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The Antimicrobial Efficacy of Different Endodontic Sealers Against Enterococcus Faecalis: An in Vitro Study

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KEYWORDS
AH plus, Enterococcus faecalis, Mineral trioxide aggregate Fillapex, Sealapex, Tubliseal.

ABSTRACT:

Objective-This in vitro research compares the effectiveness of four different endodontic sealers against Enterococcus faecalis in terms of antibiotic resistance.

Materials and Methods-Four different endodontic sealers were evaluated for their effectiveness, including resin-based (AH Plus), zinc oxide/eugenol-based (Tubliseal), calcium hydroxide-based (Sealapex), and mineral trioxide aggregate-based (MTA Fillapex). The effectiveness of antibacterial agents employing the agar diffusion technique against E. faecalis. Four wells were created by scraping off agar at evenly spaced spots, filling them with newly mixed root canal sealers, and then inoculating them with E. faecalis. All three plates were left to incubate for 72 hours in an aerobic environment at 37°C. We assessed the diameter of the inhibitory zones after twenty-four, forty- eight, and 72 hours.Unpaired t-test and one-way analysis of variance were used to statistically analyse the data.

Results-The data were statistically analysed using the unpaired t-test and one-way analysis of variance.All of the investigated sealers exhibited some E. faecalis bacterial growth inhibition. These were effective against germs in decreasing order of antibacterial activity: MTA Fillapex>Sealapex> AH Plus >Tubliseal. With longer durations of action, the root canal sealers' effectiveness somewhat declined.

Conclusion-Calcium hydroxide-based sealers had the best antimicrobial effectiveness, followed by resin-based sealers, while MTA-based sealers had the worst.

Introduction

Complete chemicomechanical preparation, irrigation, obturation, and postendodontic restoration are necessary for an effective endodontic procedurein order to attain the best outcomes, the root canal must be disinfected of bacteria.[1]One of the key elements that promotes the repair of the periapical tissues is root canal cleaning. It is difficult to completely remove all germs from the inside of root canals, which may result in the failure of endodontic therapy, despite thorough cleaning, shape, and the application of intracanal medications. The main etiological causes of pulp necrosis and apical periodontitis are thought to be microorganisms and their byproducts.[2] Gram-positive Enterococcus faecalis has frequently been discovered in root canal-treated teeth that exhibit symptoms of chronic apical periodontitis. This bacterium can generally withstand endodontic therapy. It is challenging to use root canal medications to eradicate these species when they become embedded in the dentinal tubules of the canal. According to S. Sundqvist et al.[3]E. faecalis infections were found in 38% of unsuccessful root canal procedures. Despite making up a very tiny fraction of the primary flora in infected root canals, Enterococcus species are frequently recovered following failed endodontic therapy and have also been linked to pre-existing root canal infections. The removal of germs by mechanically cleaning and shaping, together with antibacterial irrigants, sufficient filling of the empty space, and the application of antibacterial dressings between visits, if necessary, are directly connected to the effectiveness of obturation.[3]In order to increase the antibacterial effectiveness of root canal sealers, antimicrobial chemicals are added. The optimal root canal filling material, according to Grossman, should be bacteriostatic.[4] The root canal sealers should have an antibacterial action, create an airtight closure, and be tissue friendly. The antimicrobial properties of sealants may stop germs and chronic residual illness from reentering via the mouth cavity, increasing the likelihood that an endodontic treatment will be effective. According to their chemical make-up, root canal sealants may be divided into six categories: glass ionomer-based, zinc oxide-eugenol-based, calcium hydroxide-containing, epoxy resin-based, and mineral trioxide aggregate (MTA)-based. The aim of this study was to assess the in vitro antimicrobial efficacy of sealers of various bases, including zinc oxide eugenol (Tubliseal), calcium hydroxide (Sealapex), mineral trioxide aggregate (MTA Fillapex), and epoxy resin (AH Plus). This was done due to the variation in composition of the available sealers and taking into account the American National Standards Institute/American Dental Association standards.

AIM

This in vitro research compares the antimicrobial effectiveness of four endodontic sealers against E. faecalis, including resin-based AH Plus, zinc oxide/eugenol-based Tubliseal, calcium hydroxide-based Sealapex, and mineral trioxide aggregate-based MTA Fillapex.

Materials and Methods

This study examined the antibacterial effectiveness of four root canal sealers against E. faecalis. The following sealers were included in the study: [Figure 1, Table 1]

Group I: Resin-based sealers (AH plus)
Group II: Sealers based on zinc oxide and eugenol (Tubliseal)
Group III: Sealers based on calcium hydroxide (Sealapex)
Group IV: Sealers based on MTA (MTA Fillapex)
Standard strains of E. faecalis were obtained after the bacteria were cultivated on solid media and culture comprising broth suspensions was created (MTCC). 2093). To ensure their purity, microbes were subcultured in the proper culture medium. On three Petri plates containing Mueller-Hinton agar medium, aliquots of the mixture harbouring E. faecalis were distributed.Four portions were distributed into each agar plate in an equal amount. 4 mm diameter well was created in each region of each plate using a sterilised stainless steel cylinder with agar that was scraped off at evenly spaced intervals. The sealants were blended in accordance with the manufacturer's recommendations. Each sealer's freshly mixed sample was inserted into a well in each of the three plates' four regions. Zones of inhibition were evaluated after 24, 48, and 72 hours of aerobic 72-hour incubation on all plates (Figures 2–4). A one-way analysis of variance (ANOVA) and unpaired t-test were used to statistically analyse the diameter of the growth inhibition zones.

Table 1- Sealers used in the study

Materials	Trade Name	Manufacturers	Composition
Group I (resin-based sealer	AH-Plus	Dentsply, DeTrey, Konstanz, Germany	Paste A - Bisphenol A epoxy resin, bisphenol F epoxy resin, calcium tungstate, zirconium oxide, silica, and iron oxide pigments Paste B -Dibenzydiamine, aminoadamante, trycyclodecane-diamine, calcium

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			tungstate, zirconium oxide, silica, and silicone oil
Group II (zinc oxide-eugenol-based sealer)	Tubliseal	Sybron Endo	Paste A - Zinc oxide, oleoresin, bismuth trioxide, thymol iodide, oil, and waxes Paste B - Eugenol, polymerized resin, and annidalin
Group III (calcium hydroxide-based sealer)	Sealapex	Sybron Endo, Glendora, CA, USA	Calcium hydroxide, barium sulfate, zinc oxide, titanium dioxide, and zinc stearate
Group IV (MTA-based sealer)	MTA Fillapex	Angelus (Londrina/ Parana/Brazil)	After the mixture: Salicylate resin, natural resin, diluting resin, bismuth oxide, nanoparticulated silica, MTA, and pigments Paste A - Salicylate resin, bismuth trioxide, fumed silica Paste B - Fumed silica, titanium dioxide, MTA, base resin

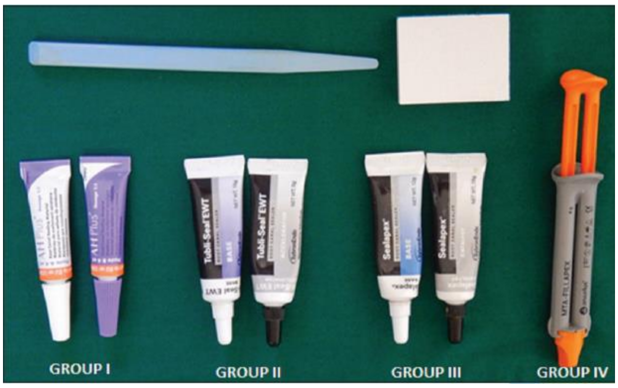

Figure 1 Sealers used in the study

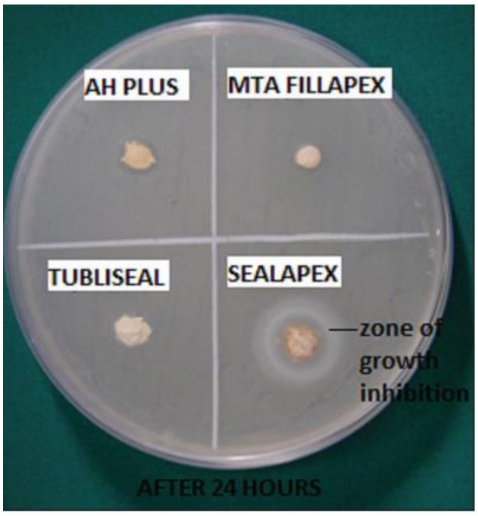

Figure 2 Zone of growth inhibition after 24 hrs

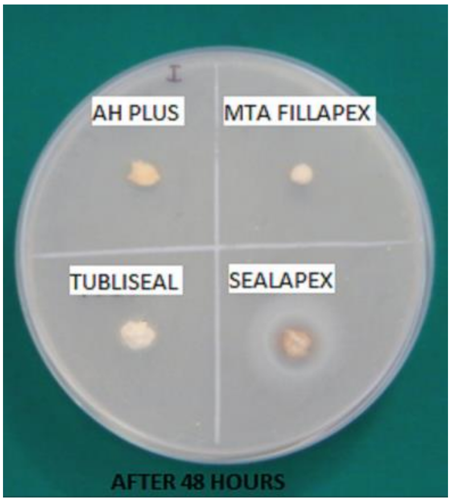

Figure 3 Zone of growth inhibition after 48 hrs

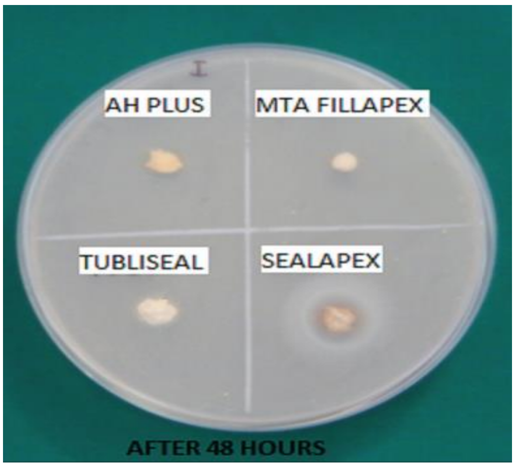

Figure 4 Zone of growth inhibition after 72

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Results

All four root canal sealers displayed growth-inhibiting zones. Table 2 displays the average diameters of the inhibition zones brought on by the four sealers put to the test. The studied microorganism was most effectively inhibited by Sealapex, followed by AH plus, while MTA Fillapex had the least impact. Additionally, it demonstrates that the zone of inhibition shrank over time, peaking at 24 hours and peaking at 72 hours. The antibacterial activity of the tested endodontic sealers was assessed using an ANOVA and pairwise comparison using an unpaired t-test with a 95% confidence level and the appropriate degree of freedom. Table 3 displays the outcomes that were attained.As seen in the table, Sealapex's average inhibition zone diameter differs considerably from that of Tubliseal, MTA Fillapex, and AH +, whereas the difference between the average inhibition zone diameters for the other pairings is not statistically significant. As a result, it was discovered that Sealapex had the greatest average diameter of the inhibitory zones.

Table 2 Mean diameter of inhibition zone in mm

Sealers	24hrs	48hrs	72hrs
AH plus	9.11	7.46	7.23
Tubliseal	8.23	4.5	0
Sealapex	14.56	13.30	11.28
MTA Fillapex	6.56	4.31	0

Table 3 Mean inhibition, standard deviation, standard error, t value and P value of the obtained readings

Pair of comparison	Mean of inhibition zone diameter	SD	SE	t	Remark and P
Sealer III and II	13.101 7.1668	1.6779 1.6498	1.9641	3.0263	0.000(significant)
Sealer III and IV	13.101 5.5835	1.6779 1.5323	1.9106	3.9402	0.000(significant)
Sealer III and I	13.101 8.1113	1.6779 0.7699	1.3055	3.8307	0.000(significant)
Sealer II and IV	7.1668 5.5835	1.6498 1.5323	2.2516	0.7033	0.725(not significant)
Sealer II and I	7.1668 8.1113	1.6498 0.7699	1.4163	-o.6667	0.752(not significant)
Sealer IV and I	5.5835 8.1113	1.5323 0.7699	1.3409	-1.8853	0.537(not significant)

Discussion

Unquestionably, one of the most crucial phases in a successful endodontic procedure is chemomechanical preparation. This does not, however, diminish the significance of the the obturation's quality, in which the sealer plays a part.Root canal sealants assist by preventing leakage, offer antibacterial action by lowering the likelihood of lingering microorganisms, and treat periapical lesions.[5] A root canal treatment's failure is frequently caused by the bacteria's persistence in the root canal system. It has been demonstrated that enterococci may persist in root canals as solitary organisms.[6]Even after root canals have been cleaned, shaped, and irrigated with antimicrobial solutions, it is challenging to entirely eradicate germs from the root canal system. In order to do this, root filler products with antibacterial activity may be used.[7] Despite the fact that aerobic and facultative bacteria typically make up a small part of primary endodontic infections, they are commonly detected in instances with extended therapy, flare-ups, and endodontic failures. E. faecalis was utilised in the current investigation since it is the most often employed microbe in several in vitro studies pertaining to chronic periapical infections.[8] It is the most drug-resistant bacterium that may last up to 12 months in the root canal, even in nutrient-poor conditions.[9] One of the tests employed in this study, the agar diffusion test, the most popular techniques for evaluating the antibacterial effectiveness of different endodontic sealers.[10] With this technique, root canal sealers may be directly compared to the microorganisms to be evaluated, and it is possible to see which sealer is most likely to get rid of bacteria in the local microenvironment of the root canal system.[11]The primary drawback of the agar diffusion test is that it is unable to distinguish between the bactericidal and bacteriostatic effects of The test material, outcomes of this approach, and the antibacterial activity of the test material for the specific microorganisms are all strongly reliant on how well the test material diffuses across the medium.[12]The size of the inhibitory zones does not, therefore, affect the sealer's overall antimicrobial effectiveness.Endodontic sealants come in a range of materials, including as zinc oxide/eugenol, calcium hydroxide, glass ionomer, silicon, resin, and bioceramic.[13,14] These Sealants have an antibacterial impact based on their chemical makeup.[15] In addition to gutta percha or silver cones, Grossman created zinc oxide-based sealers in 1936 that were based on eugenol. Tubliseal, Endomethasone, and Endofill are three frequently used zinc oxide-eugenol-based sealers.Improved biological characteristics and a solid seal of the root canal system were the goals of the introduction of calcium hydroxide-containing sealers. The ability of hydroxide-based sealers to release hydroxide ions, which results in an alkaline environment, may be the cause of their antibacterial action.[16] Among these, a paste-paste presentation is provided for Sealapex (SybronEndo, Glendora, CA, USA) and Apexit Plus (Ivoclar, Vivadent, Fürstentum, Schaan, Liechtenstein). The first resin-based sealer was introduced by Schröeder in 1954. Since then, research has been done that has helped to increase the calibre of sealants, leading to the creation of the physicochemically sound AH Plus epoxy resin-based sealant. Because they include bisphenolA diglycidyl ether or because the polymerization of the resin releases formaldehyde, epoxy resin-based sealants have antibacterial properties.[17] A newly released sealer is MTA Fillapex (Angelus, Londrina, PR, Brazil). The MTA in this sealer's chemical makeup is a key component of the production concept. Alkaline pH and consequent antibacterial activity are two characteristics of MTA that are present in the MTA Fillapex sealer.The agar diffusion test was used in this study to evaluate all sealers. Following incubation, the diameter of the inhibition zones around the sealers was measured, and the sealer with the largest inhibition zone was deemed to have the most effective antimicrobial action. According to the study's findings, MTA Fillapex had the lowest antimicrobial activity at 24, 48, and 72 hours whereas Sealapex had the highest antimicrobial activity. The bactericidal activity of all four sealers was seen to diminish with time in the current investigation, peaking at 24 hours and troughing at 72 hours. Estrela et al. [18] proposed the hypothesis that the rate at which calcium hydroxide dissociates into calcium ions and hydroxyl ions affects the antibacterial mechanism in calcium hydroxide-based sealers. This dissociated hydroxyl ion produces a high pH environment that prevents enzymatic activity necessary for the metabolism, proliferation, and cell division of microorganisms. MTA Fillapex was shown to be the least effective in this investigation. Torabinejad et al.[19]the MTA was discovered to have antibacterial action against various facultative bacteria, but not against E. faecalis, Staphylococcus aureus, Bacillus subtilis, or Escherichia coli, or against anaerobic bacteria. Stowe et al. [20] discovered that MTA suppressed the development of both E. faecalis and Streptococcus sanguis when they tested its antibacterial activities.MTA's antibacterial properties are related to the presence of calcium oxide, which when combined with water creates calcium hydroxide.[19-21] Gilberto Debelian noted that MTA is difficult to apply in narrow canals, making the material challenging to use in canals alongside guttapercha.[22] According to Kuga et al. [23], MTA Fillapex has decreased antibacterial action when compared to Sealapex due to lower pH, which might be attributed to variations in the proportion of extractable calcium hydroxide.The inclusion of epoxy resin and amine compounds in AH + may contribute to its antibacterial activity. Our findings, however, were identical to those of Zhang et al.[24] and Kayaoglu et al.,[25] who observed that freshly mixed AH + successfully killed E. faecalis. Tandon et al. [26] proposed that freshly mixed AH + had a substantial antibacterial impact, whereas set samples had little antibacterial action. Wang et al. [27] tested four endodontic root canal sealers for antibacterial effectiveness against E. faecalis biofilm in dentinal tubules. It was discovered that zinc oxideeugenolbased sealers had a poorer antibacterial impact than other sealers. The explanation was based on the sensitivity of the technology employed to evaluate materials. Similarly, Tabrizizadeh and Mohammadi's investigation found that zinc oxideeugenolbased sealers had a limited antibacterial impact.[28] It should be noted that the size of the inhibitory zones does not indicate a root canal sealer's exact antibacterial activity. As a result, when examined in vivo, the root canal sealers investigated in this study may have different zones of inhibition against E. faecalis.

Conclusion

The studies provided here are in vitro, and in vivo factors such as the presence of dentin and serum may alter the antibacterial action of sealers. As a result, more in vivo investigations are required to assess the antibacterial efficiency of sealers.

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