Comparative Evaluation Of The Antimicrobial Activity Of Different Endodontic Sealers On Enterococcus Faecalis – An Invitro Study

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Abstract: Background and objectives: To evaluate the in vitro antimicrobial activity of different root canal sealers. Materials and method: Five root canal sealers were selected for the study namely Zinc Oxide Eugenol, Endoflas FS, Endomethasone, AH plus and Sealapex . Enterococcus faecalis was obtained from American Type Culture Collection (ATCC 29212).The media used were Brain Heart Infusion Broth (BHI) and Blood Agar. 10 blood agar plates of 15 x 100 mm were prepared each inoculated with prepared E. faecalis to obtain Lawn culture. Ampicillin discs (10μg) were used as the control discs. The sealers were manipulated under the UV laminar flow chamber. 100 microlitre (0.1 ml) of each sealer was placed on the sterile paper disc with micropipettes. The zones of inhibition were measured at 24 hours and 48 hours from the edge of the paper discs with help of vernier calipers and recorded. Results : Kruskal-wallis one way ANOVA was used to calculate the overall P-value. Mann – Whitney U-test was employed to identify the significant groups at 5% level after correcting the p-values for multiple comparisons by Bonferroni correction method. Conclusion: Endomethasone showed significantly greater antimicrobial effect against E. faecalis. There was no significant difference of antimicrobial activity between Zinc Oxide Eugenol and Endoflas FS on E. faecalis. Sealapex was less effective against E .feacalis. AH plus showed no antimicrobial activity on E. faecalis. [Kothari A et al NJIRM 2013; 4(3) : 121-127]

Key Words: E. feacalis, antimicrobial efficacy, root canal sealer.

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Introduction: Bacteria and their products are considered to be the primary etiologic agent of pulpal necrosis and periapical lesions. The greatest challenge in eliminating endodontic infections is not related to microorganisms present in the lumen of the root canal but rather of those disseminated in the root canal system ramifications. Hence, the major goal of root canal treatment is the elimination of microorganisms from the root canal system and the prevention of subsequent re infection [1].

The most effective way to achieve elimination of microorganisms from root canal system is by means of adequate instrumentation and efficient and copious irrigation. However, no less important than the biomechanical preparation is an adequate filling of the canal, which enables good apical sealing and prevention of subsequent infection for long term success. [2]

Thorough debridement and complete elimination of microbes is of paramount importance before completion of obturation of the root canal. Hence, bacteriological studies were integral part of root canal treatment and two consecutive negative cultures were essential before obturation. To achieve this, the antibacterial effect of irrigants was advantageous. [2,3,4].The other chemical agents that form an inseparable part of root canal treatment are the root canal sealers. Antimicrobial properties of these sealers will ensure elimination of microbes as well as prevent re-infection particularly when bacteriological sampling before obturation is not a routine procedure. Hence the testing of antimicrobial properties of these agents is highly relevant and useful in root canal treatment and worthy of evaluation [5].

A number of cements are used as root canal sealers. Some are used for their medicament value and some for their mechanical properties and sealability. They are generally grouped as Zinc oxide eugenol based sealers, e.g. Zinc oxide eugenol, Roth sealer, Tubliseal, Endomethasone, Endoflas; Calicium hydroxide based sealers, e.g. Apexit, Sealapex; and Resin based sealers, e.g. AH 26, AH plus etc. Although *Entercoccus* spp usually constitute a small proportion of the initial flora in an untreated root canal, this genus *is* most commonly recovered from the root canals of teeth with failed root treatement and has also been implicated in persistent root canal infections [5].

Material and Methods: Five root canal sealers were selected for the study, of which Zinc Oxide Eugenol (DPI, Mumbai,India), Endoflas FS(Sanlor Laboratories,ColombiaS.A),Endomethasone(Septodont, Cedex, France) are zinc oxide eugenol based sealers, AH plus (Dentsply,Detrey,Germany) is an epoxy resin based sealer and Sealapex (SybronEndo, Orange, California) is a calcium hydroxide based sealer.

The present study is carried out following the method described by Kriby, Bauer (1966) in which the disc diffusion susceptibility test for antimicrobial resistance is detected by challenging bacterial isolates with antibiotic discs that are placed on the surface of an agar plate that has been seeded with a lawn of bacteria. When discs containing a known concentration of antimicrobial agent are placed on the surface of a freshly inoculated plate, the agent immediately begins to diffuse and establish a concentration gradient around the paper disc. The highest concentration is closest to the disc and upon incubation the bacteria grow on the surface of the plate except where the antibiotic concentration in the gradient around each disc is sufficiently high to inhibit growth [5].

Following incubation the diameter of the zone of the inhibition around each disc is measured in millimeters with the help of vernier calipers.

Enterococcus faecalis was obtained from American Type Culture Collection (ATCC 29212).The media used were Brain Heart Infusion Broth (BHI) (M210, Himedia Laboratories, Mumbai)(Fig 1) and Blood Agar (Fig 2). Brain Heart Infusion Broth is used for cultivation of Enterococcus faecalis and Blood Agar is used as enriched media for the growth of Enterococcus faecalis. Enterococcus faecalis was sub cultured in a blood agar plate from a laboratory maintained frozen culture. The plate was incubated at 37^0C in ambient atmosphere for a 24 hrs period. A pure single E. faecalis colony was isolated from the same cultured plate and inoculated into a BHI broth. The BHI broth was incubated at 37^0C in ambient atmosphere for a 24

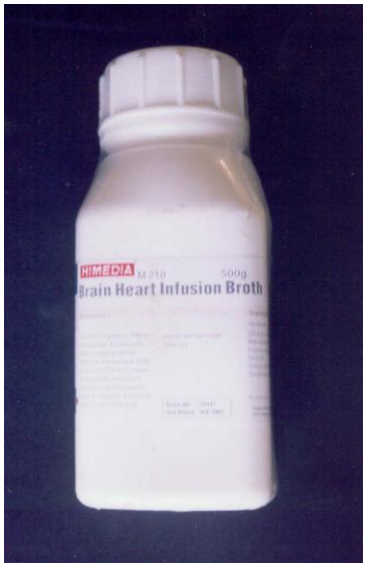
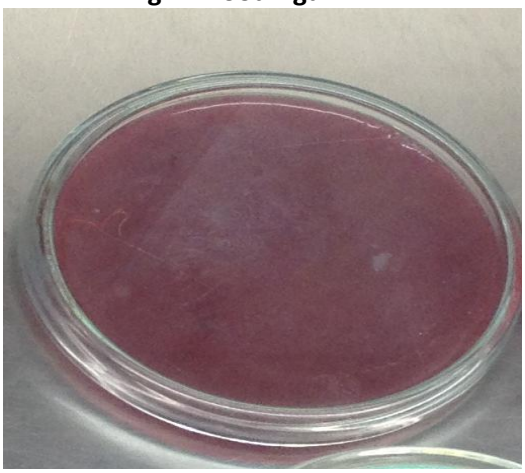


Fig 1. Brain heart infusion broth

ig 2. Blood Agar



hrs period. A culture suspension of E. faecalis in peptone water was obtained by transferring the growth from BHI broth to obtain a turbidity of 0.5 MacFarland $BaSO_4$ standard.

This scale allowed the bacterial concentration of a suspension to be estimated by its turbidity; 0.5 corresponded to a concentration of 1.0 x 10^8 colony forming units /ml. 10 blood agar plates of 15 x 100 mm were prepared for inoculation. Each plate contained 20 ml of sterilized Nutrient Agar (M001, Himedia Laboratories, Mumbai) with 5% Sheep blood. The plates were incubated at 37^0C in ambient atmosphere for a 24 hrs period to check any external contamination.

In UV Laminar Flow Chamber (Fig 3), the plates were inoculated with prepared E. faecalis suspension by evenly swabbing the plates with a

sterile non-absorbable cotton swab to obtain Lawn culture

Fig 3. UV Laminar Flow Chamber

Fig4 .Whatman Filter paper discs

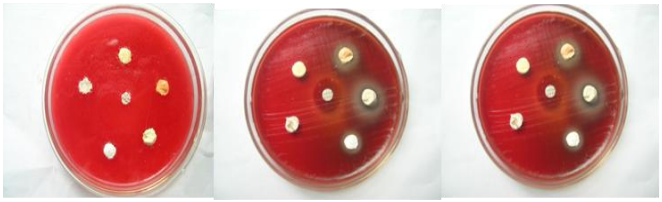
Fig 5 : Micropipette

Ampicillin discs (10µg, Himedia laboratories) were used as the control discs for Enterococcus feacalis. The filter paper discs (Whatman No.1 filter paper)(Fig 4) were standardized to 6 mm in diameter through punch. The sealers were manipulated according to manufacturers' instructions to get homogenous consistency under the UV laminar flow chamber.

After the inoculum has been dried, five sterile filter paper discs are applied with the help of sterile forceps and pressed gently to ensure even contact with the medium. 100 microlitre (0.1 ml) of each sealer is placed on the sterile paper disc with the help of micropipettes (Fig 5). The Ampicillin discs are used as controls in the Petri dishes inoculated with Enterococcus faecalis.

The plates containing the sealer impregnated discs along with control discs were kept for incubation at 37^0C in ambient atmosphere for a 24 hrs period.

Fig 6 : A sample of sealers impregnated with the zones of inhibition visible at 24 hours and 72 hours



The zones of inhibition were measured at 24 hour and 48 hours(Fig 6) from the edge of the paper discs with help of vernier calipers and recorded. The point of abrupt diminision of growth, which corresponds to the point of complete inhibition of growth, is taken as zone edge.

Result: All the values for zones of inhibition were tabulated as shown (Tables 1 & 2).A graphical representation of means of zones of inhibition is also shown (graph1).

Table:1 Table 1 : Inhibition zones in Millimeters at 24 Hours

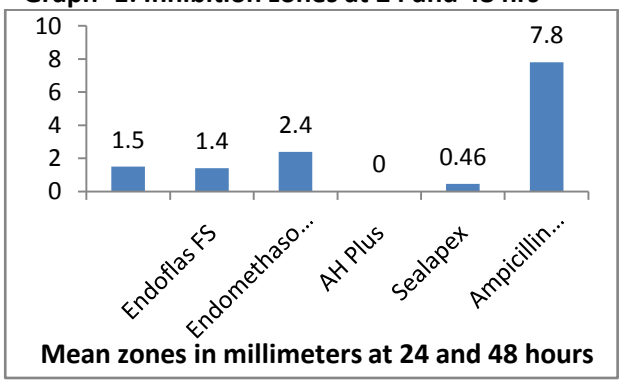
	Specimens									
Sealer	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
ZOE	2	2	1	2	1	2	1	2	1	1
Endoflas	1	2	2	1	1	1	1	2	2	2
Endomethasone	2	3	3	2	3	2	2	2	3	2
AH Plus	-	-	-	-	-	-	-	-	-	-

Sealapex	1	0.5	0.5	0.3	0.5	0.6	0.4	0.3	0.3	0.2
Ampicillin (Control)	7	8	7	8	8	8	7	9	8	8

Table:2 : Inhibition zones in Millimeters at 48 Hours

	Specimens									
Sealer	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
ZOE	2	2	1	2	1	2	1	2	1	1
Endoflas	1	2	2	1	1	1	1	2	2	2
Endomethasone	2	3	3	2	3	2	2	2	3	2
AH Plus	-	-	-	-	-	-	-	-	-	-
Sealapex	1	0.5	0.5	0.3	0.5	0.6	0.4	0.3	0.3	0.2
Ampicillin (Control)	7	8	7	8	8	8	7	9	8	8

Graph- 1: Inhibition zones at 24 and 48 hrs



Kruskal-wallis one way ANOVA was used to calculate the overall P-value. Mann – Whitney U-test was employed to identify the significant groups at 5% level after correcting the p-values for multiple comparisons by Bonferroni correction method. Note- Non-significant groups are I vs II (P=1.00)

Among the study groups, the mean value in Endomethasone (2.40±0.52) in significantly higher than the mean value in Zinc Oxide Eugenol (1.5±0.53), Endoflas (1.40±0.52), AH –plus (0.000±0.000) and Sealapex(0.46±0.24) (P<0.05). Similarly, the mean values in Zinc Oxide Eugenol (1.50±0.53) and Endoflas are significantly higher than the mean values in AH plus and Sealapex (P<0.05). Also, the mean value in Sealapex is significantly higher than AH plus (P<0.05).

It was found that the mean value in Ampicillin (control). (7.80±0.42) is significantly higher than the mean values in the entire study group (P<0.05). However, there was no significant difference in mean values between Zinc Oxide Eugenol and Endoflas (P>0.05). And the statistical analysis for the 48 hour period was no different from the 24 hour period.

Discussion: Enterococcus faecalis is a facultative anaerobic bacterium, commonly isolated in failed root canals. The ecological changes such as nutrients, oxygen tension & bacterial interrelationship that occur in the root canal during and after treatment favour these facultative anaerobic microorganisms. E. faecalis can survive with even scant amounts of substrate and as a single microorganism [5], and grow to establish mono-infections that are difficult to eradicate using conventional root canal procedures.. E. faecalis has been used extensively in studies of root canal disinfection because this bacterium is easy to grow in the culture medium and, rapidly and efficiently colonizes in medium [6]. Hence, E. faecalis was selected as the test microorganism in this study also.

Approximately one third of the canals of root filled teeth with persistent periapical lesions have shown high proportion of Enterococcus faecalis [5,7]. The probable reasons for the isolation of Enterococcus faecalis in failed root canal treated teeth may be due to (a) a small amount of enteric bacteria is already present in the infected canal at the beginning of the therapy and their relative proportion increases during the treatment as other bacteria are susceptible to therapy or (b) enteric bacteria enter the root canal during the treatment because of (i) inadequate isolation of the working area, (ii) a leaking temporary filling or (iii) the root canal has been left open for drainage.[8,9,10] Hence this experiment was designed to investigate the effect of a few commonly used root canal sealers, on the growth of Enterococcus faecalis.

Five root canal sealers were tested in this study, of which Zinc Oxide Eugenol, Endoflas FS, Endomethasone, were zinc oxide eugenol based sealers, AH plus is a resin based sealer and Sealapex is a calcium hydroxide based sealer.

In general there are three in vitro techniques most commonly used for evaluating the antimicrobial activities: the agar dilution method, which yields a quantitative result for the amount of antimicrobial agent that is required and can only be used with substances that are soluble in the culture medium: the agar diffusion method, which gives an inhibition zone around the materials tested indicating which substance has antibacterial activity but this method does not distinguish between bacteriostatic or bactericidal properties of the substances tested : and the direct exposure method, which provides qualitative information about the substance used but is highly technique sensitive.

The results of the agar diffusion method, depend upon the molecular size, solubility, and diffusion of the materials through the aqueous agar medium, the sensitivity of the drug, bacterial source (wild strains & collection species) the number of bacteria inoculated, pH of the substrates in plates, agar viscosity, storage conditions of the agar plates, incubation time and the metabolic activity of the organisms [11,12]. Therefore, the inhibition zones in agar may be related more to the materials solubility and diffusibility and not to their actual efficacy against the microorganisms [11].

Measuring zones of inhibition, as is done in agar diffusion studies helps to determine the degree of antimicrobial action of a sealer. This has been one of the predominant test strategies used to qualitate such antimicrobial activity due to the relative technical ease of this procedure, as well as the maintenance of the chemical properties of the tested sealers through out the experimental procedure [13], the agar diffusion test has been recommended by Pumarola et al [14].

The results obtained by Pumarola T [14] ,Orstavik D [15], Grossman [16], Canalda & Pumarola [17], with zinc oxide eugenol based root canal sealers showed greater zones of inhibition. In the present study, the antimicrobial activity of the zinc oxide eugenol based root canal sealers (Endomethasone, Zinc Oxide Eugenol and Endoflas FS) on Enterococcus faecalis was verified in accordance with the above studies and similar results were obtained.

Siqueira and Uzeda [18] and Barbosa et al [19] reported the inefficiency of both calcium hydroxide based sealers and pastes in inhibiting some facultative anaerobic and aerobic bacteria such as Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis and Streptococcus mutans. In the present study, the antimicrobial activity of the calcium hydroxide - based material (Sealapex) on Enterococcus faecalis was verified in accordance with the above studies and similar results were obtained. Calcium hydroxide has a low solubility; it does not diffuse well and thereby requires a long time to alkalize the culture medium. However, in a clinical situation, the buffer ability of blood, tissue fluids, and dentin might exercise the same effects [18].

AH plus, a resin based sealer was modified from AH 26, and is popular for its tissue compatibility property. AH plus does not release formaldehyde compared with its predecessor. In this study, AH plus did not show any zone of inhibition and the result was in concurrence with the study done by Mickel AK et al [20].

In several studies [21, 22, 14, 23], the antibacterial effect of calcium hydroxide containing sealers with that of zinc oxide eugenol based sealers using the agar diffusion test (ADT) was evaluated. With fresh samples, Zinc oxide eugenol based sealers showed a consistently greater inhibition regardless of microorganisms tested. It has been established that eugenol is a potent antibacterial agent [24] and it is conceivable that it plays a major role within the activity of ZOE-based sealers [25].

Most endodontic sealers have inherent antimicrobial properties. Antimicrobial compounds such as iodoform, thymoliodide, and paraformaldehyde are also added to enhance the antibacterial activities. These compounds may be responsible for the antimicrobial effects of the sealers which would maintain the sterility of root canal system and thus potentiate repair [15,17].
The incorporation of antimicrobial components into root canal sealers may become an essential factor in preventing the re-growth of residual bacteria and control of bacterial re-entry into the root canal space. Manufacturers incorporate

antibacterial components in both the powder and the liquid phase of endodontic sealers .

A gradual, continuous release of formaldehyde from the paraformaldehyde containing Endomethasone would account for the sustained antibacterial activity . Kaplan et al [26] found the most effective antimicrobial sealers contain eugenol and formaldehyde. Without these agents, sealers were absolutely ineffective against E. faecalis.

For an antimicrobial agent to be effective it should be potent and long acting without irritating the normal periapical tissue. However, to some degree, all the currently, used endodontic sealer are periapical tissue irritants [6].

No current root canal sealer provides a perfect seal with the cavity wall and there is always a micro-space at the interface between the two, along which microorganisms can penetrate. Thus, the possibility that root canal sealers may possess antibacterial properties is of great significance since the longevity of the treated tooth may be improved by the use of such sealers [27].

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, invivo 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: The conclusions drawn from this study were:

1. Endomethasone showed significantly greater antimicrobial effect against E. faecalis.
2. There was no significant difference of antimicrobial activity between Zinc Oxide Eugenol and Endoflas FS on E. faecalis.
3. Sealapex was less effective against E .feacalis
4. AH plus showed no antimicrobial activity on E. faecalis.

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