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Original Research

Antimicrobial efficacy of six endodontic root canal sealers against Enterococcus faecalis and Candida albicans: A comparative study

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ABSTRACT:
Background: *Enterococcus faecalis* remains one of the primary micro-organisms that has been associated with the failure of root canal treatments. Although few studies have shown the presence of Candida albicans also, within root canal cultures. Though various endodontic protocols have reported good anti-microbial action against *E. faecalis,* there is a need for the development of a good sealer. Also, the complete eradication of micro-organisms from within root canal systems forms an important and integral component of endodontic treatment. **Aim**: The present study aimed to compare six endodontic sealers for their anti-microbial activity against E. fecalis and C. albicans. **Material and methods**: This in vitro study was conducted to test the inhibitory activity of six sealers: AH Plus, Apexit Plus, Sealapex, Mineral Trioxide Aggregate, Endomethasone, and Endoflas FS over these organisms. ATCC strains were used for testing individual sealers for their anti-microbial efficacy. For the study, lawn cultures were prepared in agar plates by inoculating the study organisms. Filter paper discs were placed in the agar plates by punching holes and placing these filter paper discs. The selected sealers were then mixed as per operator instructions and placed onto these filter paper discs. These prepared agar plates were then incubated for 72 hours. Inhibition in the growth of the selected organism was measured by measuring the inhibitory zones using Vernier Callipers. All observations were then recorded and entered into an Excel worksheet. Mean and standard deviation values were calculated and the statistical tool, ANOVA was then applied to elicit statistical significance. A p-value of 0.05 and less was considered statistically significant while a value less than 0.001 was considered extremely significant. **Results**: Sealapex was shown to possess maximum inhibition against E. faecalis and C. albicans as compared to Eugenol-based and epoxy resin sealers. **Conclusion**: Sealapex is the best endodontic sealer as evident by the study findings followed by Eugenol-based and Epoxy resin sealers. MTA demonstrated the least inhibitory activity.
Keywords: E. fecalis, C. albicans, endodontic, sealers, biocompatible, anti-microbial, calcium hydroxide, eugenol, bioceramic, epoxy resin.

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INTRODUCTION

Oral microorganisms and their end-products are the main etiological agents causing infection in pulpal as well as periapical pathologies. [1, 2]

A basic requirement for repairing periapical tissues following root canal therapy is eliminating microbes as well as toxins from within a root canal. [3] The use of proper instrumentation and copious irrigation with the use of endodontic sealers are the various methods for achieving complete eradication of intra-canal infection. Complexities in the endodontic anatomy of a tooth, particularly at an apical two-third portion of any root canal can lead to incomplete removal of microorganisms.

At other times, the persistence of microorganisms and their reproduction in complex anatomical areas might even contribute to complete treatment failure. Improvement in the success rates of endodontic therapy may be achieved using endodontic sealers that exhibit excellent sealing as well as anti-microbial activities. [3]

Endodontic sealers that have anti-microbial functions combat any residual infection that persists and allows bacteria to re-enter the oral cavity. [4] There are a variety of endodontic sealers which have anti-microbial properties. [5]

Root canal sealers and gutta-percha are used together for the complete filling of the gap between dentin walls and gutta-percha cones. [6] Conventionally, root canal or endodontic sealers are classified on basis of chemical composition a) Zinc oxide eugenol-based; b) Epoxy resin-based, and c) Calcium hydroxide based. [7]

Mineral trioxide Aggregate or MTA is a calcium silicate-based sealer or cement which has been formed by the addition of a variety of oxide compounds. [8]

These sealers have been shown to demonstrate variability in their activities against tested microbial organisms. Also, there appears to be intra-species and genera differences with the same sealer used. Different bacteria might have variable sensitivity towards the same sealer, hence, greater than one microorganism must be studied for evaluating their anti-microbial activity. [9] These variety of micro-organisms are constituents of primary endodontic infections, however, they are also responsible for protracted treatment, flare-ups and are also, associated with failure of endodontic treatments. [10, 11]

Both Enterococcus faecalis and Candida albicans have shown resistance to various anti-microbial agents and are also responsible for persisting infection. [12] Both of these microbial organisms are now commonly traced in periodontal as well as endodontic pathologies.

There are numerous endodontic sealers available that have been developed on different chemical formulae- epoxy resin-based sealers, Calcium hydroxide-based sealers, zinc oxide eugenol, Mineral trioxide aggregate, or MTA-based sealers. Sealers based upon Epoxy resin have excellent physical properties and also, have adequate biological properties. Excellent formation of the apical seal has been reported with epoxy resin-based sealers as they have good cytological compatibility, bio-compatibility, good soft tissue tolerance, long-term stability, and good sealing capacity. [13]

Calcium hydroxide-based sealers are extensively used in the field of endodontics as they possess good anti-microbial activity and also, cause stimulation of deposition of dental hard tissue at the apical portion of the root thus, forming a biological seal. [14]

Mineral trioxide aggregate or MTA is an excellent bio-material used in the endodontic area since the early 1990s. [15] MTA is widely accepted for its biocompatibility and excellent sealing capacity. [16,17]

Thus, this study aimed to compare the anti-microbial efficacy of six endodontic root canal sealers viz., AH Plus, Apexit Plus, Sealapex, Mineral Trioxide Aggregate, Endomethasone, and Endoflas FS against Enterococcus faecalis and Candida albicans.

MATERIALS AND METHODS

A total of six endodontic (root canal sealers) were selected for this study. These included- AH Plus, Apexit Plus, Sealapex, Mineral Trioxide Aggregate, Endomethasone, and Endoflas FS.

This study was conducted by utilizing the ‘disc diffusion test for analyzing the susceptibility of Enterococcus feacalis as well as Candida albicans using antibiotics enriched discs that were placed in agar surfaces that were seeded using the lawn technique with. Faecalis and C. albicans.

When these discs which contained a known anti-microbial agent in a particular concentration were placed on a fresh inoculated agar plate, the anti-microbial agent undergoes diffusion and establishes a concentration gradient surrounding this paper disc with the highest drug concentration closest to the disc. On incubating these inoculated plates, bacterial growth occurs except in areas with a high concentration of antibiotics in a graded manner where growth was inhibited. Following a period of incubation, the diameter of the zone of inhibition surrounding each disc was measured in millimeters using Vernier Calipers.

Enterococcus faecalis strain was obtained from ATCC strain 29212. The culture media utilized were Brain Heart Infusion Broth (BHI) and Blood Agar (Himedia Laboratories, Mumbai).

Brain Heart Infusion Broth was used for cultivating and Blood Agar was used as an enriched medium for growing Enterococcus faecalis. Enterococcus faecalis was then sub-cultured in a blood agar plate which was incubated at 37° C in an ambient atmosphere for 24 hours.

Pure isolated E. faecalis colony was then isolated from the same culture plate and was then inoculated into BHI broth which was subsequently incubated at 37° C for 24 hours. Suspension of E. faecalis culture in peptone water was prepared by transference of growth from BHI broth with 0.5 MacFarland $BaSO_4$ standard turbidity.

Each blood agar plate contained 20 ml of sterilized Nutrient Agar (Himedia Laboratories, Mumbai) containing 5 % Sheep blood which had undergone incubation at 37 °C for 24 hours for eliminating any contamination.

Plates were then inoculated with E. faecalis suspension by swabbing them using sterile cotton swabs for preparing Lawn culture.

Ampicillin discs (Himedia Laboratories) were used as a control for testing Enterococcus feacalis. Filter paper discs (Whatman No.1 filter paper) were then standardized at 6 mm diameter. All endodontic sealers were then mixed as per manufacturers' guidelines under an Ultraviolet laminar flow chamber.

After drying the inoculum, six sterile filter paper discs were placed with help of sterile forceps and were then gently pressed. 100 micro-liters of each of the prepared sealer was then placed over sterile paper discs using micropipettes. Ampicillin paper discs were used as controls. These plates which contained these discs were impregnated using sealer along with the control discs and were incubated at 37 degrees for 24 hours.

C. albicans strain (ATCC 10231) was similarly obtained and dilution was performed for obtaining suspension in a sterile Trypticase Soy Broth. Petri-dishes measuring 120 mm diameter which contained a double layer of Mueller-Hinton agar were then prepared. Following this fungal suspension was then inoculated using sterile cotton swabs. As in the method followed for Enterococcus faecalis, six sealer containing filter paper discs were placed in the lawn culture and subsequently, incubated at 37^{o} centigrade for twenty-four hours.

Zones of inhibition were then measured at 24 and 48 hours duration from the edge of the filter paper discs with vernier calipers and observations were then recorded. 'Zone edge' was defined as the 'point of abrupt decrease in growth which corresponded with point of complete growth inhibition.

RESULTS AND OBSERVATIONS

All the root canal sealers i.e., AH Plus, Apexit Plus, Sealapex, Mineral Trioxide Aggregate (MTA), Endomethasone and Endoflas FS tested in this study demonstrated 'zones of Inhibition'. The average diameters of all zones of inhibition were as follows:

Sealapex was reported with the largest zone of inhibition which was followed by AH plus, Apexit Plus, endomethasone, Endoflas FS respectively while MTA recorded the least inhibitory effect on tested micro-organisms. Also, zones of inhibition were found to decrease with time. The highest inhibitory activity was observed at 24 hours while the lowest zone of inhibition was noted at 72 hours. (Table 1)

Table 1: Mean ± Standard deviation in diameters of zones of inhibition

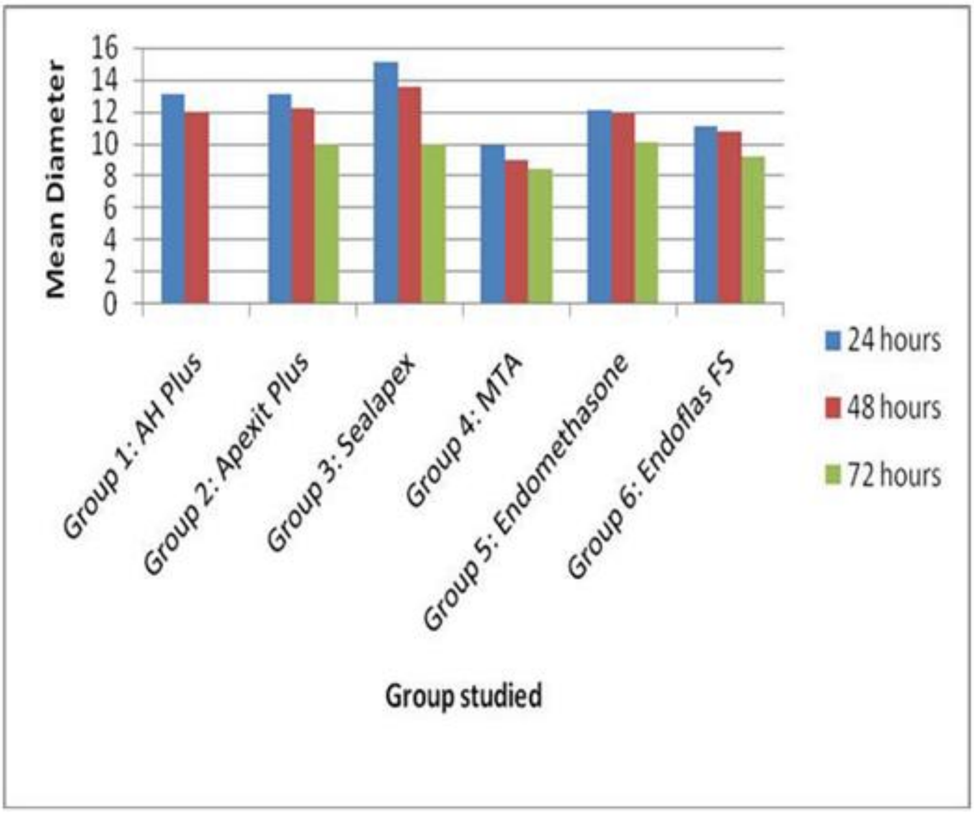
Endodontic sealers	Mean ± SD diameters at different periods of incubation		
	24 hours	**48 hours**	**72 hours**
Group 1: AH Plus	13.12 ± 0.09	12.01 ± 0.03	10.01 ± 0.01
Group 2: Apexit Plus	13.09 ± 0.03	12.19 ± 0.08	10.05 ± 0.06
Group 3: Sealapex	15.11 ± 0.13	13.56 ± 0.02	10.05 ± 0.06
Group 4: MTA	10.01 ± 0.07	09.03 ± 0.02	08.4 ± 0.12
Group 5: Endomethasone	12.08 ± 0.3	11.9 ± 0.12	10.12 ± 0.9
Group 6: Endoflas FS	11.09 ± 0.01	10.8 ± 0.02	9.2 ± 0.1

For statistical evaluation of the anti-microbial activity of endodontic sealers, statistical tests- ANOVA at 95% confidence level was performed. Statistical significance at 24-hour observation was observed between groups 1 and 3; 2 and 3; 1 and 6; 3 and 5; 3 and 6 and 4 and 5. A highly significant statistical difference at 24 hours was noted between groups 1 and 4 (AH Plus and MTA) and Groups 3 and 4 (Sealapex and MTA). (Table 2)

Table 2: Showing *P* values after inter-group comparison between various endodontic sealers used (ANOVA test for statistical analysis)

Intra-group comparison	At 24 hours	At 48 hours	At 72 hours
Group1 and 3	0.00	0.06	0.07
Group 2 and 3	0.00	0.05	1.00
Group 2 and 4	0.05	0.00	0.00
Group 1 and 4	0.001	0.00	0.00
Group 1 and 5	0.06	0.07	1.00
Group 1 and 6	0.05	0.05	0.09
Group 1 and 2	1.00	1.00	1.00
Group 2 and 5	0.08	0.07	1.00
Group 2 and 6	0.06	0.05	0.07
Group 3 and 4	0.001	0.001	0.001
Group 3 and 5	0.05	0.08	1.00
Group 3 and 6	0.04	0.05	0.08
Group 4 and 5	0.03	0.05	0.04
Group 4 and 6	0.06	0.07	0.07

Graph 1: Showing mean diameters with different endodontic sealers



DISCUSSION

A good root canal therapy protocol includes- proper biomechanical preparation of root canals along with good hermetic sealing of root canal using a dimensionally stable, inert as well as a bio-compatible material. [18]

An ideal root canal sealer must fulfill all requirements of having properties of an ideal physic-chemical as well as biological material. Microorganisms and their end-products are chief etiological agents of pulpal diseases. [19] Hence, the primary objective of endodontic treatment is eliminating microbial organisms from within root canal systems and preventing re-infection.

Bacteriological and fungal i.e., mixed microbial infections in root canal systems have a close association with post-endodontic treatment failures. Endodontic or root canal sealers exhibit anti-microbiological activity and are also, expected to possess biological compatibility. Both of these properties are extremely important and are requirements for any successfully performed endodontic or root canal treatment. Conventionally used root canal (endodontic) sealers have been shown to possess anti-bacteriological roles against a plethora of Gram-negative bacteria, for example, P. gingivalis as well as P. endodontalis. Both of these micro-organisms- P. gingivalis along with P. endodontalis are closely linked with the primary source of infection which is existent inside root canal systems. On the other hand, Enterococcus faecalis has been detected mainly in peri-apical and periodontal diseases in teeth that are endodontically treated.

The Agar diffusion testing method is one of the commonly used techniques for assessing the anti-microbial activities of various root canal sealers. [20, 21]

The present study demonstrated that Sealapex reported the largest inhibition zone. Epoxy resin-based sealers (AH plus and Apexit Plus) showed slightly reduced inhibitory activity followed by Zinc oxide Eugenol-based sealers (endomethasone and Endoflas FS) respectively while MTA recorded the least inhibitory effect on tested micro-organisms. These zones of inhibition were demonstrated to decrease with increasing duration time i.e., the greatest inhibition was observed after 24 hours whereas, the lowest inhibition was observed after 72 hours.

In the present study, zinc oxide eugenol-based sealers demonstrated comparative efficacy with calcium hydroxide-based sealers. This finding has been supported by Siquiera et al in their study. [22]

However, in the present study, the Zinc oxide eugenol-based sealers were found to be less effective when compared with Epoxy resin-based sealers which is in contradiction to findings reported by Gomes et al [23] and Shakourie et al. [24]

Abduljabbar along with Abumostafa (2021) in a cross-sectional, experimental, and in vitro study evaluated three endodontic sealers- a) Ceraseal, b) BioRoot RCS (calcium hydroxide-calcium silicate complex-based sealer) and c) Endosequence/BC sealer. In this study, the agar plates were inoculated with the organism, Enterococcus faecalis. Selected sealers were then placed within the agar plates. Then, the agar plates were incubated at 37 degrees temperature under an anaerobic environment for a total duration lasting up to one week. Inhibition zones were then measured at intervals of 24 hrs, 48 hrs, 72 hrs, and on the seventh day. The highest amount of bactericidal activity was shown with BioRoot RCT whereas a minimal amount of zone of inhibition was seen with CeraSeal. A statistically significant difference between all recorded zone with inhibition was observed on the comparison at 24 hours; 48 hours; 72 hours and on the 7th day. [25]

Shin et al in 2017 have shown microbicidal activity of Endoseal sealer against Gram-negative microorganisms (P. gingivalis and P. endodontalis) along with Gram-positive, E. faecalis due to a high concentration of oxide compounds which results in deeper penetrability of calcium hydroxide that leads to DNA and protein denaturation. [26]

Bodrumulu and Seniz (2006) performed testing of different endodontic sealers by agar diffusion technique wherein agar inoculated with Enterococcus faecalis was incubated at 37°centigrade for 72 hours under aerobically maintained laboratory conditions. Zones of inhibition were measured at 24, 48, and 72-hour intervals. Inhibition in the growth was demonstrated with decreasing order of anti-bacterial efficacy for Endomethasone < Sultas < Sealapex < Diaket < Epiphany and AH26. [27]

Sealapex functions by releasing hydroxyl ions in the micro-environment which causes an increase in pH above 12.5. As the final setting of calcium hydroxide-based sealer takes place, a gradual decline in pH up to 9.14 is seen, where it becomes ineffective in activity. [28]

Since endodontic sealers maintain close contact with periapical tissues, the choice of an endodontic sealer must be such that it remains biocompatible with the adjacent tissues for a long period.

In the present study, a bioceramic-based sealer demonstrated maximal antibacterial and antifungal activity when compared to other epoxy resin-based and eugenol-based endodontic sealing materials. However, more representative and large samples must be studied to study the most useful root canal sealing material.

CONCLUSION

Selecting an ideal endodontic sealer is an arduous and important step in maintaining the sterility of a root canal after biomechanical preparation as it helps in combating microbial activity. This contributes largely to a good and successful endodontic treatment. The direct contact method or agar diffusion method is one of the commonly used microbiological techniques that have been tested for evaluating the efficacy of any dental material for its bacteriostatic or bactericidal properties. The present study used the agar diffusion method for evaluating the anti-microbial effect of various selected endodontic sealers. Maximal inhibition in growth was evident at the 24th hour with a gradual decline in microbicidal activity.

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