Original Article

Antimicrobial Efficacy of Different Endodontic Sealers against *Enterococcus faecalis*: An *In vitro* Study

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Abstract

Objective: The aim of this *in vitro* study is to compare the antimicrobial efficacy of four different endodontic sealers against *Enterococcus faecalis.*
Material and Method: Four different endodontic sealers, namely, resin based (AH Plus), zinc oxide-eugenol based (Tubliseal), calcium hydroxide based (Sealapex), and mineral trioxide aggregate (MTA Fillapex) based were tested for their antimicrobial efficacy against *E. faecalis* using agar diffusion method. Four wells were made by the removal of agar at equidistant points and filled with freshly mixed respective root canal sealers and were inoculated with *E. faecalis*. All the three plates were incubated for a period of 72 h at 37°C under aerobic conditions. The diameter of inhibition zones was measured at 24, 48, and 72 h time intervals. Data obtained were statistically analyzed using one-way analysis of variance and unpaired *t*-test.
Results: All the tested sealers showed some bacterial growth inhibition of *E. faecalis*. Their efficacy in descending order of antibacterial activity was as follows: Sealapex > AH Plus > Tubliseal > MTA Fillapex. The efficacy of the root canal sealers decreased marginally with increase in their duration of action.
Conclusion: Antimicrobial efficacy of calcium hydroxide-based sealer was highest followed by resin-based sealer and was the least with MTA based sealer.

Keywords: *AH plus, Enterococcus faecalis, Mineral trioxide aggregate Fillapex, Sealapex, Tubliseal*

Introduction

For a successful endodontic treatment, complete chemicomechanical preparation, irrigation, obturation, and postendodontic restoration are essential to achieve optimal results, thus eliminating bacteria from the root canal.[1] Root canal disinfection is one of the main determinants which aids in the healing of the periapical tissues. Irrespective of thorough cleaning, shaping, and the use of intracanal medicaments, it is difficult to completely eradicate all microorganisms from the root canal system, which may lead to the failure of endodontic treatment. Microorganisms and their by-products are considered as primary etiological factors for pulp necrosis and apical periodontitis.[2]

Enterococcus faecalis is Gram-positive bacterium that can mostly resist endodontic therapy and has been frequently found in root canal-treated teeth with signs of chronic apical periodontitis. When lodged in the dentinal tubules of the canal, it is difficult to remove these species through root canal medicaments. Sundqvist *et al*.[3] related that 38% of failed root canal treatments were infected by *E. faecalis*. Although Enterococcus species comprise a small proportion of the initial flora in infected root canal, they are most commonly recovered from unsuccessful endodontic treatment and has also been associated with existing root canal infections. The success of obturation is directly related to the elimination of microorganisms through mechanical cleaning and shaping, supplemented by antibacterial irrigants, adequate filling of the empty space, and the use of antimicrobial dressings between

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appointments, if necessary.[3] Therefore, antimicrobial agents are incorporated to root canal sealers to enhance their antibacterial efficacy.

Grossman advocated that the ideal root canal filling material should be bacteriostatic.[4] The root canal sealers should be tissue compatible, provide an airtight seal, and possess antimicrobial effect. The antimicrobial activity of sealers may prevent persistent residual infection and microorganisms from re-entering through the oral cavity, thereby increasing the chances of a successful endodontic treatment outcome.

Root canal sealers can be classified according to the chemical composition as zinc oxide-eugenol-based, calcium hydroxide-containing, glass ionomer-based, epoxy resin-based, and mineral trioxide aggregate (MTA)-based sealers. Due to the variation in composition of the available sealers and considering the American National Standards Institute/American Dental Association standards, the objective of this study was to evaluate *in vitro* antimicrobial efficacy of sealers of different bases: zinc oxide eugenol (Tubliseal), calcium hydroxide (Sealapex), mineral trioxide aggregate (MTA Fillapex), and epoxy resin (AH Plus).

Aim

The aim of this *in vitro* study is to compare the antimicrobial efficacy of four endodontic sealers, i.e. resin based (AH Plus), zinc oxide-eugenol based (Tubliseal), calcium hydroxide based (Sealapex), and mineral trioxide aggregate based (MTA Fillapex) based against *E. faecalis*.

Material and Method

In this study, antimicrobial efficacy of four root canal sealers was tested against *E. faecalis*. The sealers included in the study were as follows [Table 1 and Figure 1]:

- Group I – Resin-based sealer (AH plus)
- Group II - Zinc oxide-eugenol-based sealer (Tubliseal)
- Group III - Calcium hydroxide-based sealer (Sealapex)
- Group IV - MTA-based sealer (MTA Fillapex).

The microorganisms were grown in solid media, and culture containing broth suspensions was prepared and, thus, standard strains of *E. faecalis* were obtained (MTCC 2093). Microorganisms were subcultured in appropriate culture media to confirm their purity. Aliquots of the suspension containing *E. faecalis* were spread on three Petri dishes containing Mueller-Hinton agar medium.

Each agar plate was equally divided into four sections. In each section of each plate, a 4 mm diameter well was prepared with a sterile stainless steel cylinder by the removal of agar at equidistant points. The sealers were mixed according to the manufacturer's instructions. A freshly mixed sample of each sealer was placed into the wells in all the four sections of the three plates. All plates were incubated for 72 h at 37°C under aerobic conditions, and zones of inhibition were measured at 24, 48, and 72 h [Figures 2-4]. The diameter of the growth inhibition zones was analyzed statistically using one-way analysis of variance (ANOVA) and unpaired *t*-test.

Results

All four root canal sealers showed zones of growth inhibition. The mean diameters of inhibition zones caused by the four tested sealers are presented in Table 2. Sealapex exhibited the largest inhibition zone followed by AH plus while MTA Fillapex had the least effect on the tested microorganism. It also shows that the zone of inhibition decreased with time, the highest being at 24 h and the lowest at 72 h.

To evaluate the antimicrobial activity of the tested endodontic sealers, ANOVA and pairwise comparison were carried out using unpaired *t*-test at 95% confidence level and relevant degree of freedom. The obtained results are shown in Table 3.

From the table, it can be observed that the average diameter of inhibition zone of Sealapex differs significantly when compared to Tubliseal, MTA Fillapex, and AH plus while, for all the remaining pairs, the difference in the average diameter of inhibition zones is not significant. Thus, it was seen that the highest value of the average diameter of the inhibition zones was for Sealapex.

Discussion

Chemomechanical preparation is undoubtedly one of the most important steps in successful endodontic treatment. However, this does not negate the importance of the quality of the obturation, in which the sealer has a role to play.

Root canal sealers help by minimizing leakage, provide antimicrobial activity by reducing the possibility of residual bacteria, and resolve periapical lesion.[5] The persistence of bacteria in the root canal system often leads to failure of the root canal treatment. *Enterococci* have been shown to survive in root canals as single organisms.[6] It is difficult to completely remove microorganisms from the root canal system, even after debridement, shaping, and irrigation of the root canals with antimicrobial agents. Therefore, the use of root filling materials with antimicrobial activity might help to achieve this goal.[7]

Table 1: Sealers used in the study

Material	Trade name	Manufacturer	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 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

MTA=Mineral trioxide aggregate

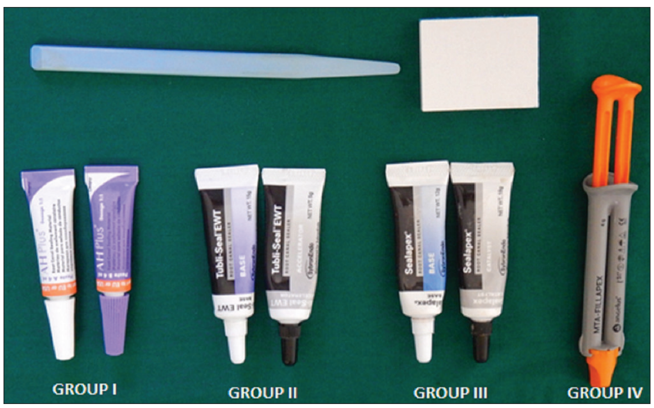

Figure 1: Sealers used in the study

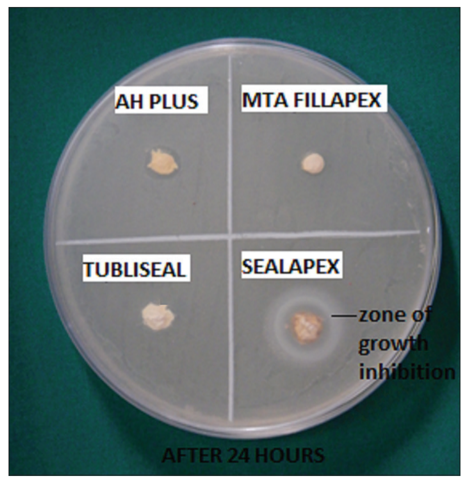

Figure 2: Zones of growth inhibition after 24 h

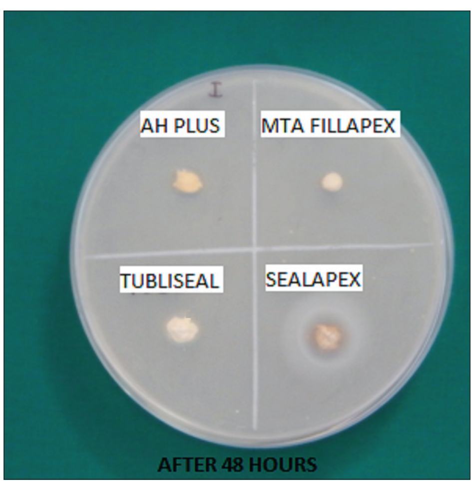

Figure 3: Zones of growth inhibition after 48 h

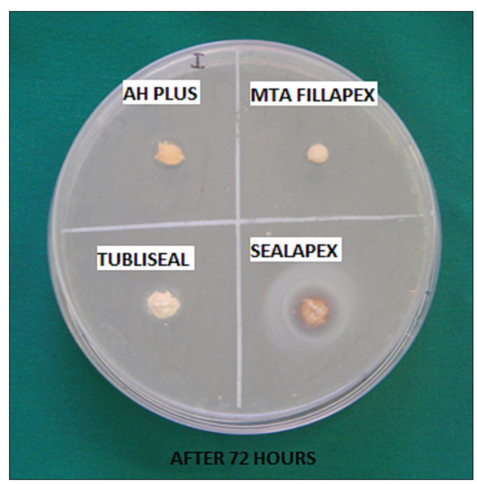

Figure 4: Zones of growth inhibition after 72 h

Although aerobic and facultative microorganisms usually constitute a minor proportion of primary endodontic infections, they are frequently found in cases with prolonged treatment, in flare-ups, and in endodontic failures. In the present study, *E. faecalis* was used as it is the most commonly used microorganism in various *in vitro* studies relevant to persistent periapical infections.[8] It is the most drug-resistant bacteria which has the ability to survive up to 12 months in the root canal even under nutrient-deficient conditions.[9]

The agar diffusion test used in this study is one of the most commonly used methods to determine the antimicrobial efficacy of various endodontic sealers.[10] This method allows direct comparison of the root canal sealers against the microorganisms to be tested and the visual indication of which sealer has the potential to eradicate microorganisms in the local microenvironment of the root canal system.[11] The main disadvantage of agar diffusion test is that it cannot differentiate between bactericidal and bacteriostatic effect of the material, and results of this method are dependent on the antimicrobial activity of the test material for the particular microorganisms and is highly influenced by the diffusibility of the material across the medium.[12] Therefore, the absolute antimicrobial efficacy of the sealer is not determined by the size of the inhibition zones.

A variety of endodontic sealers are available in the market which include zinc oxide-eugenol, calcium hydroxide, glass ionomer, silicon, resin, and bioceramic.[13,14] These sealers have antimicrobial effect depending on their chemical composition.[15]

Grossman, in 1936, introduced zinc oxide-eugenol-based sealers, to be used as root canal filling material along with gutta-percha or silver cones. Commonly used zinc oxide-eugenol-based sealers are Tubliseal, Endomethasone, and Endofill.

The aim of introducing calcium hydroxide-containing sealers was to improve the biological properties and to ensure a good seal of the root canal system. The antimicrobial activity of hydroxide-based sealers may be attributed to its hydroxide ion-releasing property which creates an alkaline environment.[16] Among these, Apexit Plus (Ivoclar, Vivadent, Fürstentum, Schaan, Liechtenstein) and Sealapex (SybronEndo, Glendora, CA, USA) are available in a paste–paste presentation.

Table 2: Mean diameter of inhibition zone

Sealers	Mean diameter (SD) of inhibition zone (mm) Duration of incubation		
	24 h	48 h	72 h
AH Plus	9	7.66	7.33
Tubliseal	8.33	4.2	0
Seal apex	14.66	13.33	11.33
MTA Fillapex	6.66	4.3	0

MTA=Mineral trioxide aggregate, SD=Standard deviation

Schröeder, in 1954, introduced the first resin-based sealer. Since then, studies were conducted which contributed to the improvement of the quality of sealers, which lead to the development of an epoxy resin-based sealer, AH Plus which has good physicochemical properties. Epoxy resin-based sealers have antimicrobial activity due to the presence of either bisphenol A diglycidyl ether or due to the release of formaldehyde during polymerization.[17]

MTA Fillapex (Angelus, Londrina, PR, Brazil) is a recently introduced sealer. The philosophy behind manufacturing this sealer is the presence of MTA in its chemical structure. One of the properties of MTA that is present in the MTA Fillapex sealer is the alkaline pH and subsequent antibacterial activity.

In this study, all sealers were tested using the agar diffusion test. After incubation, the diameter of zones of inhibition around the sealers was measured and the sealer which exhibited the maximum zone of inhibition was considered as having the most efficient antimicrobial activity.

The results of this study showed that Sealapex showed highest antimicrobial activity whereas MTA Fillapex showed lowest antimicrobial activity measured at 24, 48, and 72 h. From the present study, it is also observed that the antibacterial activity of all the four sealers decreased with time, i.e. it was highest at 24 h and lowest at 72 h. Estrela *et al*.[18] hypothesized that the antimicrobial mechanism in calcium hydroxide-based sealers is influenced by its speed of dissociation into calcium ions and hydroxyl ions. This dissociated hydroxyl ion

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 Sealer II	13.111 7.1667	1.6778 1.6499	1.9642	3.0264	0.000 (significant)
Sealer III and Sealer IV	13.111 5.5834	1.6778 1.5321	1.9105	3.9403	0.000 (significant)
Sealer III and Sealer I	13.111 8.1111	1.6778 0.7698	1.3053	3.8306	0.000 (significant)
Sealer II and Sealer IV	7.1667 5.5834	1.6499 1.5321	2.2515	0.7032	0.726 (not significant)
Sealer II and Sealer I	7.1667 8.1111	1.6499 0.7698	1.4164	−0.6668	0.751 (not significant)
Sealer IV and Sealer I	5.5834 8.1111	1.5321 0.7698	1.3408	−1.8852	0.536 (not significant)

SD=Standard deviation, SE=Standard error creates a high pH environment, which inhibits enzymatic activities which are essential for microbial metabolism, growth, and cellular division.

In the present study, MTA Fillapex was found to be the least effective. Torabinejad *et al*.[19] detected the antimicrobial efficacy of MTA against some facultative bacteria; however, no antimicrobial activity was found against *E. faecalis*, *Staphylococcus aureus*, *Bacillus subtilis,* and *Escherichia coli* or against anaerobic bacteria. However, on assessing the antimicrobial properties of MTA, Stowe *et al*.[20] found that MTA inhibited the growth of both *E. faecalis* and *Streptococcus sanguis*. The antibacterial property of MTA is due to the presence of calcium oxide which forms calcium hydroxide on contact with water.[19,21] Gilberto Debelian stated that MTA is hard to apply in narrow canals, thus making the material difficult to apply along with gutta-percha in canals.[22]

MTA Fillapex had lower antimicrobial activity when compared to Sealapex due to lower pH which may be due to the differences between the percentage of extractable calcium hydroxide in accordance with the study conducted by Kuga *et al*.[23]

The antibacterial efficacy of AH plus could be due to the presence of epoxy resin and amine ingredients. However, our results were similar to that of Zhang *et al*.[24] and Kayaoglu *et al*.,[25] who reported that freshly mixed AH plus killed *E. faecalis* effectively. Tandon *et al*.[26] suggested that freshly mixed AH plus had significant antibacterial effect whereas set samples did not show any antibacterial activity.

Nirupama *et al*.[27] evaluated the antimicrobial efficacy of four endodontic biomaterials against *E. faecalis, Candida albicans,* and *S. aureus* and found that Tubliseal has shown maximum antimicrobial activity against *C. albicans* and *S. aureus*, which could be due to eugenol which is effective against mycotic and vegetative forms of bacteria. Antimicrobial effect of zinc oxide-eugenol sealer was mainly attributed to the action of eugenol, and a phenolic compound acts on microorganisms by protein denaturation, whereby the protein becomes nonfunctional.[10]

The size of the inhibition zones depends mainly on two factors which include: (1) toxicity of the material to a particular strain of bacteria and (2) the ability of the material to diffuse through the particular medium. In turn, the diffusibility is mainly affected by three factors which include: (1) hydrophobicity or hydrophilicity of the material, (2) the rate of release from the matrix in which the material is placed, and (3) size. These factors are difficult to control in *in vitro* studies. The test results may vary according to the above-mentioned factors.[28] Wang *et al*.[29] evaluated the antibacterial efficacy of four endodontic root canal sealers on *E. faecalis* biofilm in dentinal tubules. It was found that zinc oxide-eugenol-based sealer has weaker antibacterial effect when compared to other sealers. The reason explained was on the basis of sensitivity of the technique used to test materials. Similarly, the study conducted by Tabrizizadeh and Mohammadi also demonstrated low antibacterial effect of zinc oxide-eugenol-based sealer.[30] It should be noted that the size of the inhibition zones does not determine the exact antimicrobial efficacy of a root canal sealer. Therefore, the root canal sealers tested in the present study may show differences in the zones of inhibition against *E. faecalis* when tested *in vivo*.

The data presented here relate to *in vitro* conditions, and *in vivo* conditions such as the presence of dentin and serum might modify the antimicrobial activity of sealers. Hence, further *in vivo* studies are needed to evaluate the antimicrobial efficacy of sealers.

Conclusion

Under the limitations of this study, it can be concluded that Sealapex showed the highest antimicrobial activity against *E. faecalis* while MTA Fillapex showed the least. Moreover, the efficacy of the root canal sealers against *E. faecalis* decreased with time. Further research is required to compare their efficacy *in vivo*.

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Conflicts of interest

There are no conflicts of interest.

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