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ORIGINAL RESEARCH

Assessment of Antimicrobial Activity of Endodontic Sealers on *Enterococcus faecalis*: An *in vitro* Study

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ABSTRACT

Microorganisms invading the root canal system of a tooth may interact with the host tissue and cause pulpo-periapical pathosis. The goals of root canal treatment are to disinfect the root canal system and to prevent subsequent reinfection. The disinfection is attempted with endodontic instruments, irrigants, and medications. Due to the complex canal anatomy, micro-organisms can persist even after thorough disinfection regimens are used. After disinfection, the canal is sealed with a root filling material along with sealers. To curtail residual microorganisms, root-filling materials should ideally be bactericidal, as well as biocompatible. In addition, the root filling materials are expected to act as a physical barrier to prevent the leakage of substrate to any residual microorganisms and the ingress of additional microorganisms.

The aim of the present study was to assess the antimicrobial activity of various Endodontic sealers on the *Enterococcus faecalis*. *Enterococcus faecalis* is a microorganism commonly detected in asymptomatic, persistent endodontic infections. In the changing face of dental care, continued research on *E. faecalis* and its elimination from the dental apparatus may well define the future of the endodontic specialty.

Keywords: Antimicrobial activity, Endoflas, Metapex, AH plus, AH 26.

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INTRODUCTION

The success of endodontic treatment depends mainly on elimination of infecting microorganisms. This is achieved through chemomechanical preparation of root canals and leaving antimicrobial dressings in the root canal between appointments. However, microorganisms might still survive these challenges.[1,2] The chemical agents that form an inseparable part of root canal treatment are the cement sealers along with gutta-percha. Antimicrobial properties of these sealers will ensure elimination of microbes as well as prevent reinfection particularly when bacteriological sampling before obturation is not a routine procedure. Hence, along with biocompatibility of the cement, its antimicrobial properties are highly relevant and useful in root canal treatment and worthy of evaluation. Therefore, root canal sealers with good sealing ability and antimicrobial activity are desired to entomb and kill the surviving microorganisms.

Microorganisms infecting the root canal dentine might adhere superficially to the dentinal wall or penetrate deeper into the dentinal tubules.[3,4] *Enterococcus faecalis* is a resilient bacterium frequently recovered from obturated root canals with signs of apical periodontitis.[5] The presence of *Enterococcus faecalis* at the time of obturation can significantly reduce the success rate of root canal treatment. When established in the dentinal tubules, it is difficult to eliminate this species through root canal medication. Therefore, it might be advantageous if the sealer exerts some antimicrobial activity as the last element in the treatment regimen.

The purpose of this study is to evaluate the antimicrobial activity of zinc oxide eugenol-based sealer, calcium hydroxide-based sealer and resin-based sealer on *Enterococcus faecalis* which is most commonly isolated in failed root canal cases.

MATERIALS AND METHODS

Preparation of the Medium for *Enterococcus faecalis*

The strains of microorganisms used for the study were standard strains of *Enterococcus faecalis* which were obtained from ATCC29212 and were subcultured in blood agar plate and were incubated at 37°C for 24 hours (Fig. 1). A pure, single *Enterococcus faecalis* colony was isolated from the same cultured plate and Gram's staining was done to confirm its growth, which was observed under microscope and then inoculated with a brain heart infusion (BHI) broth. The BHI broth was incubated at 37°C for a 24-hour period and checked for bacterial growth by changes in turbidity. A drop of BHI containing *Enterococcus faecalis* was placed into saline solution and checked for correct bacterial concentration with a spectrophotometer. The density of the bacterial suspension is standardized by comparing the broth at a density equivalent to the barium sulfate standard of 0.5 McFarland units, which is equivalent to 1.5×10^8 colony forming units per milliliter (CFU/ml).

Preparation of the Disks

Seventeen blood agar plates of 15 × 100 mm were prepared for inoculation. The plates were incubated at 37°C in ambient atmosphere for 24 hours period to check any external contamination.

Fig. 1: Growth of *Enterococcus faecalis* on blood agar plate after 24 hours

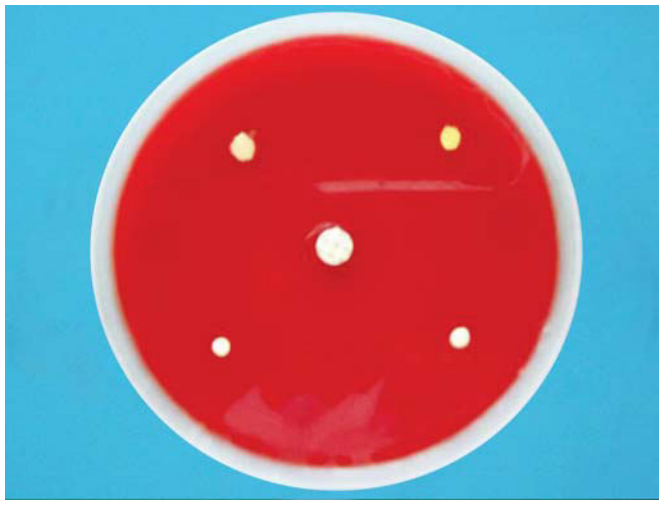


Fig. 2: Sealers impregnated on blood agar plate inoculated with *Enterococcus faecalis*: Top row left—endoflas FS, bottom row left—AH plus, top row right—metapex, bottom row right—AH 26

These 17 blood agar plates were inoculated with prepared *Enterococcus faecalis* suspension by evenly swabbing the plates with a sterile cotton swab to obtain a lawn culture. Amoxiclav disks (10 micrograms) were used as the control disks for *Enterococcus faecalis*. The filter paper disks (Whatman No. 1 filter paper) were standardized to 3 mm in diameter. The sealers were manipulated according to manufacturer's recommendation to get homogeneous consistency using a glass slab and spatula or paper mixing pad and agate spatula. Sterile filter paper disks were applied with the help of sterile forceps and pressed gently to ensure even contact with the medium. Around 100 microliter (0.1 ml) of each sealer is placed with the help of micropipettes.

The plates containing the sealer impregnated disks along with control disks were kept for incubation at 37°C in ambient atmosphere for a 24 hours period (agar diffusion method) (Fig. 2). The Petri dishes containing the sealer impregnated disks along with the microorganisms namely *Enterococcus faecalis* were incubated for 48 hours at 37°C in an incubator. Zones of inhibition were measured at the end of 24 hours and 48 hours period. The diameter of the zone of inhibition of growth was measured in millimeters with the help of an inhibition zone measuring scale and the values recorded. The point of abrupt diminution of growth, which corresponds to the point of complete inhibition of growth, is taken as the zone edge.

Sealers used in the study were Endoflas, Metapex, AH Plus, AH26. The composition of these are mentioned in Table 1.

RESULTS

The results were tabulated in Tables 2 and 3 after 24 and 48 hours respectively and statistically analyzed by Kruskal-Wallis one-way ANOVA used to calculate the p-value and

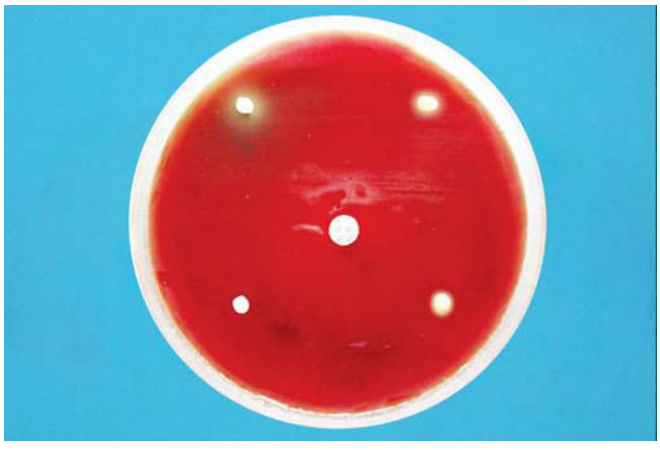
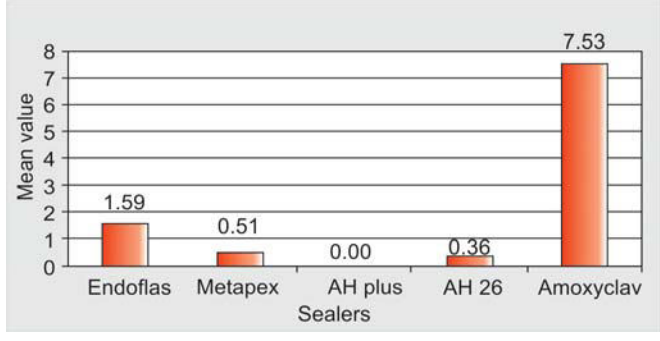
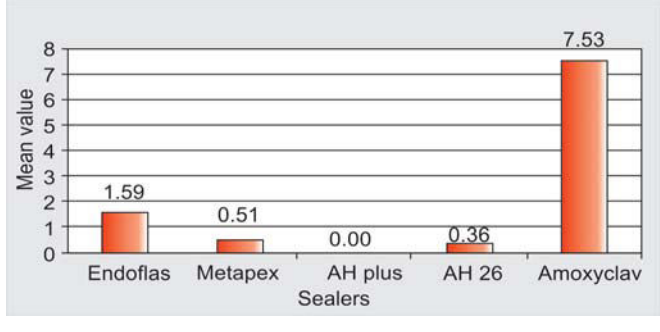


Fig. 3: Top row left—endoflas FS, bottom row left—AH plus, top row right—metapex, bottom row right—AH 26



Graph 1: Mean value of sealers at 24:00 hours



Graph 2: Mean value of sealers at 48:00 hours

Table 1: Endodontic sealers—components and composition

Sealers	*Components*	*Composition*	*Manufacturer*
Endoflas	Powder	Barium sulfate, zinc oxide, calcium hydroxide	Sanlor
	Liquid Accelerator	Eugenol Zinc acetate	
Metapex	Paste	Calcium hydroxide iodoform	Meta Biomed
AH plus	Paste A	Bisphenol-A epoxy resin, bisphenol-F epoxy resin, calcium tungtate, zirconium oxide, silica, iron oxide pigments	Dentsply
	Paste B	Dibenzyldiamine, aminoadamantane, tricyclodecane-diamine, calcium tungstate, zirconium oxide, silica, silicone oil	
AH26	AH26 silver free powder AH26 resin	Bismuth oxide, methenamine Epoxy resin	Dentsply

Table 2: Zone of inhibition measured after 24 hours (measured in mm)

Sealers	Specimens																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Endoflas	1	2	2	2	1	1	1	2	1	2	2	2	1	2	1	2	2
Metapex	1	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.2	0.5	0.5	0.2
AH Plus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AH26	0.5	0.5	0.2	0.2	0.5	0.5	0.2	0.2	0.2	0.2	0.5	0.5	0.5	0.2	0.2	0.5	0.5
Amoxiclav	8	7	8	8	8	7	7	8	7	8	8	8	7	8	7	7	7

Table 3: Zone of inhibition measured after 48 hours (measured in mm)

Sealers	Specimens																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Endoflas	1	2	2	2	1	1	1	2	1	2	2	2	1	2	1	2	2
Metapex	1	0.5	0.5	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.2	0.5	0.5	0.2
AH Plus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
AH26	0.5	0.5	0.2	0.2	0.5	0.5	0.2	0.2	0.2	0.2	0.5	0.5	0.5	0.2	0.2	0.5	0.5
Amoxiclav	8	7	8	8	8	7	7	8	7	8	8	8	7	8	7	7	7

Mann-Whitney U-test was used to identify the significant groups at 5% level after correcting the p-values for comparison by Bonferroni correction method (Table 4). Graphs were plotted presenting mean values of sealers at 24 and 48 hours (Graphs 1 and 2). The disk impregnated with the sealer, which exhibited the maximum zone of inhibition was considered as having the most efficient antimicrobial activity (Fig. 3).

DISCUSSION

Chemomechanical preparation is of paramount importance 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. According to Grossman, a requirement and characteristic of a sealer should be bacteriostatic or at least not encourage bacterial growth.[6] The most common reason for the failures in conservative root canal therapy are problems in instrumentation, however, occasionally bacteria resistant to conservative therapy of good quality may also be involved.[7] Hence, a three-dimensional seal with the antimicrobial property of the sealer is critical for endodontic success. It is generally believed that the significant cause of root canal treatment

Table 4: Multiple comparisons

Dependent variable: Inhibition zone

			Mean difference			95% confidence interval	
	Sealers (I)	Sealers (J)	(I-J)	Std. error	Sig.	Lower bound	Upper bound
Tukey HSD	Endoflas	Metapex	1.0824*	0.1182	0.000	0.7524	1.4123
		AH Plus	1.5882*	0.1182	0.000	1.2583	1.9182
		AH26	1.2294*	0.1182	0.000	0.8995	1.5594
		Amoxyclav	–5.9412*	0.1182	0.000	–6.2711	–5.6112
	Metapex	Endoflas	–1.0824*	0.1182	0.000	–1.4123	–0.7524
		AH Plus	0.5059*	0.1182	0.000	0.1759	0.8358
		AH26	0.1471	0.1182	0.726	–0.1829	0.4770
		Amoxyclav	–7.0235*	0.1182	0.000	–7.3535	–6.6936
	AH Plus	Endoflas	–1.5882*	0.1182	0.000	–1.9182	–1.2583
		Metapex	–0.5059*	0.1182	0.000	–0.8358	–0.1759
		AH26	–0.3588*	0.1182	0.026	–0.6888	–2.8874E-02
		Amoxyclav	–7.5294*	0.1182	0.000	–7.8594	–7.1995
	AH26	Endoflas	–1.2294*	0.1182	0.000	–1.5594	–0.8995
		Metapex	–0.1471	0.1182	0.726	–0.4770	0.1829
		AH Plus	0.3588*	0.1182	0.026	2.887E-02	0.6888
		Amoxyclav	–7.1706*	0.1182	0.000	–7.5005	–6.8406
	Amoxyclav	Endoflas	5.9412*	0.1182	0.000	5.6112	6.2711
		Metapex	7.0235*	0.1182	0.000	6.6936	7.3535
		AH Plus	7.5294*	0.1182	0.000	7.1995	7.8594
		AH26	7.1706*	0.1182	0.000	6.8406	7.5005
Dunnett t (2-sided)[a]	Endoflas	Amoxyclav	–5.9412*	0.1182	0.000	–6.2357	–5.6466
	Metapex	Amoxyclav	–7.0235*	0.1182	0.000	–7.3181	–6.7290
	AH Plus	Amoxyclav	–7.5294*	0.1182	0.000	–7.8240	–7.2349
	AH26	Amoxyclav	–7.1706*	0.1182	0.000	–7.4651	–6.8760

*The mean difference is significant at the 0.05 level

[a]Dunnett t-tests treat one group as a control and compare all other groups against it

failure is the persistence of microorganisms in the apical third of root filled teeth.[8] Approximately, apical one-third of the canals of root filled teeth with persistent periapical lesions have shown high proportion of *Enterococcus faecalis*.[9] Significant increase in *Enterococcus faecalis* contamination in retreatment cases has been shown as they gain entry into the canal during root canal treatment, due to poor temporary seals between appointments or inadequate aseptic techniques.[10]

And other probable reasons for the isolation of *Enterococcus faecalis* in failed root canal treated teeth may be due its survive even with scant amounts of substrate and without the support of other microorganisms, and grow to establish monoinfections.

A sealer with an antimicrobial activity can be considered advantageous, in order to eliminate the remaining microbes present in the root canal after chemomechanical preparation of the root canal system and to prevent reinfection. Zinc-oxide eugenol-based sealers have been traditionally the most commonly employed sealants. They have served as the benchmark with which other sealers are compared, as it reasonably meets most of Grossman's requirements for sealers.[11]

In order to improve the antimicrobial efficacy of zinc oxide eugenol sealers, known bactericidal agents, such as iodoform have been incorporated resulting in modified zinc oxide eugenol-based sealers, such as Endoflas FS and medicated canal sealer (MCS). Luebke and Ingle in 1976 forecast a new paradigm for endodontics involving a broader use of calcium hydroxide in medicating and sealing the root canal.[11] This has led to the introduction of several calcium hydroxide-based sealers namely Metapex, Sealapex and Apexit.

Epoxy resin-based sealers (AH26) were introduced because of its advantages, such as high radiopacity, low solubility, slight shrinkage and antimicrobial efficacy.[11] The antimicrobial efficacy of AH26 is attributed to the release of formaldehyde. However, formaldehyde is a known mutagenic and carcinogenic agent.[12] Hence, this sealant has been replaced by AH Plus an improved epoxy resin sealant. AH Plus has retained the epoxy resin 'glue' of AH26 and also is free of formaldehyde release.[13]

Hence, the objective of this *in vitro* study was to analyze the antimicrobial effect of a traditional zinc oxide eugenol-based sealer with a iodoform incorporated zinc oxide eugenol based sealer (Endoflas FS), a calcium hydroxide-based sealer (Metapex) and the epoxy resin-based sealers (AH Plus and AH26), against the microorganisms *Enterococcus faecalis* using a agar disk diffusion test. The techniques employed to assess antimicrobial efficacy

include broth dilution, agar disk diffusion, agar disk dilution, spiral gradient test, E-test and automated antimicrobial testing systems. Each of these techniques has their own inherent advantages and disadvantages.[14]

Traditionally, agar diffusion method and agar dilution method are commonly employed for detecting antimicrobial susceptibility. In our study, Kirby-Bauer method (agar disk diffusion method) was chosen instead of the agar dilution method. The disadvantage of the agar dilution method is that this technique can alter some of the properties of the sealers being tested. Moreover, some sealers cannot be homogeneously dissolved and is a difficult and slow technique. Hence, the agar disk diffusion method was used in this method. The chemical properties of the sealers are not changed[15] and the antimicrobial resistance can be detected by challenging bacterial isolates with antimicrobial disks. Moreover, this is an easy and less technique-sensitive method.

Amoxyclav was chosen as the control against *Enterococcus faecalis* as it is a potent bactericidal causing lysis of the bacterial cell wall.[16] Among the test groups, Endoflas FS showed statistically significant antimicrobial efficacy against *Enterococcus faecalis*.

The superior antimicrobial efficacy of Endoflas FS could be attributed to the presence of eugenol and iodoform in its composition. Eugenol, a phenolic compound acts on microorganisms by protein denaturation whereby the protein becomes nonfunctional. The antimicrobial effect of zinc oxide eugenol sealers can be gauged by the results of the following studies. Andre Mickel et al found that zinc oxide eugenol-based sealant exhibited larger zone of inhibition against *Enterococcus faecalis* when compared with calcium hydroxide-based sealer Metapex and epoxy resin-based sealer AH Plus and AH26. Iodoform acts by the liberation of iodine, which is an oxidizing agent.[17] Oxidizing agents like iodine can irreversibly oxidize and thus inactivate essential metabolic compounds like protein, which has been accounted for the antimicrobial action.[18]

The calcium hydroxide-based sealer, Apexit showed zones of inhibition against *Enterococcus faecalis*, which was statistically lesser than the zones of inhibition produced by both the zinc oxide eugenol-based sealers. Esterela et al[19] hypothesized that in calcium hydroxide, the antimicrobial mechanism is influenced by its speed of dissociation into calcium ions and hydroxyl ions.[20]

This dissociation into hydroxyl ions creates a high pH environment, which inhibits enzymatic activities that are essential for microbial metabolism, growth and cellular division. The resin-based sealers AH Plus and AH26 showed no zones of inhibition against *Enterococcus faecalis*. The elimination of formaldehyde release from AH Plus has made it an ineffective antimicrobial sealant.

This result was in concurrence with Andre Mickel et al who found AH Plus to be ineffective against *Enterococcus faecalis*.

The rationale for performing this *in vitro* antibacterial activity test is to offer the clinician valuable information regarding the antimicrobial properties of various root canal sealers. Consequently, to determine the true antimicrobial effectiveness, *in vivo* testing is essential. With this in mind, the findings from this study show that the various endodontic sealers differ in their antimicrobial activity as indicated by their zones of inhibition.

CONCLUSION

1. Endoflas FS, zinc oxide-based sealer showed significantly greater antimicrobial effect against *Enterococcus faecalis*.
2. There was no significant difference between the antimicrobial activity of AH26 and AH Plus sealer on *Enterococcus faecalis*.
3. Metapex, a calcium hydroxide-based sealer is less effective against *Enterococcus faecalis*.
4. AH Plus, a resin-based sealer showed no antimicrobial activity on *Enterococcus faecalis*.
5. There was no difference in the zones of inhibition between 24 hours and 48 hours time periods.

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