



ANTIMICROBIAL ACTIVITY OF APEXIT AND RC SEAL AGAINST ENTEROCOCCUS AND LACTOBACILLUS USING ZINC OXIDE EUGENOL AS CONTROL

Sneka S¹, Dr. Revathi Duraiswamy*²

¹Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences,
Chennai- 600077, Tamil Nadu, India

²Associate Professor, Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai- 600077, Tamil Nadu, India

CORRESPONDING AUTHOR: Dr. Revathi Duraiswamy, Associate Professor, Department of Prosthodontics, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai- 600077, Tamil Nadu, India

ABSTRACT:

Introduction: The sealer is a calcium hydroxide based sealer. These sealers along with the use of gutta percha cones are one of the most reliable methods for filling the root canal space. The major function of sealers apart from holding gutta percha points together should be to exhibit antimicrobial properties. Endodontic sealers with antimicrobial properties can be beneficial to decrease or eliminate microorganisms from the root canal space and thereby provide a better root canal therapy. **Aim:** The aim of the study is to evaluate the antimicrobial activity of apexit and RC seal against enterococcus and lactobacillus using zinc oxide eugenol as control. **Materials and methods:** In this study, Antimicrobial viability of two root waterway sealers was tested against enterococcus and lactobacillus, the two stronger bacteria seen in the root canal. Standard strains of enterococcus and lactobacillus are grown on enriched media and incubated for 12 hours. Then using a sterile metal tube of 6 mm diameter 3 wells were cut on the media. The wells are filled with the sealers, the sealers used were RC seal, apexit and zinc oxide eugenol. The plates were taken and in each sealer was placed and the plates were incubated aerobically at 37 degree celsius overnight. After incubation the zone of inhibition was measured in millimeters and tabulated. **Results and discussion:** In this study which compared the Antimicrobial activity of three sealants against *enterococcus* and *lactobacillus*, it was found that rc seal shows significantly higher Antimicrobial activity compared with the other two. **Conclusion:** From this study, it was concluded that apexit does not show any antimicrobial activity against both *enterococcus* and *lactobacillus* but RC seal have potential antibacterial activity against *enterococcus* and *lactobacillus* species.

KEY WORDS: Apexit, RC seal, Enterococcus, Lactobacillus, innovative technique

INTRODUCTION:

Root canal sealer is usually a calcium hydroxide based sealer(1). These sealers along with the use of gutta percha cones are one of the most reliable methods for filling the root canal space. The major function of sealers apart from holding gutta percha points together should be to exhibit antimicrobial properties(1,2). Endodontic sealers with antimicrobial properties can



be beneficial to decrease or eliminate microorganisms from the root canal space and thereby provide a better root canal therapy. Root canal sealer is used along with Gutta Percha for obturation of root canals(3). Some root canal sealers can be complete sealers where no Gutta Percha is necessary. Endodontic sealers have varying base compositions, some may be Calcium Hydroxide, epoxide-amine resins, Barium Sulfate, Bismuth Oxychloride and Zinc Oxide. Endodontic sealers can be categorized as containing Eugenol or not containing Eugenol(3,4). The component Zinc Oxide Eugenol can be placed in the root canal cavity temporarily to reduce inflammation and sensitivity. Application of endodontic sealers can be via syringe, hand mix and premeasured capsules(5)

Enterococcus faecalis is Gram-positive bacterium that can mostly resist endodontic therapy and has been frequently found in root canal-treated teeth with signs of chronic apical periodontitis. When lodged in the dentinal tubules of the canal, it is difficult to remove these species through root canal medicaments(6). Antimicrobial agents are incorporated to root canal sealers to enhance their antibacterial efficacy. The root canal sealers should be tissue compatible, provide an airtight seal, and possess antimicrobial effect. The antimicrobial activity of sealers may prevent persistent residual infection and microorganisms from re-entering through the oral cavity, thereby increasing the chances of a successful endodontic treatment outcome(6,7).

Lactobacillus is one of a number of probiotics considered to be biological therapeutics and host immune-modulating biologicals that are generally recognized as safe (GRAS). *Lactobacillus* can produce lactic acid, acetic acid, formic acid and other acids to reduce intestinal pH, which may be the most important mechanism.(8)

In this in vitro study the antimicrobial activity of RC sealer on enterococcus and lactobacillus species is measured based on zone of inhibition in the time interval of 12, 24 and 48hrs. The aim of the study is to evaluate the antimicrobial activity of apexit and RC seal against enterococcus and lactobacillus using zinc oxide eugenol as control

MATERIALS AND METHOD:

In this study, Antimicrobial viability of two root waterway sealers was tested against enterococcus and lactobacillus, the two stronger bacteria seen in the root canal.

Standard strains of enterococcus and lactobacillus are grown on enriched media and incubated for 12 hours. These fresh cultures were used to make a suspension in saline with turbidity matching McFarland standard. 50 microliter of the suspension was pipetted and placed on surface of mueller hinton agar and uniformly spread using a sterile swab. Then using a sterile metal tube of 6 mm diameter 3 wells were cut on the media. The wells are filled with the sealers, the sealers used were RC seal, apexit and zinc oxide eugenol. The plates were taken and in each sealer was placed and the plates were incubated aerobically at 37 degree celsius overnight. After incubation the zone of inhibition was measured in millimeters and tabulated.

RESULTS :

TABLE 1: shows the zone of inhibition of the sealants against *Enterococcus faecalis*

ENTEROCOCCUS	PLATE 1	PLATE 2	PLATE 3	Mean value
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APEXIT	0mm	0mm	0mm	0
RC SEAL	17mm	15mm	14mm	15.3
ZINC OXIDE EUGENOL	8mm	8.2 mm	8 mm	8

TABLE 2: shows the zone of inhibition of the sealants against lactobacillus

LACTOBACILLUS	SAMPLE 1	SAMPLE 2	SAMPLE 3	Mean value
APEXIT	0mm	0mm	0mm	0
RC SEAL	14mm	13mm	13mm	13.3
ZINC OXIDE EUGENOL	15mm	15.2 mm	15 mm	15

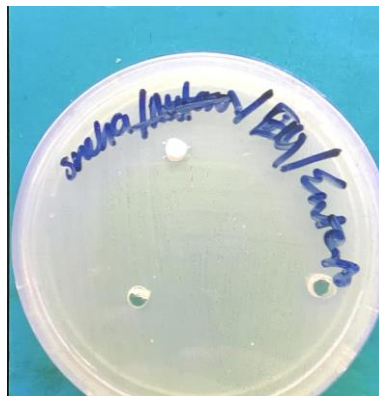


Figure 1: Zone of inhibition of zinc oxide eugenol against enterococcus



Figure 2: Zone of inhibition of apexit against *lactobacillus*

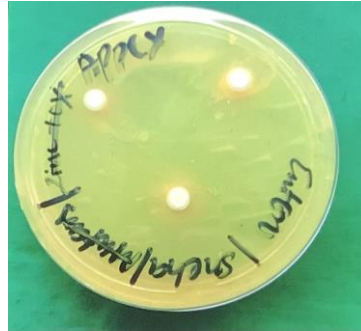


Figure 3: Zone of inhibition of apexit against *enterococcus*



Figure 4: Zone of inhibition of zinc oxide eugenol against *lactobacillus*



Figure 5: Zone of inhibition of Rc seal eugenol against *lactobacillus*



Figure 6: Zone of inhibition of RC seal eugenol against *enterococcus*



DISCUSSION:

In this study which compared the Antimicrobial activity of three sealants against *enterococcus* and *lactobacillus*, it was found that rc seal shows significantly higher Antimicrobial activity compared with the other two. Estrela et al hypothesized that the antimicrobial mechanism in calcium hydroxide-based sealers is influenced by its speed of dissociation into calcium ions and hydroxyl ions. This dissociated hydroxyl ion creates a high pH environment, which inhibits enzymatic activities which are essential for microbial metabolism, growth, and cellular division.

Root canal sealers facilitate by minimizing outflow, give antimicrobial activity by reducing the chance of residual bacterium, and resolve periapical lesion. Enterococci are shown to survive in root canals as single organisms. It is difficult to take away microorganisms from the root canal system even after debridement, shaping and irrigation of the root canals with antimicrobial agents. Therefore, the utilization of root filling materials with antimicrobial activity may facilitate this goal.(9)

Although aerobic and facultative microorganisms sometimes represent a minor proportion of primary dentistry infections, they're frequently found in cases with prolonged treatment, in flare-ups, and in Endodontic failures. Within the Present study, Enterococcus and Lactobacillus was used because it is the most commonly used microorganisms in various in vitro studies relevant to persistent periapical infections. It is the foremost drug-resistant bacterium that has the ability to survive up to twelve months within the Root canal even under nutrient-deficient conditions.(10)

The agar diffusion Test utilized in this study is one amongst the most Commonly used methods to see the antimicrobial effectivity of varied Endodontic sealers. This methodology permits direct comparison of the root canal sealers against the microorganisms to be tested and therefore the visual indication of that sealer has the potential to eradicate microorganisms within the native microenvironment of the root canal system(11,12).The main disadvantage of agar diffusion test is that it cannot differentiate between bactericidal and bacteriostatic effect of the material, and results of this are dependent with the antimicrobial activity of the test material for a particular microorganisms and is highly influenced by the diffusibility of the material across the medium. Therefore, absolutely the antimicrobial effectivity of the sealer is not determined by the area of the inhibition zones.(11)

The probable reasons for the isolation of Enterococcus faecalis in failed root canal treated teeth may be due to (a) a small amount of enteric bacteria is already present in the infected canal at the beginning of the therapy and their relative proportion increases during the treatment as other bacteria are susceptible to therapy or (b) enteric bacteria enter the root canal during the treatment because of (i) inadequate isolation of the working area, (ii) a leaking temporary filling or (iii) the root canal has been left open for drainage (13). Hence the ideal objective of the root canal treatment is not only the elimination of infection but also preventing reinfection of the treated root canal system.

A sealer with an antimicrobial activity can be considered advantageous, in order to eliminate the remaining microbes present in the root canal after chemomechanical preparation of the root canal system and to prevent reinfection.(14)



Our team has extensive knowledge and research experience that has translated into high quality publications(16–27),(28–32).

CONCLUSION:

Based on the results the accompanying end could be drawn:Resin based sealer delivered the biggest inhibitory zones in both enterococcus and lactobacillus species when compared to apexit using zinc oxide eugenol as control.The present study concluded that resin based sealers have good potential against both the microorganisms. Therefore Rc sealer is a better choice for use in RCT.

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REFERENCE

1. Rahman H, Chandra R, Chowdhary D, Singh S, Tripathi S, Anwar SZ. Antimicrobial Activity of MTA Fillapex, Real Seal SE, Acroseal and Zinc oxide Eugenol Sealers against Enterococcus Faecalis and Candida Albicans [Internet]. Vol. 16, IOSR Journal of Dental and Medical Sciences. 2017. p. 66–9. Available from: <http://dx.doi.org/10.9790/0853-1601046669>
2. Haghgoo R, Ahmadvand M, Nyakan M, Jafari M. Antimicrobial Efficacy of Mixtures of Nanosilver and Zinc Oxide Eugenol against Enterococcus faecalis [Internet]. Vol. 18, The Journal of Contemporary Dental Practice. 2017. p. 177–81. Available from: <http://dx.doi.org/10.5005/jp-journals-10024-2012>
3. S H, Hanumitha S, Mahalakshmi V, Abirami S, Madras Christian College, Chennai, et al. Biosynthesis of Zinc Oxide Nanoparticles Using Bacillus Species Potentiates Anticancer and Antimicrobial Activity [Internet]. Vol. -2, International Journal of Trend in Scientific Research and Development. 2018. p. 1796–802. Available from: <http://dx.doi.org/10.31142/ijtsrd17137>
4. Chandak M, Thosar N, Basak S. Evaluation of Antimicrobial Activity of Two Endodontic Sealers: Zinc Oxide with Thyme Oil and Zinc Oxide Eugenol against Root Canal Microorganisms—An in vitro Study [Internet]. Vol. 11, International Journal of Clinical Pediatric Dentistry. 2018. p. 79–82. Available from: <http://dx.doi.org/10.5005/jp-journals-10005-1489>
5. Rajamanickam K, Yang J, Sakharkar MK. A Novel Antimicrobial–Phytochemical Conjugate With Antimicrobial Activity Against Streptococcus uberis, Enterococcus faecium, and Enterococcus faecalis [Internet]. Vol. 10, Frontiers in Pharmacology. 2019. Available from: <http://dx.doi.org/10.3389/fphar.2019.01405>



6. Ameliana Y, Herawati H, Pradopo S. Daya antibakteri penambahan Propolis pada zinc oxide eugenol dan zinc oxide terhadap kuman campur gigi molar sulung non vital (The antibacterial effect of propolis additional to zinc oxide eugenol and zinc oxide on polybacteria of necrotic primary molar) [Internet]. Vol. 47, Dental Journal (Majalah Kedokteran Gigi). 2014. p. 198. Available from: <http://dx.doi.org/10.20473/j.djmk.v47.i4.p198-201>
7. Dragland IS, Wellendorf H, Kopperud H, Stenhagen I, Valen H. Investigation on the antimicrobial activity of chitosan-modified zinc oxide-eugenol cement [Internet]. Vol. 6, Biomaterial Investigations in Dentistry. 2019. p. 99–106. Available from: <http://dx.doi.org/10.1080/26415275.2019.1697621>
8. Teixeira PCM. LACTOBACILLUS | Lactobacillus Brevis [Internet]. Encyclopedia of Food Microbiology. 1999. p. 1144–51. Available from: <http://dx.doi.org/10.1006/rwfm.1999.0900>
9. 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 [Internet]. Vol. 8, Journal of International Society of Preventive and Community Dentistry. 2018. p. 104. Available from: http://dx.doi.org/10.4103/jispcd.jispcd_29_18
10. Kour S, Chaudhury T, Pradeep PR. Comparative Evaluation of Antimicrobial Efficacy of Oxum Solution and Other Endodontic Irrigants Against Enterococcus Faecalis - An In vitro Study [Internet]. Vol. 4, Acta Scientific Dental Sciencs. 2020. p. 02–8. Available from: <http://dx.doi.org/10.31080/asds.2020.04.0801>
11. Varada SL, Veetil JE, Nair GC. Antimicrobial Efficacy of Simarouba glauca (Lakshmi Taru) Plant Extract against Enterococcus faecalis Biofilm: An in vitro Study [Internet]. Vol. 2, Conservative Dentistry and Endodontic Journal. 2017. p. 43–7. Available from: <http://dx.doi.org/10.5005/jp-journals-10048-0025>
12. Arora DR, Arora R, Department of Pedodontics and Preventive Dentistry / RUHS. INDIA, 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.” [Internet]. Vol. 13, IOSR Journal of Dental and Medical Sciences. 2014. p. 90–3. Available from: <http://dx.doi.org/10.9790/0853-13349093>
13. Chakraborty T, Taneja S. An assessment of Antimicrobial Activity of Three Endodontic Sealers on Enterococcus faecalis, Candida albicans and Staphylococcus aureus by a Direct Contact Test: An In Vitro Study [Internet]. Available from: <http://dx.doi.org/10.21203/rs.2.16983/v2>
14. Shakouie S. Antimicrobial efficacy of AH-Plus, adseal and endofill against Enterococcus faecalis- An in vitro study [Internet]. Vol. 6, African Journal of Microbiology Research. 2012. Available from: <http://dx.doi.org/10.5897/ajmr-11-1054>
15. Kumar PS, Santosh Kumar P, Vidhya S, Mahalaxmi S. Antimicrobial Efficacy of Various Concentrations of Bamboo Salt against Enterococcus faecalis and Candida albicans: An in vitro Study [Internet]. Vol. 2, Journal of Operative Dentistry & Endodontics. 2017. p. 65–8. Available from: <http://dx.doi.org/10.5005/jp-journals-10047-0039>
16. Priyadharsini JV, Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. In silico analysis of virulence genes in an emerging dental pathogen A. baumannii and related



- species [Internet]. Vol. 94, Archives of Oral Biology. 2018. p. 93–8. Available from: <http://dx.doi.org/10.1016/j.archoralbio.2018.07.001>
17. Vijayashree Priyadharsini J. In silico validation of the non-antibiotic drugs acetaminophen and ibuprofen as antibacterial agents against red complex pathogens. *J Periodontol*. 2019 Dec;90(12):1441–8.
 18. Paramasivam A, Vijayashree Priyadharsini J, Raghunandhakumar S. N6-adenosine methylation (m6A): a promising new molecular target in hypertension and cardiovascular diseases. *Hypertens Res*. 2020 Feb;43(2):153–4.
 19. Vijayashree Priyadharsini J, Smiline Girija AS, Paramasivam A. An insight into the emergence of *Acinetobacter baumannii* as an oro-dental pathogen and its drug resistance gene profile - An in silico approach. *Heliyon*. 2018 Dec;4(12):e01051.
 20. Paramasivam A, Vijayashree Priyadharsini J. Novel insights into m6A modification in circular RNA and implications for immunity. *Cell Mol Immunol*. 2020 Jun;17(6):668–9.
 21. Paramasivam A, Priyadharsini JV, Raghunandhakumar S. Implications of m6A modification in autoimmune disorders. *Cell Mol Immunol*. 2020 May;17(5):550–1.
 22. Girija ASS, Shankar EM, Larsson M. Could SARS-CoV-2-Induced Hyperinflammation Magnify the Severity of Coronavirus Disease (CoViD-19) Leading to Acute Respiratory Distress Syndrome? *Front Immunol*. 2020 May 27;11:1206.
 23. Jayaseelan VP, Arumugam P. Exosomal microRNAs as a promising theragnostic tool for essential hypertension. *Hypertens Res*. 2020 Jan;43(1):74–5.
 24. Ushanthika T, Smiline Girija AS, Paramasivam A, Priyadharsini JV. An in silico approach towards identification of virulence factors in red complex pathogens targeted by reserpine. *Nat Prod Res*. 2021 Jun;35(11):1893–8.
 25. Ramalingam AK, Selvi SGA, Jayaseelan VP. Targeting prolyl tripeptidyl peptidase from *Porphyromonas gingivalis* with the bioactive compounds from *Rosmarinus officinalis*. *Asian Biomed*. 2019 Oct 1;13(5):197–203.
 26. Kumar SP, Girija ASS, Priyadharsini JV. Targeting NM23-H1-mediated inhibition of tumour metastasis in viral hepatitis with bioactive compounds from *Ganoderma lucidum*: A computational study. *pharmaceutical-sciences* [Internet]. 2020;82(2). Available from: <https://www.ijpsonline.com/articles/targeting-nm23h1-mediated-inhibition-of-tumour-metastasis-in-viral-hepatitis-with-bioactive-compounds-from-ganoderma-lucidum-a-comp-3883.html>
 27. Mathivadani V, Smiline AS, Priyadharsini JV. Targeting Epstein-Barr virus nuclear antigen 1 (EBNA-1) with *Murraya koengii* bio-compounds: An in-silico approach. *Acta Virol*. 2020;64(1):93–9.
 28. Samuel SR, Kuduruthullah S, Khair AMB, Shayeb MA, Elkaseh A, Varma SR. Dental pain, parental SARS-CoV-2 fear and distress on quality of life of 2 to 6 year-old children during COVID-19. *Int J Paediatr Dent*. 2021 May;31(3):436–41.
 29. Samuel SR. Can 5-year-olds sensibly self-report the impact of developmental enamel defects on their quality of life? *Int J Paediatr Dent*. 2021 Mar;31(2):285–6.
 30. Barma MD, Muthupandiyani I, Samuel SR, Amaechi BT. Inhibition of *Streptococcus mutans*, antioxidant property and cytotoxicity of novel nano-zinc oxide varnish. *Arch Oral Biol*. 2021 Jun;126:105132.



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31. Teja KV, Ramesh S. Is a filled lateral canal - A sign of superiority? J Dent Sci. 2020 Dec;15(4):562–3.
 32. Reddy P, Krithikadatta J, Srinivasan V, Raghu S, Velumurugan N. Dental Caries Profile and Associated Risk Factors Among Adolescent School Children in an Urban South-Indian City. Oral Health Prev Dent. 2020 Apr 1;18(1):379–86.