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Original Research

Comparison of antimicrobial activity of three different root canal sealers: An in vitro study

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ABSTRACT:
Background: The purpose of this study was to evaluate the antimicrobial activities of 3 endodontic sealers (AH Plus, sealpex, and ZOE) against E. fecalis. **Methodology**: The antimicrobial activities of selected endodontic sealers were assessed by direct contact test (DCT) on a total of 300 study samples equally distributed in three groups using spectrophotometer readings. Collected data were analyzed using Two-way analysis of variance and Newman–Keuls *post hoc* tests. **Result**: ZOE was demonstrated to exhibit greater and long-term efficacy while AH Plus showed the best antimicrobial effect. **Conclusion**: Of the studied sealants, AH Plus is best followed by ZOE while Sealapex showed minimal inhibitory activity.
Keywords: AH Plus, Sealapex, ZOE, antimicrobial.

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INTRODUCTION

Bacteriological infections within root canals have been closely linked with post-endodontic failures. Endodontic sealers which exhibit anti-microbiological activity and are also, biologically compatible are essential for successful root canal therapy. Conventional root canal sealers have demonstrated anti-bacterial properties against numerous Gram-negative bacteria, P. gingivalis, and P. endodontalis. Both P. gingivalis as well as P. endodontalis have been related to primary infection within root canals whereas Enterococcus faecalis is detectable in apical periodontal pathologies in endodontically treated teeth. [1]

Sometimes, root canal systems demonstrate anatomical variations such as- isthmus, fins, and lateral or accessory canals while treating a root canal alongside mechanical or manual cleaning, variety of root canal irrigating medicaments such as sodium hydroxide, calcium hydroxide, and chlorhexidine for eradication of bacteria in infected root canals. Hermetic sealing of root canals is a requisite for the entombment of any residual bacteria while destroying them in root canal-treated teeth. Despite this, many bacterial species remain entrapped within root canals. Root canal sealers are used along with gutta-percha cones to fill the gap existing between dentinal walls and gutta-percha used. [2] Traditionally, root canal sealers have been categorized based on their composition into- a) Zinc oxide eugenol; b) Epoxy resin and c) Calcium hydroxide. [3] A calcium silicate-based cement has been formulated by adding various other oxide compounds termed as "Mineral trioxide Aggregate" or MTA has been introduced as an endodontic sealer. [4]

Endodontic sealers contain constituents like- thymol, eugenol, and paraformaldehyde which help in providing anti-microbiological efficacy. [5]

Of the commonest root canal sealers used in dentistry, AH Plus (Dentsply, Germany) is an epoxy-based resinous sealer while MTA Fillipex (Angelus, Brazil) is an MTA-based endodontic sealer and contains silica

nanoparticles, bismuth oxide, synthetic Portland cement, and butyl ethylene glycol di-salicylate. Smart paste Bio (Smart Seal DRFP, England) is a calcium silicate-based sealer material. It is insoluble, injectable, non-resorbable, hydrophilic, and radio-opaque in nature. During the process of polymerization, it causes the release of calcium hydroxide and hydroxyapatite. It is highly biocompatible when it completely sets. [6,7] Sealapex was the first commercially available endodontic sealer based on calcium hydroxide. [8] Toramaru et al in 2004 demonstrated that MTA demonstrated anti-bacterial activity against almost every strain except for P. aeruginosa while Acroseal was found to be effective only against S. aureus and M.luteus. A similar, activity was noted for Sealapex and Endo CPM sealer. [8]

Holland and Souza in 1985 had demonstrated that this sealant possesses excellent bio-compatible properties and induced excellent apical sealing with newly formed mineralized tissue. [9]

The "direct contact test" was introduced by Weiss et al for evaluating the anti-microbial activity of both root canal sealing as well as root-end filling substance. It is both a quantitative as well as reproducible study assay that allows for analyzing various insoluble substances. Additionally, it can be used under standardized laboratory settings. [10]

An ideal root canal or endodontic sealer is biocompatible, dimensionally stable, is capable of sealing adequately, and should have longer-lasting anti-microbicidal activity. [11]

Thus, based upon existing scientific evidence the present study aimed to do in vitro comparative analysis of antimicrobial activities of three different root canal sealers namely, AH Plus, Sealapex, and Zinc Oxide Eugenol against Enterococcus faecalis.

MATERIALS AND METHODS

Present *in vitro* experimental study was conducted by analyzing the antimicrobial efficacy of three endodontic sealers- a) Group I: AH Plus, a resin-based sealer (n=100); b) Group II: Sealapex, a calcium hydroxide-based sealer (n=100) and c) Group III: Zinc oxide eugenol (n=100). All test sealers were prepared as per the manufacturer's instructions.

Test organism, *E. faecalis* was obtained from the Department of Microbiology that was grown under anaerobic conditions in brain–heart infusion broth at a temperature, 37°C. Test inoculum
was prepared by re-suspending washed cells to previously determined optical densities. The suspension was adjusted under a spectrophotometer at 800 nm wavelength. Direct Contact Test was performed for turbidimetric determination of bacterial proliferation in 96 welled micro-titer plates. Cell Kinetics in each of the well was monitored at 630 nm at 37°C and was recorded using a spectrophotometer. (Stat Fax 2100; Awareness Technology).

Out of 96 microtiter plate wells, two sets of four wells were used per sealer: (i) Subgroup 1 (with the sealer) and (ii)Subgroup 2 (without sealer). The wells' surface was kept perpendicular to the well floor and the side walls were coated with freshly prepared sealer using a cavity liner applicator.

At the recommended setting time of sealer, bacterial suspension (108 CFU) was then placed on the test material. BHI broth was then added to each of these wells and gently mixed for 2 minutes.

A small quantity of broth was transferred from Subgroup 1 wells to adjacent Subgroup wells which already were containing fresh BHI medium. Thus, two sets of four wells for each tested material containing an equal volume of liquid medium were prepared. The microtitre plate was then incubated at 37°C following this, it was kept in a microplate reader where OD of each well was measured at 630 nm at regular intervals at-1 hour, on the first, third, fifth, and seventh days.

STATISTICAL ANALYSIS

Data was collected by recording the optical densities (OD) and measuring turbidity by using a spectrophotometer. Obtained data were statistically analyzed using Two-way analysis of variance and Newman–Keuls *post hoc* test.

RESULTS

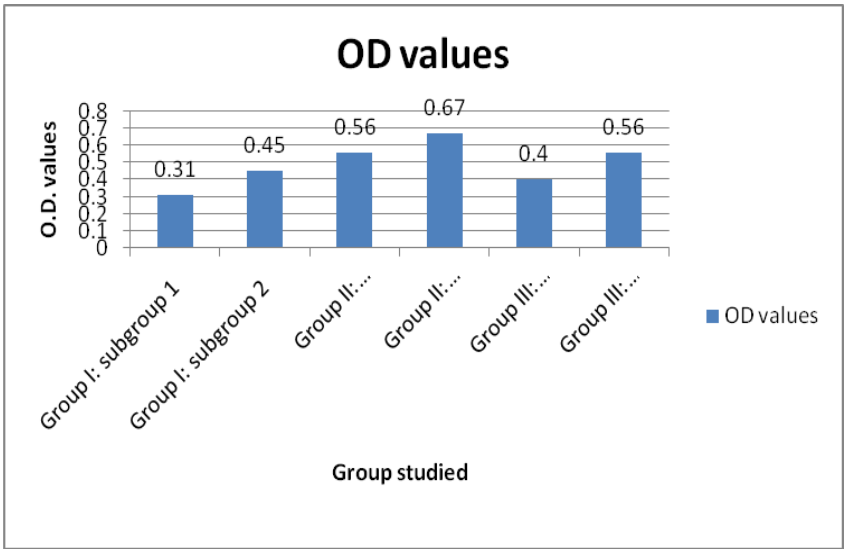
Table 1: Table demonstrating composition, the manufacturer tested root canal sealant materials

Group	Material used	Composition
I	AH Plus	Paste A containing epoxy resins, calcium tungstate, zirconium oxide, silica and iron oxide pigment. Paste B containing amines, calcium tungstate, zirconium oxide, silica, and silicone oil.
II	Sealapex	Polymeric calcium hydroxide root canal sealant
III	Zinc Oxide Eugenol	Powder: zinc oxide, silver Liquid: Eugenol

On inter-group comparisons, Group I (AH Plus) demonstrated the greatest Inhibitory activity of bacterial growth at 1-hour interval which was followed by a decrease in inhibition on the 5th and 7th day while Group II

(Sealapex) showed the least bacterial inhibition at 1-hour interval which further decreased on 1st, 3rd, 5th and 7th days. Group III (ZOE) showed an antibacterial efficacy at 1 hour. On 1st day, it demonstrated the greatest antibacterial activity compared to other groups. A statistically significant difference was obtained in comparing the antimicrobial activity of the sealants used.

Graph 1: Graph demonstrating antibacterial activity on day 1, 3rd, 5th, and 7th day



DISCUSSION

Elimination of micro-organisms from root canal systems is an essential part of endodontic treatment. *E. faecalis* is the most commonly isolated microorganism from refractory periapical periodontitis.

Abduljabbar and Abumostafa in 2021 in their cross-sectional experimental in vitro study compared three endodontic sealers- Ceraseal, BioRoot RCS (calcium hydroxide—calcium silicate complex based sealer), and Endosequence/BC sealer. Agar plates were infused using Enterococcus faecalis in which these sealers were placed. Following this, these agar plates were incubated at a temperature of 37 degrees centigrade under an anaerobically treated environment for 1 week. Incubation zones were measured at the duration of 24 hours, 48 hours, 72 hours, and on the 7th day. The highest antibacterial activity was exhibited by BioRoot RCT while the minimal zone of inhibition was exhibited by CeraSeal with significant statistical difference observed on comparing zone of inhibition at 24 hours in comparison with 48 hours, 72 hours, and 7th-day measurement. [5]

Gholamhoseini et al in 2018 studied the bactericidal effect of MTA-Fillapex, SureSeal, and Endoseal-MTA against S. aureus and Enterococcus faecalis using the agar diffusion technique. Mean diameters of zone of inhibition for S. aureus were observed at 11.56 mm; 11.62 mm; and 13.68 mm for MTA-Fillipex, SureSeal, and Endoseal MTA, respectively. However, for E. faecalis, mean zones of inhibition were observed to be 13.65 mm, and no zone of inhibition was seen for any other sealer tested. A statistically significant P value (P = 0.00) was noted for both the organisms and sealers used. [6]

Alzardy et al in 2018 demonstrated that endodontically sealing material comprised of nanosilver demonstrated statistically significant (P = 0.000) difference when compared to other sealers tested with higher anti-microbial efficacy against E. faecalis using the agar diffusion testing method. [7]

Shin et al (2017) evaluated that Endoseal sealer cement demonstrated anti-microbiological activity against various Gram-negative micro-organisms, P. gingivalis, as well as P. endodontalis in addition to Gram-positive bacterial organism, E. faecalis whereas Endosequence BC sealer, has shown weaker anti-bacteriological activity against the bacterial cell wall. Endoseal in comparison contains higher amounts of oxide compounds thereby, resulting in deeper penetration of calcium hydroxide which causes denaturation of DNA and proteins. [11,12]

Poggio et al in 2017 evaluated different endodontic sealers based on "direct contact test" results after the complete set of cement. It was found that both the TotalFill BC sealer and EasySeal exhibited bactericidal activity against E. faecalis. [13] Endodontic treatment failure can be largely contributed to a variety of intra-radicular and secondary infections. This persistence of pathogenic microorganisms may be attributed to less effective intra-canal irrigation and inadequate biomechanical preparation which may be due to anatomical variations or limitations. [14]

Gjorgveska et al in 2017 in their experimental study compared quaternary ammonium compounds released from root canal sealers that were admixtures of benzalkonium chloride or cetylpyridinium chloride

incorporated at 2 % of total weight. The effectiveness of these compounds was assessed after some time of 1 and 4-week intervals. It was noted that when both benzalkonium chloride and cetylpyridiym chloride were added, a significant reduction in compressive strength of the endodontic sealer used was seen when compared to the use of cetylpyridium chloride, a quaternary ammonium compound, as the sole constituent of endodontic sealer. In contrast, it was observed that there was an increase in compressive strength values due to the enhancement of the curing reaction. Cetylpyridium chloride is a broad-spectrum anti-microbial agent with bactericidal activity on Gram-positive bacterial organisms and yeast. [15]

Guel et al in 2016 evaluated antimicrobial activities of endodontic sealers namely, AH Plus, MTA Fillapex, and Smartpaste Bio. Antimicrobicidal activity was evaluated by utilizing the agar diffusion technique. The agar plates were infused with S. aureus, C. Albicans, E.coli, Ps. Aeruginosa, and Enterococcus faecalis. Selected sealers were placed within prepared wells in agar plates for a duration of 24, 48, and 72 hours. Smart paste Bio demonstrated the largest zone of inhibition whereas, the lowest zone of inhibition was exhibited by MTA Fillipex. Significant statistical difference ($P < 0.05$) was found on comparing Smartpaste Bio and Mineral Trioxide Aggregate (MTA) Fillipex but no statistical significance was observed on comparing Smartpaste Bio and AH Plus ($P > 0.05$). [16]

Gurel et al (2016) additionally, demonstrated in their study that Smartpaste Bio has a superior anti-fungal activity which can be attributed to a high level of pH. Similarly, Doharthem et al (2011) have also shown that calcium silicate-based endodontic sealers possess antifungal activity. [17]

Zhang et al in 2009 reported that fresh iRoot SP was capable of destroying bacteria in 2 minutes duration while AH Plus (an epoxy resin-based sealer), EndoRez, Sealapex, and Epiphany destroyed all bacterial species in 5 minutes, 20minutes, 60 minutes each duration, respectively. [18]

Bodrumulu and Seniz in 2006 tested various root canal sealers by using the agar diffusion technique by incubating agar which was inoculated with Enterococcus faecalis at a temperature of 37-degree centigrade for 72 hours under aerobic environmental conditions while the zones of inhibition were measured in durations of 24, 48 and 72 hourly intervals. Inhibition of bacterial growth was observed in subsequently decreasing antibacterial efficacy as Endomethasone, Sultas, sealapex, Diaket, epiphany, and AH26. [19] The mechanism of action of Sealapex is based on the release of hydroxyl ions. There is an increase in the level of pH above 12.5. With the setting of calcium hydroxide, there is a decline in pH to an approximate value of 9.14, which ultimately, renders it ineffective. [19]

Gomes et al in 2004 in their analysis using the 'direct contact method' demonstrated that Endomethasone, as well as Endo-Fill, exhibited the greatest anti-microbiological activity, however, no statistically significant difference was noted while Sealer 26 showed the least anti-microbial properties.[20]

The results of the present study are in conformance with other studies. The consistent anti-microbiological effectiveness over 1st, 3rd, 5th, and 7th day especially, in case of ZOE, the surface hydrolysis of zinc eugenolate causes a release of eugenol, which explains its effectiveness beyond 72 hours.

Sealapex demonstrated the least inhibitory activity at a 1-hour interval which underwent further reduction on the first day and subsequent reduction. However, AH Plus was the best antibacterial sealant used.

CONCLUSION

SEALERS exhibit different microbial inhibitory activity on *E. faecalis*. Bacteria population show higher susceptibility for AH Plus as compared to Sealapex and ZOE. Though, sustained antimicrobial activity was demonstrated by ZOE.

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