

“A Comparative Evaluation of Antimicrobial Efficacy of Three Endodontic Sealers: Endoflas FS, AH Plus and sealapex against *Enterococcus faecalis* - an in vitro study.”

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Abstract:

Aims and Objective: To compare the antimicrobial properties of three endodontic sealers such as Sealapex (calcium hydroxide based), Endoflas FS (zinc oxide eugenol based) and AH Plus (resin based) against *Enterococcus faecalis*. **Materials and Methods:** The materials required for the study were sealers like Sealapex, AH plus and Endoflass FS, incubator, tweezer, scale and autoclave. The study was conducted on agar plates. Three wells were made by removal of agar at equidistant points and filled with root canal sealers and inoculated with *Enterococcus faecalis*. The plates were maintained at room temperature for 2 hours for prediffusion of materials and then incubated at 37°C for 24 hours, 48 hours and 72 hours. After incubation, the diameter of zones of inhibition around the plates was measured. The readings obtained were statistically analysed using ANOVA and Bonferroni test. **Results:** In all determined intervals, the antibacterial activity of Endoflas FS was significantly greater than other test materials ($P < 0.001$). AH Plus sealer had moderate effect, whilst Sealapex showed the lowest antibacterial activity against *Enterococcus faecalis*. **Conclusion:** The antibacterial activity of Endoflas FS was highest followed by AH Plus and Sealapex.

Key-words: AH Plus, Endoflas FS, *Enterococcus faecalis*, Sealapex.

I. Introduction

Microorganisms and their by-products are considered as the primary etiological agents of necrotic pulps and apical periodontitis and endodontic therapy is an invaluable measure to preserve teeth that would otherwise need to be extracted. Though the main goal of endodontic therapy is the elimination of microorganisms from the root canal system and the prevention of subsequent reinfection, most of treatment failures are caused by microorganisms surviving the treatment procedures and causing re-infection of the root canal system [1].

The endodontic microflora is typically a polymicrobial flora consisting of gram positive and gram negative bacteria. Microorganisms and their products are the most frequent etiologic factors in causing pathologic conditions of pulpal and periapical origin. In the normal oral flora, there are about three hundred different bacterial species, of which a great number of microorganisms can colonize in the root canal system [2].

Enterococcus faecalis is a gram positive, group D streptococci and a facultative anaerobe that occur singly, in pairs and in short chains and are capable of surviving in harsh environment [3]. The ability of *Enterococcus faecalis* to cause periapical disease and chronic failure of an endodontically treated tooth is due to its ability to bind to the collagen of the dentinal tubule and remain viable within the tubule. In addition, several studies have shown that it is resistant to the most common inter appointment medicament such as, calcium hydroxide, both in vivo and in vitro [4,5,6,7].

Enterococcus faecalis have been frequently found in obturated root canals exhibiting signs of chronic apical periodontitis. A recognized pathogen in post-treatment endodontic infections, *E. faecalis* is frequently isolated both in mixed flora and in monocultures. It is also apparent from the dental literatures that *E. faecalis* is often difficult to eradicate from the root canal system with current intracanal medications [6,8].

The root canal sealers should be tissue compatible, provide an airtight seal, and should possess antimicrobial effect because a good root canal sealer with antimicrobial activity might better cope with a persistent residual infection and microorganisms reentering via the oral cavity, thereby, increasing the chances of a successful endodontic treatment outcome [9].

The aim of the study was to compare the antimicrobial efficacy of three root canal sealers AH Plus resin based sealer, Endoflas fs zinc oxide eugenol based sealer and Sealapex calcium hydroxide based sealer against *Enterococcus faecalis*.

II. Materials and Method

The study was carried out using three root canal sealers namely Sealapex, AH Plus, and Endoflas FS.

Standard strains of *Enterococcus faecalis* MTCC 439 was obtained from Institute of Microbial Technology (IMTECH), Chandigarh were used in this study.

The method used to investigate the antimicrobial activity of the sealers was Agar diffusion method. All microorganisms were sub-cultured in appropriate culture media to confirm their purity. The study was conducted on Mueller Hinton agar plates. Three wells were made by removal of agar at equidistant points and filled with root canal sealers after being mixed according to manufacturer’s instructions.

All microorganisms were grown at 37°C for 24 hours in Mueller Hinton Broth. This MH broth was used as second layer. This broth was then added over the wells after the insertion of sealer cements.

The plates were maintained at room temperature for 2 hours for prediffusion of materials and then incubated at 37°C under appropriate gaseous conditions for 24 hours 48 hours and 72 hours. After incubation, the diameters of zones of inhibition around the plates were measured using Antibiotic zone scale. ANOVA test and Bonferroni test were employed for statistical analysis.

III. Results

All sealers were tested using the agar diffusion test. After incubation, the diameters of zones of inhibition around the plates were measured and the sealer which exhibited the maximum zone of inhibition was considered as having the most efficient antimicrobial activity.

Table I shows the materials with trade name and their composition.

Table II shows that highest inhibition is recorded with Endoflas sealer (28.33 ± 2.51) followed by AH Plus (19.87 ± 2.15) and Sealapex (11.47 ± 0.94) respectively and it also shows that inhibition decreased with time highest being at 24 hrs and lowest at 72 hrs.

There was a highly significant difference found between AH Plus and Endoflas FS ($P < 0.001$) as well as between Ah Plus and Sealapex ($P < 0.001$) and Endoflas FS and Sealapex ($P < 0.001$) at all the time intervals.

Tables Table No. I: Materials and their Composition

Material	Manufacturer	Trade name	Composition
SEALER I	SANLOR	ENDOFLOSS FS	Iodoform, zinc oxide eugenol
SEALER II	DENTSPLY	AH PLUS	Diepoxide, Calcium tungstate, Zirconium oxide, Pigments and Aerosil, 1-adamantane amine
SEALER III	SYBRON KERR	SEAL APEX	Calcium oxide, Barium sulphate, Silica Titanium dioxide, Zinc stearate.

Table No. II: Mean inhibition with different Sealers at different time intervals

		24 Hours	48 Hours	72 Hours
E. Faecalis	Endoflas FS	25.60 (± 0.97)	24.00 (± 1.49)	22.10 (± 1.37)
	AH Plus	19.30 (± 0.95)	17.90 (± 1.37)	16.20 (± 1.62)
	Sealapex	11.60 (± 0.97)	10.80 (± 1.03)	10.10 (± 0.32)

IV. Discussion

Antibacterial activity is one of the desirable properties of an ideal sealer [10]. The incorporation of antimicrobial components into root canal sealers may become an essential factor in preventing the re-growth of residual microorganisms and control of bacterial reentry into the root canal system. Because the antimicrobial components do not have selective toxicity against microorganisms, they may also exert toxic effects on host cells therefore biocompatibility is important factor when selecting a sealer during obturation [11].

The techniques employed to assess antimicrobial efficacy include Broth dilution, Agar diffusion, Spiral Gradient test, E-test and Automated Antimicrobial testing systems. **Gopikrishna AV et al. (2006)** [9]. In the present study, the agar diffusion test was used for testing all the sealers which is one of the most frequently used in vitro method to test the antimicrobial activity of dental materials. The advantages of this method is the creation of direct comparisons of the root canal sealers against test microorganisms and the visual indication of which sealer has the potential to eliminate microorganisms in the local microenvironment of the root canal system [9,12,13,14]. After incubation, the diameters of zones of inhibition around the agar plates were measured and the sealer which exhibited the largest zone of inhibition was considered as having the most efficient antimicrobial activity.

Analyzing the antimicrobial activity of different sealers against microorganism provided the information regarding the effectiveness of the tested sealers in preventing the organisms from re-infecting the root canal system. When communication exists between the internal and external environment, or when residual microorganisms remains from inadequate cleaning and shaping, the antimicrobial activity of various

components of root canal sealers plays an important role in the success of endodontic treatment **Mickel AK et al. (2003)** [15].

The results of this study showed that iodoformized zinc oxide eugenol based sealer (Endoflas FS) showed the highest antimicrobial activity against *Enterococcus faecalis* followed by (AH Plus) a resin based sealer and (Sealapex) calcium hydroxide based sealer showed the least antimicrobial efficacy. This study was in accordance with study done by **Sabyasachi S et al. (2010)** [16] and **Gomes BPF et al. (2004)** [17].

Endoflas FS showed the highest antimicrobial activity because known bactericidal agents such as iodoform have been incorporated in order to improve the antimicrobial efficacy of zinc oxide eugenol sealers, resulting in modified zinc oxide eugenol based sealers such as Endoflas FS and Medicated Canal Sealer (MCS) [9]. Iodoform is a mild antiseptic because of the slow liberation of iodine when in contact with body fluids. It has bactericidal and fungicidal effects through its iodine content. Iodoform acts by the liberation of iodine, which is an oxidizing agent. Oxidizing agents like iodine can irreversibly oxidize and thus inactivate essential metabolic compounds like protein, which has been accounted for its antimicrobial action. The studies done by **Kayaoglu G et al. (2005)** [18] and **Gopikrishna AV et al. (2006)** [9] also showed the similar findings.

Zinc oxide eugenol based sealers (Endoflas FS) have been traditionally the most commonly employed sealers during root canal treatment because it reasonably meets most of the Grossmann's requirements for sealers. The antimicrobial effect of zinc oxide eugenol cement was mainly attributed to the action of eugenol. Eugenol, a phenolic compound acts on microorganisms by protein denaturation whereby the protein becomes non-functional [9,19,20,21]. The results of the various studies performed by **Markowitz K et al. (1992)** [19] and **Saggar V et al. (1996)** [20] also confirms that zinc oxide eugenol containing sealers were more superior in inhibiting the microorganisms.

The present study also showed that the antimicrobial activity of AH Plus was inferior to zinc oxide eugenol based sealer but superior to the calcium hydroxide based sealer, Sealapex. This is because AH Plus (DENTSPLY) is a resin based sealer containing epoxy resin and due to the discreet liberation of formaldehyde [12].

The results of the present study also demonstrated that Sealapex showed the minimal antimicrobial activity on the tested microorganism. The findings of the present study was in accordance with the earlier studies done by **Miyagak DC et al. (2006)** [12] and **Sabyasachi S et al. (2010)** [16] who also demonstrated that calcium hydroxide based sealers are not a potent a microbial growth inhibitor. The least antimicrobial effect of this sealer stems from dissociative ability of this sealer into calcium and hydroxyl ions which increases the medium pH [12,22,23].

From the present study it was also observed that the antibacterial activity of all the three sealers decreased with time i.e. it was highest at 24 hours and lowest at 72 hours. The fact that the in vitro antimicrobial activity reduces with time is also important, because it could mean that the sealers become more stable in contact with the body fluids. This observation was in accordance with study done by **Gomes BPF et al. (2004)** [17].

The results of this study showed statistically highly significant difference between all the sealers tested and showed that Endoflas FS exhibited the largest zones of inhibition and found to be superior to AH plus and Sealapex in inhibiting the microorganism.

The rationale for performing this in vitro study is to offer information to clinicians that Endoflas FS has the highest antimicrobial efficacy followed by AH Plus and Sealapex root canal sealers against *Enterococcus faecalis*. However, it should be taken into considerations that 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 efficacy of sealers. Hence, further in vivo studies are needed to evaluate the antimicrobial efficacy of sealers.

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