetres)

Microorganism	Test	SEALERS			
		ZOE	Epoxy resin	MTA	Bioceramic
E. coli	1	8.3	9.6	6.2	6.2
	2	9.3	11.4	5.8	7.0
	3	9.4	11.0	6.0	6.8
C. Albicans	1	9.7	15.2	14.6	9.8
	2	9.1	15.8	15.1	10.2
	3	7.2	16.0	12.3	10.0
E. Faecalis	1	6.9	11.1	9.8	10.4
	2	6.0	10.8	8.2	9.5
	3	5.1	12.1	8.0	10.1
Mean± Standard Deviation		7.88±1.65	12.55±2.43	9.55±3.64	8.88±1.69
p-value	p<0.0026 Statistically Significant				

Table 3: Inhibition zones of sealers in triplicates after 72 hours (in millimetres)

Microorganism	Test	SEALERS			
		ZOE	Epoxy resin	MTA	Bioceramic
E. coli	1	7.4	9	6.2	5.0
	2	8.0	10.2	4.8	6.0
	3	8.6	9.8	6.0	6.0
C. Albicans	1	7.8	15.0	14.5	9.8
	2	7.1	14.0	14.4	10.2
	3	6.1	15.0	12.1	10.0
E. Faecalis	1	5.8	9.9	9.6	9.8
	2	5.1	10.0	7.4	9.2
	3	4.1	11.1	8.0	9.0
Mean± Standard Deviation		6.66±1.48	11.55±2.41	9.22±3.65	8.33±2.05
P-Value	p<0.0031 Statistically Significant				

Discussion

Residual microbes in the root canal often lead to failure of root canal treatment. They have the ability to invade dentinal tubules, resistance to deprived nutrition, and to compete with different microorganisms. The commonest residual microorganism which leads to recurrent persistent infections is Enterococcus faecalis, Escherichia coli, Candida albicans. [2-6]

Therefore these organisms are selected for this study. The antimicrobial activity of endodontic sealers against these microorganisms may help in controlling infection Agar diffusion method is the most common technique used for evaluation of antimicrobial activity. [2,15-18] This method allows direct comparison of sealers. Variations in agar medium, number of specimens per plate, bacterial strains, diffusion capacity of inhibitory agents, physical properties of test materials and cellular density may interfere with the formation of

inhibition zones around materials used in antimicrobial testing. It does not differentiate between bactericidal and bacteriostatic effect. [2,10,12] Agar diffusion method has its own advantages and disadvantages. Based on various chemical compositions numerous root canal sealers are available.

Zinc oxide eugenol-based sealers are the most commonly used. The sealer consists of base paste and catalyst paste. [2,6] Eugenol is a phenolic compound that acts by protein denaturation.9 Eugenol is also lipophilic which acts on lipids in the cell membrane and increases cell membrane permeability of the microorganisms. [2-7,16] Some studies have reported that ZOE along with paraformaldehyde has shown higher antibacterial activity.[3] Zinc oxide eugenol sealers exhibit bactericidal effect when freshly mixed, but the effect declines with time. [2-7,16] Shin JH et al, [5] Arora S et al, [6] Villagomez PC et al [16] and other studies showed least microbial growth inhibition zone for ZOE sealer. In the present study ZOE sealer showed least inhibition zones compared to other sealers. ZOE sealer showed greater inhibition zones for E. coli and Candida albicans then E. faecalis. This goes in support of previous studies.

Epoxy resin-based sealer is a pack of two pastes. Paste A is epoxy paste and Paste B is amine paste. [6,15] Epoxy resin-based sealer after setting adapts closely to canal walls due to good flow with minimal shrinkage and long-term dimensional stability. [2] High antibacterial activity of this sealer may be due to unpolymerized residues like bisphenol A, diglycidyl ether and formaldehyde during polymerization. This penetrates bacteria and inhibits its metabolism. Oxygen inhibition layer of the surface of any polymerizing resin leaves an uncured monomer layer, which could be another reason. [5,9,10,15, 16,18] Ramachandra PKM et al, [2] Kumar S etal, [7] Pallavi P et al, [15] and other studies showed epoxy resin-based AH Plus sealer exhibited the greatest microbial growth inhibition zone. This goes in support of our present study. In present study, Epoxy Resin sealer showed the highest antimicrobial activity with an inhibition zone of 13.88±2.96 millimetres among all the sealers.

Epoxy Resin based showed greater inhibition zones for Candida albicans than for E. faecalis, and E. coli. Effectiveness of Epoxy based and Zinc Oxide Eugenol based sealers declined gradually with time because freshly mixed sealer diffuses better than the set sealer, and depended on the microbial susceptibility to them, which goes in support of previous studies. [4,10,15,16]

Mineral trioxide aggregate (MTA), a tricalcium silicate was developed in early 1990s. MTA is widely used for its excellent sealing capacity, enhanced consistency, radiopacity, easy handling and great working time and biocompatibility. MTA based sealer contain calcium oxide, which when mixed with water, forms calcium hydroxide, which increase pH by dissociation of calcium and hydroxide ions which in turn causes the lysis of the microbial cell membrane. [7-12,15,18] Antimicrobial effect of MTA has shown controversial results in different studies. [10] Kumar s et al, [7] Huang Y et al, [10] Simsek et al, [12] Pallavi P et al, [15] Kaul M et al, [18] and others showed some antibacterial activity against microorganisms. In the present study MTA sealer showed inhibition zone of 9.66±3.84 millimetres after 24 hours. Greater inhibition zones were shown for Candida albicans followed by E. faecalis and E. coli.

BioCeramic sealer is a newer sealer available in premixed calibrated syringes, with intracanal tips which minimises wastage of material and time, consists of calcium trisilicates, tricalcium aluminate, calcium phosphate monobasic, zirconium oxide, calcium hydroxide, radio opacifiers and various filling and thickening agents. [11] Bioceramic sealers have gained importance because of their alkaline pH (above 12), biocompatibility, bioactivity, non-toxicity, dimensional stability, sealing ability etc. Setting reaction of this hydrophilic sealer is catalysed by absorption of moisture present in dentinal tubules contributing to hydration of cement and it does not shrink on setting. Its antibacterial activity is due to diffusion of calcium hydroxide resulting in formation of crystalline structure similar to tooth apatite materials.

Bioceramic sealer forms chemical adhesion to dentine by setting expansion that prevents microbial penetration. Its antimicrobial effect may be due to combination of its high pH, hydrophilic nature, and active calcium hydroxide diffusion. [8,11, 12, 13, 17] Few studies showed that Calcium silicate-base sealers containing oxide compounds may show the strong antimicrobial activity against Gram negative and positive bacteria. [5] Shin JH et al, [5] Mangat P et al , [11] Villagomez Pc et al, [16] Dagna A et al, [17] and others concluded Bioceramics sealer showed greatest inhibition zones compared to other sealers in their study. But in present study Bioceramics sealer showed a inhibition zone of 9.22±1.75 millimeters after 24 hours. Bioceramics sealer showed greater inhibition zones on E. faecalis, followed by Candida albicans and E. coli. Bioceramics sealer and MTA sealers did not show much change at the end of 48 and 72 hours.

Limitation of the study

The variation in the zone of inhibition of sealers is related to chemical composition, degree and time

taken to set, and diffusibility. Agar diffusion method has its own limitations. A material that easily diffuses will produce larger zones of inhibition.

Conclusion

All the sealers showed antimicrobial activity against the selected microorganisms. Epoxy based sealer showed highest antimicrobial effect than all other tested sealers. The antimicrobial action of Epoxy Resin sealer was superior to that of Mineral trioxide aggregate sealer followed by Bioceramics sealer and Zinc Oxide Eugenol sealer. Highest antimicrobial effect was observed for Candida albicans. Effectiveness of Epoxy based and Zinc Oxide Eugenol based sealers declined gradually with time. Bioceramics sealer and Mineral trioxide aggregate sealers did not show much change at the end of 48 and 72 hours. There is a need for development of new methods that does not interfere with diffusivity and solubility in culture medium.

Acknowledgement: My sincere thanks to all HIMS Dentistry and Microbiology Department staff, Principal, Director, statistician for their kind support, encouragement and cooperation.

Reference

1. Wainstein M, Morgental RD, Waltrick SBG, Oliveira SD, Vier-Pelisser FV, Figueiredo JAP, Steier L, Tavares CO, Scarparo RK. In vitro antibacterial activity of a silicone-based endodontic sealer and two conventional sealers. Braz Oral Res [online] 2016; 30: e18;1-5.
2. Ramachandra PKM, Krishnegowda SC, Jaganath BM, Rudranaik S, Manjula CG, Kurup NB, Madanan S. In vitro Comparative Evaluation of the Antibacterial and Antifungal Activities of Different Root Canal Sealers against Endodontic Pathogens. Int J Prev Clin Dent Res 2016; 3(4):261-266.
3. 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(6): e743-8.
4. Tandon B, Uppin VM, Hogade SA, Chaudhry S, Bansal S, Bansal S. Antibacterial activity of four root canal sealers against Enterococcus faecalis at 1st, 3rd, 5th, and 7th day duration: A In vitro study. Indian J Dent Sci 2017; 9:98-104.
5. Shin JH, Lee DY, Lee SH. Comparison of antimicrobial activity of traditional and new developed root sealers against pathogens related root canal. Journal of Dental Sciences 2018; 13: 54-59.
6. Arora S, Mir S, Gautam A, Batra R, Soni S, Lata K. Evaluation of Antimicrobial Efficacy of Root Canal Sealers against Enterococcus faecalis: A Comparative Study. J Contemp Dent Pract 2018; 19(6):680-683.
7. Kumar S, Saurav S, Kumar M, Kumari K, Prasad R, Kumar A. Comparison of antimicrobial activity of three root canal sealers: An in vitro study. International Journal of Applied Dental Sciences 2019; 5(4): 108-111.
8. Simundic Munitic M, Poklepovic Pericic T, Utrobicic A, Bago I, Puljak L. Antimicrobial efficacy of commercially available endodontic bioceramic root canal sealers: A systematic review. PLoS ONE 2019;14(10):1-20.
9. Rajisha KR, Sujatha I, Komal N, Vamshi Krishna V**.** Comparative evaluation of the antimicrobial efficacy of three endodontic sealers against Enterococcus faecalis with and without incorporation of Chitosan nanoparticles. RGUHS Journal of Dental Sciences 2019;11(1): 21-28.
10. Huang Y, Li X, Mandal P, Wu Y, Liu L, Gui H, Liu J. The in vitro antimicrobial activities of four endodontic sealers. BMC Oral Health 2019;19(118);1-7.
11. Mangat P, Dhingra A, Muni S, Bhullar HK. To compare and evaluate the antimicrobial activity of three different root canal sealers: An In Vitro Study. J Conserv Dent 2020; 23:571-6.
12. Simsek M, Kanik O. Antimicrobial activity of root canal sealers against some standard strains and clinical isolates. Medicine Science 2021; 10(2):586-91.
13. Rodriguez-Niklitschek C, Chuhuaicura P, Oporto GH. Antimicrobial activity of bioceramic root canal sealers: A systematic review. Int. J. Odontostomat 2021; 15(2):348-355.
14. Mukorera TF, Ahmed S, Maboza E, Kimmie-Dhansay F. In vitro antibacterial activity of three root canal sealers against Enterococcus Faecalis. SADJ 2022; 77(7):413-422.
15. Pallavi P, Bansal A, Kukreja N, Kaur N, Chhabra S, Ahuja J. Comparative evaluation of antimicrobial efficacy of different endodontic sealers against Enterococcus Faecalis: An in-vitro study. International Journal of Health Sciences 2022; 6(S1):9838-9845.
16. Paola Castillo-Villagomez, Elizabeth Madla-Cruz, Fanny Lopez-Martinez, Idalia Rodriguez-Delgado, Jorge Jaime Flores-Treviño, Guadalupe Ismael Malagon-Santiago & Myriam Angelica de La Garza-Ramos. Antimicrobial effectiveness of root canal sealers against Enterococcus faecalis. Biomaterial Investigations in Dentistry 2022; 9(1): 47-51.
17. Dagna A, Colombo M, Poggio C, Russo G, Pellegrini, M. Pietrocola G, Beltrami, R. In Vitro Antibacterial Activity of Different

Bioceramic Root Canal Sealers. Ceramics 2022; 5: 901–907.
18. Kaul M, Kavar DR, Gite S, Gutte N, Deosarkar B, Deshpande S. Comparison of antimicrobial activity of three root canal sealers: An in vitro study. International Journal of Health Sciences 2022; 6(S4): 8610–8617.