ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 10, pp.74685-74689, October, 2018

DOI: https://doi.org/10.24941/ijcr.32753.10.2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

IN VITRO EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF FOUR ENDODONTIC SEALERS ON THREE BACTERIAL SPECIES

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ARTICLE INFO

Article History:
Received 24th July, 2018
Received in revised form
15th August, 2018
Accepted 29th September, 2018
Published online 31st October, 2018

Key Words:

Sealers, Endodontic, Antimicrobial Action, Microorganism, Committee.

Glossary of Abbreviations (3rd Page)
(In alphabetical order)

BHI: Brain heart Infusion
C m: Centimeter
ml: Milliliter
mm: Millimeter
MTA: Mineral trioxide aggregator
pH: Power of hydrogen

ABSTRACT

Aim: The aim of this in vitro study was to evaluate the antimicrobial effectiveness of four endodontic sealers (AH Plus, Enseal, MTA fillapex and Sealapex) after 48 hours. **Materials and Method:** The freshly mixed sealers used were AH Plus, Enseal, MTA fillapex and Sealapex. The sealers were prepared according to manufacturer's instruction and placed in prepared wells of 36 agar plates which were inoculated with Streptococcus viridans, Staphylococcus aureus and Enterococcus faecalis. (10 samples were made for each Microorganisms). Four cavities, each one measuring 5 ml in diameter and 4 ml in depth were made in each agar plate using cork poorer. Agar diffusion method on Muller Hinton agar was employed and zones of inhibition were measured after 2 day. **Results:** Sealapex proved to be the most effective against all microorganisms tested. This was followed by Enseal. AH Plus showed antibacterial activity on all tested microorganisms slightly higher than that of MTA fillapex which showed the least action on all tested microorganisms. **Conclusion:** All the sealers evaluated in this study showed different inhibitory effect

Citation: Dr. Priya Horatti, Dr. Tanvi Dugge and Dr. Balaram Naik, 2018. "In Vitro Evaluation of the Antimicrobial Activity of Four Endodontic Sealers on Three Bacterial Species.", *International Journal of Current Research*, 10, (10), 74685-74689.

INTRODUCTION

The main aim of endodontic therapy is to prevent and control the root canal infections. This initial control is set by biomechanical preparation. It is believed that biomechanical preparation of a root canal, irrigation, intracanal medication eliminate the greatest amount of microorganisms and their by-products from the canal (Kobayashi et al., 1990). However, the bacteria's present inside the root canal system have shown a significant impact on this success rate. When a tooth gets infected prior to treatment, the success of root canal treatment drops to 86%, which is a true compromise from the 96% success rate of root canal treated teeth without apical periodontitis (Friedmans et al., 2003). A few bacterial species, especially the facultative anaerobes are responsible for causing apical periodontitis seen in root canal failure (Molander, 1998).

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These microorganisms that have leaked into the canal after its obturation or from bacteria that are not eliminated during therapy (Siqueira, 2001) since removing all bacteria in the canal prior to obturation has proved to be difficult even after chemo mechanical preparation (Bystram et al., 1981). The proliferation and growth of remaining intra-canal microorganisms may possibly destroy the periapical tissues resulting in periapical pathosis. Furthermore, if the access cavity is not adequately sealed, bacteria may penetrate into an obturated root canal within few days; persisting or reinfecting bacteria may induce apical periodontitis. Therefore, it is said endodontic filling materials should be antibacterial/antimicrobial. Adding anti-microbial agents to root canal sealers is a method that can add antimicrobial properties to the sealers (Yazdan Shantiaee1 et al., 1973). Endodontic treatment can be assisted by clarification of the pathogenic bacteria present inside the infected pulp, to these endodontic sealers that have different antibacterial activities against various microorganisms which are present inside diseased pulp. These differences in antimicrobial activities are attributed to their chemical constituents and additives

incorporated within the sealers. The most desirable chemical would be the one that combines maximum antibacterial effect withminimum toxicity. Therefore, one has to choose the one that combines antimicrobial effect with low toxic effect (Sangberg et al., 1973; Orstavik, 1981).

MATERIALS AND METHODS

Three standard bacterial strains obtained from the clinical laboratories of SDM College of Medical Sciences And Hospital, Dharwad were used in this study which were Streptococcus viridans, Staphylococcus aureus and Enterococcus faecalis (Figure 1). 30 samples were used in this study which were divided into 3 groups consisting of 10 plates for each group. 10 plates were inoculated with *Streptococcus viridans* containing 4 types of sealers as group 1. 10 plates inoculated with *Staphylococcus aureus* containing 4 types of sealers as group 2 and remaining 10 plates inoculated with *Enterococcusfaecalis again* containing 4 types of sealers as group 3. 3 plates were inoculated with 4 types of sealers without any bacteria as a negative control group and 3 plates with inoculums without any sealer as a positive control group. The tests for the three types of bacteria (Streptococcus viridian, Staphylococcus aureus and Enterococcus faecalis) were done using Agar Diffusion Method. Four sealers used in this study were Enseal (Figure 2), MTA Fillapex (Figure 3), AH – plus (Figure 4) and Sealapex (Figure 5).

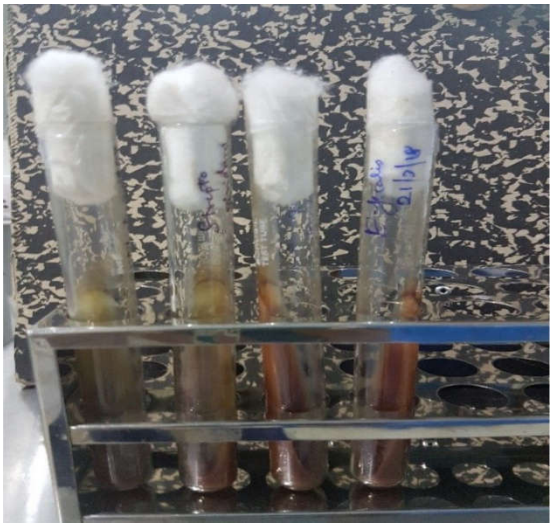

Figure 1. Streptococcus viridans, Staphylococcus aureus and Enterococcus faecalis

Figure 2. Enseal Sealer

Figure 3. MTA Fillapex Sealer

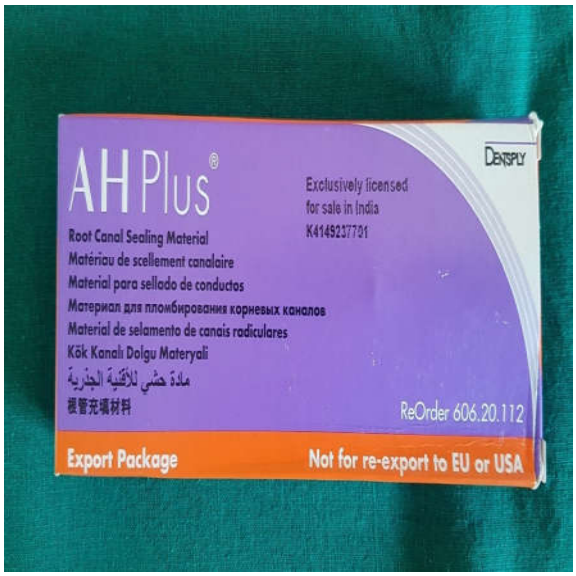

Figure 4. AH Plus Sealer

Figure 5. Sealapex Sealer

Four to five pure colonies of each bacterial strain were taken by a sterile loop. These colonies were inoculated in 10ml of BHI broth (Figure 6) in a small screw cap tubes. Incubation of these tubes were done for 24 hour at 37 ºC. Turbid suspensions were noticed at the next day. 5 ml of a sterile 0.85% normal saline solution in screw cap tubes were prepared. Bacterial strains were individually inoculated into the tubes and the suspension were adjusted visually to match the turbidity of a McFarland 0.5 scale. This number of standard contains approximately 1.5 × 108/ml of bacterial cell density. A 9 cm diameter plates with 25 ml of Mueller Hinton Agar media in each were prepared. 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 plate's four wells measuring 4 mm in depth and 5 mm in diameter were made in each agar plate using cork poorer. Each was filled completely with the four types of sealers after being mixed according to the manufacturer's instructions. The plates were preincubated in culture media at environmental temperature for two hours before incubation to allow dissociation and diffusion of sealers. The plates were incubated at 37 °C for 24 hours in the incubator (9). The agar plates were examined for bacterial inhibition zones at the next to next day. With a digital zone reader the diameter of these zones were measured. Inhibition zones were recorded at 48 and 96 hours for each sealers for each bacterial strain.

RESULTS

Effect of four sealers on Streptococcus viridans, Staphylococcus aureus and Enterococcus faecalis: From Figure (8,9,10), it is clear that Sealapex exhibited the highest mean of inhibition zone followed by Enseal .The least mean value of the antibacterial action was shown by MTA fillapex followed by AH Plus.

DISCUSSION

ADT (agar diffusion test) is the most commonly used method for evaluating antimicrobial activity of dental materials (Schmalz, 1988; Siquiera et al., 2000)**.** 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 diffusible material can produce a large growth inhibition zone (Cobankara et al., 2004). The method of measuring antimicrobial activity used here was to determine the size of the zone of bacterial growth inhibition around the specimen. The 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.

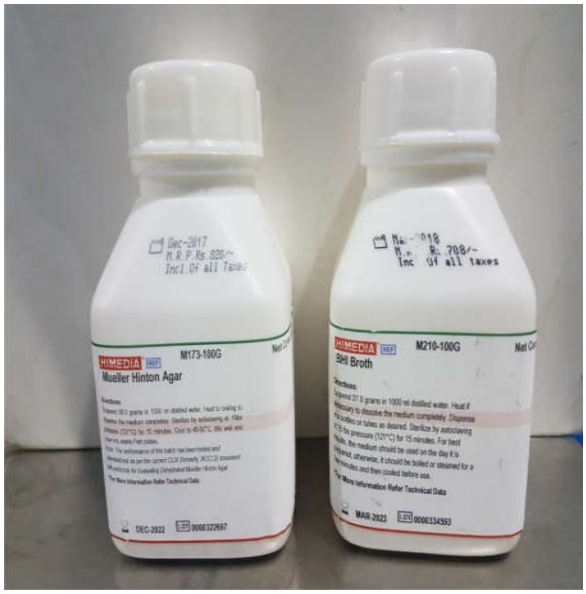

Figure 6. Muellar Hinton agar and BHI broth

Figure 7. Agar diffusion method of Endodontic sealer on *Streptococcus viridans*on Mueller Hinton Agar media

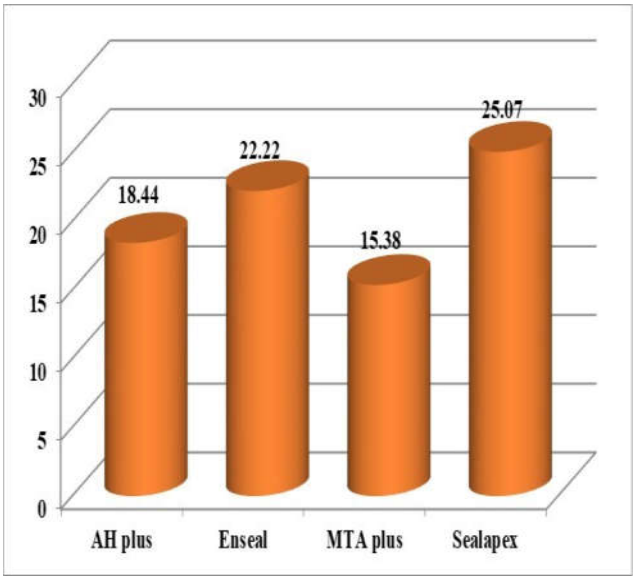

Figure 8. Comparison between the mean of inhibition zones of endodontic sealers produced against Streptococcus viridans after 24 hours

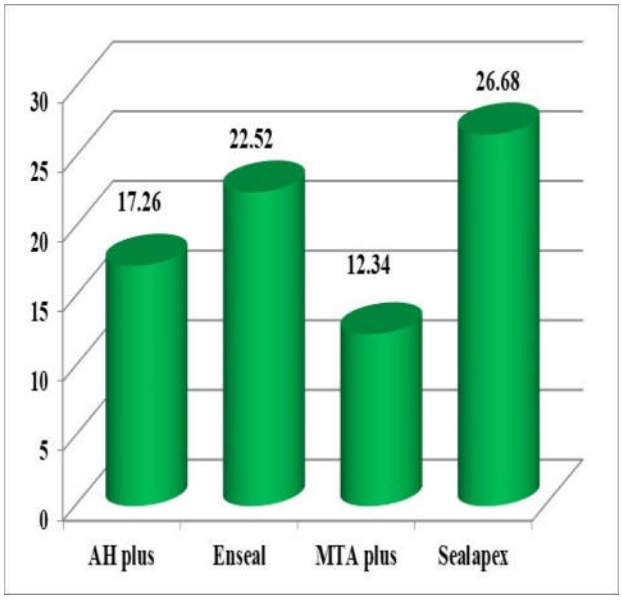

Figure 9. Comparison between the mean of inhibition zones of endodontic sealers produced against Staphylococcus aureus after 24 hours

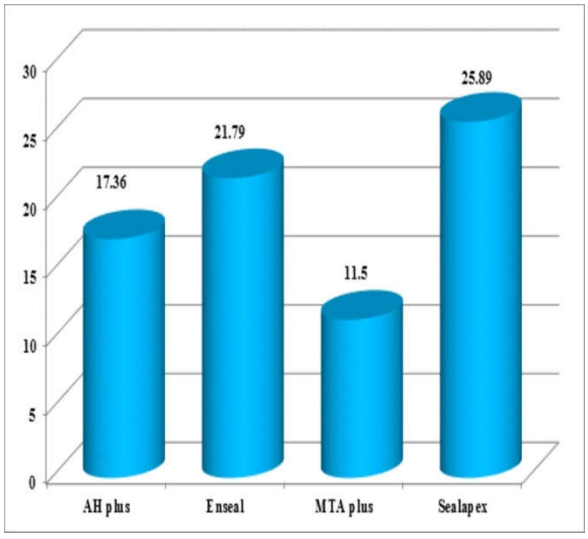

Figure 10. Comparison between the mean of zones of endodontic sealers produced against Streptococus faecalis after 24 hours

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 (Barry, 1980). However, great care was taken to keep the plates for 2 hours at room temperature so that the diffusion of the agents could take place through the agar and then it was incubated at 37°c under appropriate gaseous condition (Sipert, 2005)**.** Sealapex endodontic sealer had thehighest mean value among the others in inhibiting *Streptococcus viridans* growth and Enterococcus faecalis growth. The bactericidal effect of this sealer can be attributed to the antibacterial component in epoxy resin. This property helps to determine the higher penetrability and spreading of this endodontic sealer (Wayman, 1992; Spangberg, 1989). The antimicrobial effect of this resin based sealers can be related to bisphenol Adiglycidyl ether that was identified as a mutagenic component of the resin based material. In addition, formaldehyde release in thepolymerization process may also assist its antimicrobial properties (Siqueira et al., 2000). Formaldehyde is a phenolic compound that has a strong antibacterial activity in vitro (Ohara, 1993).

AH plus was more effective in inhibiting the growth of Staphylococcus aureus than Streptococcus viridans since Streptococcus viridans is less sensitive to formaldehyde than Staphylococcusaureus(18). MTA fillapex sealer is a new calcium silicate based root canal sealer containing MTA, salicylate resin, bismuth oxide and silica. MTA fillapex sealer came in the last stage in inhibition of bacterial growth for Streptococcus viridans, Staphylococcus aureus and Enterococcus faecalis. A possible explanation for the high antibacterial activity of this sealer could be the chemical reaction that takes place during setting which results in the formation of calcium hydroxide which subsequently dissociates into calcium and hydroxyl ions which increase the pH of the area (Holland, 2001).The reason for the difference between MTA fillapex pH and sealapex pH may be related to differences in the percentage of extractable calcium hydroxide in the content of sealers or to the intrinsic properties of these which may lead to different chemical reactions interfering in hydroxyl and calcium ions release in their solubility (Zandbiglari, 2000) It has been shown that set sealapex has a poorly formed matrix and this porous material permits ingress of water over time, promoting continued reaction between calcium powder and binder which could explain its greater release of hydroxyl ions. It is known that there is similarity in the chemical composition of sealapex and MTA fillapex Both the materials contain salicylate resin, bismuth trioxide and silica (Von Fraunhofer, 2003). MTA fillapex and sealapex showed greater pH and calcium release compared to AH plus. Recent study also showed that MTA fillapex has alkaline pH (Keine, 2012). Also, there was an extensive calcium release from AH plus and Sealapex than MTA Fillapex (in accordance to our study) that has been shown to favor a more alkaline pH of the environment. This high calcium release by Sealapex sealer could beanother reason to explain the high level of antibacterial activity (Shipper et al., 2005). AH26 and AH-Plus are basically the same material. The difference between them lies in the presence of silicone and aerosol in the formula as well as the elimination of formaldehyde release from the latter material (Shipper et al., 2005). AH-plus which is a new resin based sealers showed an antibacterial activity lower than that of other sealers against all bacteria's present. This lower antibacterial activity could most 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 (Azar, 2000). On the other hand, it could be due to minute amount of formaldehyde from the sealer or by the release of the amine and epoxy resin components of the sealer (Cohen, 1998) since AH-plus sealer is based on polymerization reaction of epoxy resin amines (Cohen, 2000). 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, its' safe to say that, 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. Therefore, one has to choose the one that combines a reasonably high antibacterial effect with a low toxic effect (Sangberg et al., 1973).

Conclusion

On the basis of the results, observations, and statistical analysis, the following conclusion could be drawn:

All materials showed antimicrobial activity against the tested strains of which, Sealapex showed the highest antimicrobial activity against all microorganisms used in this study. The least antimicrobial activity was showed by both AH plus followed by MTA fillapex. All sealers were distinctly different from each other in their antimicrobial activity depending on the types and bacterial strains, suggesting different physicochemical properties and potentially diverse clinical applications. They showed different inhibitory effects depending on their types and bacterial strains tested. Moreover the effectiveness of sealers decreased gradually with time.

Conflicts of interest: There are no conflicts of interest.

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