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Bioceramics: Not Just a Seal, But also an Antibacterial Deal

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Abstract: Introduction and aim: The main goal of endodontic therapy is prevention and control of root canal infection. Removing all bacteria in the canal prior to obturation has proven to be difficult even after chemomechanical preparation.Therefore a sealer with adequate antibacterial properties is essential in ensuring the success of the endodontic treatment. The aim of this study is to compare the in vitro antibacterial activity of various bioceramic sealerson *Enterococcus faecalis* after 24 hours and 48 hours.**Materials and Methods:** The antibacterial activity of four sealers (Guttaflow Bioseal, BioRoot RCS, MTA fillapex and AH plus) was evaluated by

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employing the diffusion method on Muller-Hinton agar. A base layer was made using Muller-Hinton agar and wells were made by removing the agar at equidistant points. The sealers were placed into the wells immediately after manipulation according to the manufacturer's instructions. *Enterococcus faecalis* ATCC 29212 was seeded by pour plate. The plates were kept at room temperature for 2 hours for pre-diffusion and then incubated at 37 °C. And the zones of inhibition were measured after 24 hours and 48 hours. **Results:** BioRoot RCS showed maximum antibacterial property followed by MTA fillapex and Guttaflow Bioseal which showed similar inhibitory effects. AH Plus showed the least antibacterial effect.**Conclusion:** Bioceramic sealers showed a greater antibacterial efficacy when compared to resin based sealers. Even though, it is not advisable to depend on the antimicrobial activity of the sealer alone in the treatment of infected root canals, it is safe to say, if a test material consistently induces a strong antibacterial effect in the sensitivity tests, it is very likely also to exert antibacterial action in living tissues.

Key words: Bioceramic sealers, Antibcaterial efficacy, Agar disk diffusion, Guttaflow Bioseal, BioRoot RCS, MTA fillapex, AH plus.

Introduction: The main goal of endodontic therapy is prevention & control of root canal infection. The initial control is set by bio-mechanical preparation. This eliminates the greatest amount of microorganisms and their by-products from the canal.[1] However it is not feasible to completely eliminate all the bacteria. Therefore, it is essential for the root canal filling materials to have some antibaterial properties.

A few bacterial species, predominantly facultative anaerobes, are responsible for causing apical periodontitis observed in root canal failure.[2] These microorganisms might have either leaked into the canal after its obturation or they might be the bacteria that were not eliminated during therapy.

Endodontic sealers have different antibacterial activities against various microorganisms' present inside the diseased pulp. These differences in the antimicrobial activities are attributed to their chemical constituents and the additives incorporated within the sealers. The most desirable constituent would be the one that combines maximum antibacterial effect with minimum toxicity. Therefore, one has to choose the sealer that combines maximum antimicrobial effect with minimal toxic effect.[3,4]

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Aim: The aim of this study is to compare the in vitro antibacterial activity of various bioceramic sealers on *Enterococcus faecalis* after 24 hours and 48 hours.

Materials: The four sealers that were used were GuttaFlow BioSeal, BioRoot RCS, MTA Fillapex and AH Plus (control). Their antibacterial efficacy was checked against *E. faecalis* ATCC 29212 which was incubated in a BHI broth and later seeded on plates containing Muller-Hinton Agar. 20 such samples were included in the study.

Method: The antibacterial efficacy of the 4 endodontic sealers were determined by employing the agar disk diffusion method using Muller-Hinton Agar.

Four to five pure colonies of the bacterial strain were taken by a sterile loop. These colonies were inoculated in 10ml of BHI broth in small screw cap tubes. Incubation of these tubes were done for 24 hours at 37℃. Turbid suspensions were noticed the next day. 5 ml of sterile 0.85% normal saline solution in screw cap tubes were prepared. Bacterial strains were individually inoculated into the tubes and the suspensions were adjusted visually to match the turbidity of a McFarland 0.5 scale. This standardized number contains approximately 1.5×10^8/ml of bacterial cell density.

10 cm diameter plates were prepared with 25 ml of Mueller Hinton Agar media in each plate. A sterile spreader was used to inoculate the microorganisms from the prepared normal saline tubes inoculated with microorganisms that had been fit to 0.5 McFarland standards. With an adjustable micropipette, 0.1 ml of each bacterial suspension was added to the surface of the plates that were inoculated by spreading the suspension in three directions, and a final spreading was done over the outer rim of the plate.

After that, the plates were allowed to dry for 3-5 minutes. Within 15 minutes, after inoculation of the plates, four wells measuring 4 mm in depth and 13 mm in diameter were made in each agar plate. Each was filled completely with the four types of sealers after being mixed according to the manufacturer's instructions. The plates were pre-incubated in culture media at environmental temperature for two hours before incubation to allow dissociation and diffusion of sealers. The plates were incubated at 37℃ for 48 hours in the incubator.[5]The agar plates were examined for bacterial inhibition zones at the next day. With a scientific ruler (with accuracy of 0.5 mm) the diameter of these zones were measured by passing the ruler through the center of the wells.

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Inhibition zones were recorded at 24 and 48 hours for each sealer.

Observation: The zones of inhibition were measured for each sealer, on every plate at the baseline at 0 hours (Fig, 1), after 24 hours (Fig.2) and 48 hours (Fig.3). The diameters were measured using a metallic scale and were tabulated.

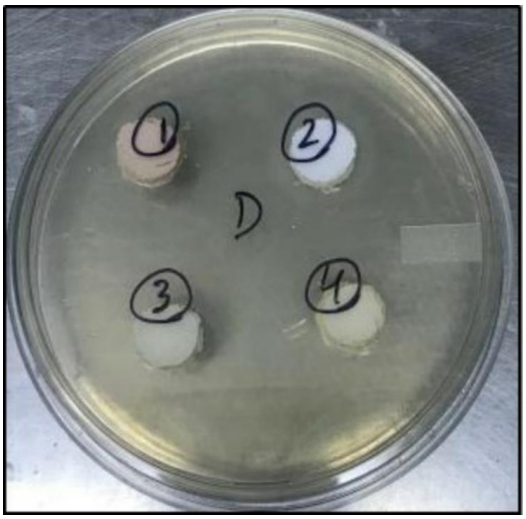

Fig. 1: Agar plates at baseline (zero hours) with 4 sealers; 1=Guttaflow Bioseal 2=BioRoot RCS, 3= MTA Fillapex & 4= AH Plus sealers respectively.

Fig. 2: Agar plates showing zones of inhibition for various sealers after 24 hours; 1=Guttaflow Bioseal 2=BioRoot RCS, 3= MTA Fillapex & 4= AH Plus sealers respectively.

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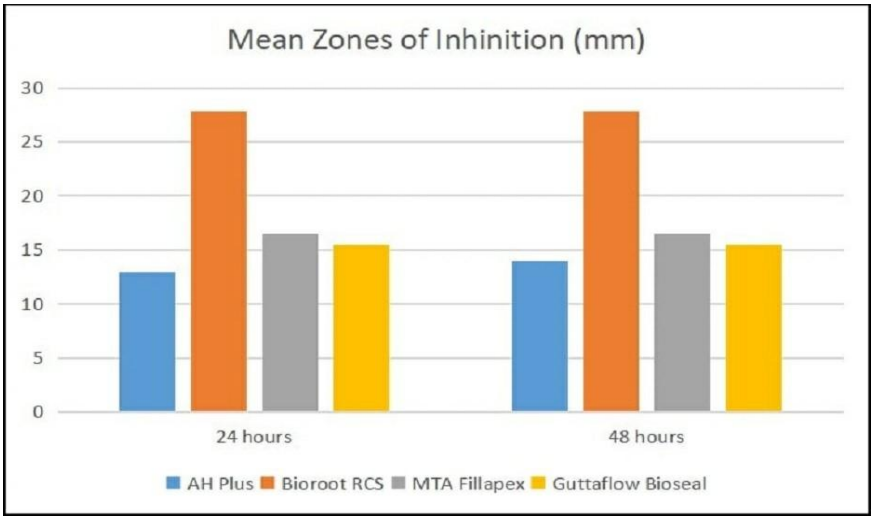
Fig. 3: Agar plates showing zones of inhibition for various sealers after 48 hours; 1=Guttaflow Bioseal 2=BioRoot RCS, 3= MTA Fillapex & 4= AH Plus sealers respectively.

Result: The zones of inhibition were measured for each sealer after 24 and 48 hours (Fig.s 1-3). Their arithmetic means were calculated. After 24 hours, the zone of diffusion for BioRoot RCS had a diameter of 27.83 mm was significantly greater than the remaining sealers. MTA Fillapex and GuttaFlow Bioseal showed similar zones of inhibition, i.e. 16.5 mm and 15.5 mm respectively. The smallest zone of inhibition was seen around AH Plus sealer 13 mm. Only AH plus showed an increase in diameter (14mm) after 48 hours. Remaining sealers showed no change. These results were compared as depicted in as shown in Graph 1.



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Discussion: ADT (agar diffusion test) is the most commonly used method for evaluating antimicrobial activity of dental materials.[6, 7] The results of this method are influenced by the contact between a material and agar, the possibility of material diffusion into agar (depends on the setting time), agar viscosity, incubation, temperature etc.

The main drawback of this method is that it cannot differentiate bactericidal from bacteriostatic effect of a material. Test results are influenced not only by material toxicity, but also by the possibility of dissolving the material in the water component of agar and the diffusion that depends on material solubility and setting time. Highly diffusable material can produce a large growth inhibition zone. Many authors have agreed that this method can be used to compare materials and show which one has the greater antimicrobial effect in the root canal.[8]

The method of measuring antimicrobial activity used here was to determine the size of the zone of bacterial growth inhibition around the specimen. This size of this zone will depend on at least two major factors. The first is the toxicity of the components of the material under study. The second is the diffusibility of any toxic factors released from the specimen. This diffusibility is a function of the hydrophilicity or hydrophobicity of the substances being released and the rate of which these substances are released from the matrix of the specimen under study.[9] However, great care was taken to keep the plates for 2 hours at room temperature to allow the diffusion of the agents through the agar and then incubated at 37℃ under appropriate gaseous condition.[5]

E. faecalis is used as a target microorganism for the determination of result of antibacterial agents on it. *E. faecalis* is more susceptible to infected failed endodontic treated teeth than with a primary infected teeth. There are many reasons for the isolation of *E. faecalis* in failed root canal treated teeth and could be as follows:

i. Enteric bacteria was already present in the infected canal at the initiation of the treatment.
ii. Enteric bacteria was introduced the root canal during the treatment due to:
    a) Inadequate isolation
    b) A leaking due to temporary filling
    c) The root canal has been left open for drainage.[9]

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Therefore, it is important to keep in mind that the objective of the root canal treatment is complete elimination of infection and also prevention of reinfection of the treated root canal system.

The antimicrobial activity of a root canal sealer increases the success rate of endodontic treatments by eliminating residual intraradicular infections that might have survived root canal treatment or have invaded the canal later through microleakage.[10, 11] Bioceramic sealers are newly introduced endodontic sealers. They have an alkaline pH, high calcium ions release, and suitable radiopacity and flow capacity.[11] They also exhibit antibacterial activity and biocompatibility.[12] These sealers are highly hydrophillic which allows them to spread easily over the root canal walls and fill the lateral microcanals too. During setting, these sealers expand and form chemical bond with the canal walls.[13] According to the literature, the key antimicrobial properties of root canal sealers lie in their alkalinity and release of calcium ions [14] which stimulates repair via the deposition of mineralized tissue.[15]

The antimicrobial effect of resin based sealers may be related to bisphenol A diglycidyl ether that was identified as a mutagenic component of the resin based material. In addition, formaldehyde release in the polymerization process may also assist its antimicrobial properties.[15] Formaldehyde is a phenolic compound that has a strong antibacterial activity in vitro.[16, 17] AH-plus sealer, which is resin based showed an antibacterial activity lower than that of other sealers. This lower antibacterial activity could probably be due to its low contents of water-soluble toxic compounds such as formaldehyde and short setting time that may induce milder antibacterial activity.[18, 19]

Conclusion: BioRoot RCS showed maximum antibacterial property followed by MTA fillapex and Guttaflow Bioseal which showed similar inhibitory effects. AH Plus showed the least antibacterial effect.There is probably no absolute way of determining the effectiveness of any sealer via in vitro studies. The results of such antibacterial tests may not highly correlate with in vivo data, however, if a test material consistently induces a strong antibacterial effect in the sensitivity tests, it is very likely also to exert antibacterial action in living tissue. The most desirable endodontic sealer would be one that combines maximal antibacterial effect with minimal toxicity. One has to choose the one that combines a reasonably high antibacterial effect with a low toxic effect.[3] Therefore, according to these results it is advisable to not depend on the antimicrobial activity of the sealer alone in the treatment of infected root canals.

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References:

1. Kobayashi T, Hayashi A, Yoshikawa K, Okuda K, Hara K. The microbial flora from root canals and periodontal pocketsof non vital teeth associated with advanced periodontitis.Int Endod J, 1990; 23: 100- 106.
2. Molander Ak, Reit C, Dahlen G, Kvist T. Microbiological status of root-filled teeth with apial periodontitis. International Endodontic Journals 1998, 31: 1-7.
3. Sangberg L, Langeland K. Biologic effect of dental materials I. Toxicity of root canal sealer filling materials on Hella cells in vitro. Oral surgery 1973, 35: 407.
4. Orstavik D. Antibacterial properties of root canal sealers, cements and pastes. International Endodontic Journals 1981, 14: 125-133.
5. Sipert C. R, Hussne R. P, Nishiyama C. K, Torres S.A. In vitro antimicrobial activity of fill canal, Sealapex, Mineral trioxide aggregate, Protland cement and Endorez. International Endodontics Journals 2005, 38: 539-543.
6. Schmalz G.Agar overlay method. Int Endod J, 1988; 21: 59-66
7. Siquiera J F, Favieri A, Gahyva S M M, Moraes S R, Lima, Lopes HP.Antimicrobial activity and flow rate of newer and established root canal sealers, J of Endod, 2000; 26:5, 274-7
8. Cobankara FK,Altinoz HC,Erganis O,Kav K,Belli S. In vitro antibacterial activities of root canal sealers by using two different methods, J of Endod, 2004; 30:1, 57-60.
9. Barry AL-Thorusberry C. Susceptibility test procedures: diffusion test procedure. American Society for Microbiology 1980, 46: 463-79.
10. G. S. P. Cheung, “Endodontic failures—changing the approach,” International Dental Journal, vol. 46, no. 3, pp. 131–138, 1996.
11. U. Sjogren, D. Figdor, S. Persson, and G. Sundqvist, “Influence of ¨ infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis,” International Endodontic Journal, vol. 30, no. 5, pp. 297–306, 1997.
12. Cohen BI, Pagnillo MK, Musikant BL, Deutsch AS. An in vitro study of the cytotoxicity of two root canal sealers. Journals of Endodontics 2000, 26(9):228.
13. Wayman B.F. A bacteriological and histological evaluation of 58 periradcular lesions. Journals of Endodontics 1992, 18: 152.

14. Spangberg LSW, Acierno TG, Cha Y. Influence of enterappted air on the accuracy of leakage studies using dye penetration methods J of Endod, 1989; 15: 548-551
15. S. Desai and N. Chandler, “Calcium hydroxide-based root canalsealers: a review,” Journal of Endodontics, vol. 35, no. 4, pp. 475–480, 2009.
16. T. Okabe, M. Sakamoto, H. Takeuchi, and K. Matsushima, “Effects of pH on mineralization ability of human dental pulp cells,” Journal of Endodontics, vol. 32, no. 3, pp. 198–201, 2006.
17. Siqueira JF Jr, Favieri A, Gahyva SM, Moraes SR, Lima KC, Lopes HP. Antimicrobial activity and flow rate of newer and established root canal sealers. J Endod 2000;26:274-7
18. Ohara PK, Torabinejad M, Kettering JD. Antibacterial effects of various endodontic irrigants on selective anaerobic bacteria. Endodontic Dental Traumatology. 1993, 9: 95-100.
19. Versiani MA, Carvalho-Junior JR, Padilha MI, Lacey S, Pascon EA, Sousa-neto MD. A comparative study of physic chemical properties of AH-plus and Epiphany root canal sealant. International Endodontics Journals 2006, 39: 464-71.