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ORIGINAL RESEARCH

Comparison of the antimicrobial efficacy of different root canal sealers against Enterococuus faecalis

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ABSTRACT:

Background: Endodontic root canal sealers provide antimicrobial activity and help to resolve periapical lesion. The aim of the present study was to evaluate the antimicrobial efficacy of different root canal sealers against enterococuus faecalis. **Method:** Five root canal sealers were selected Zinc Oxide Eugenol, Endoflas FS, Endomethasone, AH plus, Apexit and ampicillin as control group. The present study is carried out following the method described by Kriby, Bauer (1966). Entercoccus faecalis organisms zone of inhibition was measured at 24 and 48 hours with the help of vernier caliper. ANOVA and Whitney U test were used to compare the data. **Results:** 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). and 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 Apexit (0.46±0.24) (P<0.05). **Conclusion:** Endomethasone showed significantly greater antimicrobial effect against E. faecalis. Apexit was less effective against E .feacalis

Key words: Irrigation, Enterococuus faecalis

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INTRODUCTION

Bacteria and their products are considered to be the primary etiologic agent of pulpal necrosis and periapical lesions and the closed root canal network can serve as an incubator for bacterial growth. Due to the low oxygen content of a closed root space, anaerobic bacteria in particular are provided an ideal atmosphere to live, grow, multiply and ultimately activate the vast immunological defense systems that result in host destruction. Hence, the major goal of root canal treatment is the elimination of microorganisms from the root canal system and the prevention of subsequent infection.[1]

The most effective ways to achieve elimination of microorganisms from root canal system are by means of instrumentation and irrigation. [2] 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. Instrumentation mechanically removes microbes along with shaping of the canal and irrigants are adjunct for proper cleaning and washing away of the debris.[3] Intracanal medicaments were however, used regularly for disinfection. With the development of good instruments, the canal could be cleaned and shaped more efficiently thereby reducing the need for routine intracanal medication. The use of intracanal medication as a routine because of the belief that microorganisms need not be drowned in caustic drugs to eliminate them when they can be readily be mechanically or physically removed.[4] Quoting Cohen "We Do Not Need To Fire a Cannon to Kill a Flea".

To obtain the objective of cleaning, use of irrigants is an essential step and sodium hypochlorite has remained most popular due to its pulp tissue solvent action, antimicrobial activity and lubricant action. 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. Hence, the antibacterial effect of irrigants has been the most important aspect of many earlier studies.[5]

The other chemical agents that form an inseparable part of root canal treatment are the cement 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 along with biocompatibility of the cement its antimicrobial properties are highly relevant and useful in root canal treatment and worthy of evaluation. The antimicrobial spectrum and duration of cement sealers will have a direct influence on the health and longevity of the treated tooth.

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.[6] The most common spectrum of bacteria found in non-vital teeth are Peptostreptococcus, Eubacterium, Prevotella intermedia, Fusobacterium nucleatum, Actinomyces, Streptococcus mitis, Lactobacillus, Enterococcus faecalis etc. In a failed root canal treated tooth, the most common microbe isolated and attributed to is the Enterococcus faecalis particularly from the root tip.[7]

Hence a study was conducted with an objective to evaluate the antimicrobial activity of endodontic sealers on Enterococcus faecalis.

MATERIALS AND METHODS

This study was done in the Department of Conservative Dentistry & Endodontics, Adesh Institute of Dental Sciences & Research, Bathinda, in association with the Department of Microbiology, Adesh Institute of Medical College & Hospital, Bathinda. Five root canal sealers were selected for the study, of which Zinc Oxide Eugenol, Endoflas FS, Endomethasone, were zinc oxide eugenol based sealers, AH plus is a resin based sealer and Apexit is a calcium hydroxide based sealer. The present study is carried out following the method described by Kriby, Bauer (1966). In this Kriby-Bauer method, 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) and Blood Agar. 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 370C 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 370C in ambient atmosphere for a 24 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 BaSO4 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 with 5% Sheep blood. The plates were incubated at 370C in ambient atmosphere for a 24 hrs period to check any external contamination. In UV Laminar Flow Chamber, 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. Ampicillin discs were used as the control discs for Enterococcus feacalis. The filter paper discs (Whatman No.1 filter paper) where 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. 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 370C in ambient atmosphere for a 24 hrs period. The zones of inhibition were measured at 24 hour and 48 hours 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.

RESULTS

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 Apexit (0.46+0.24) ($P<0.05$).

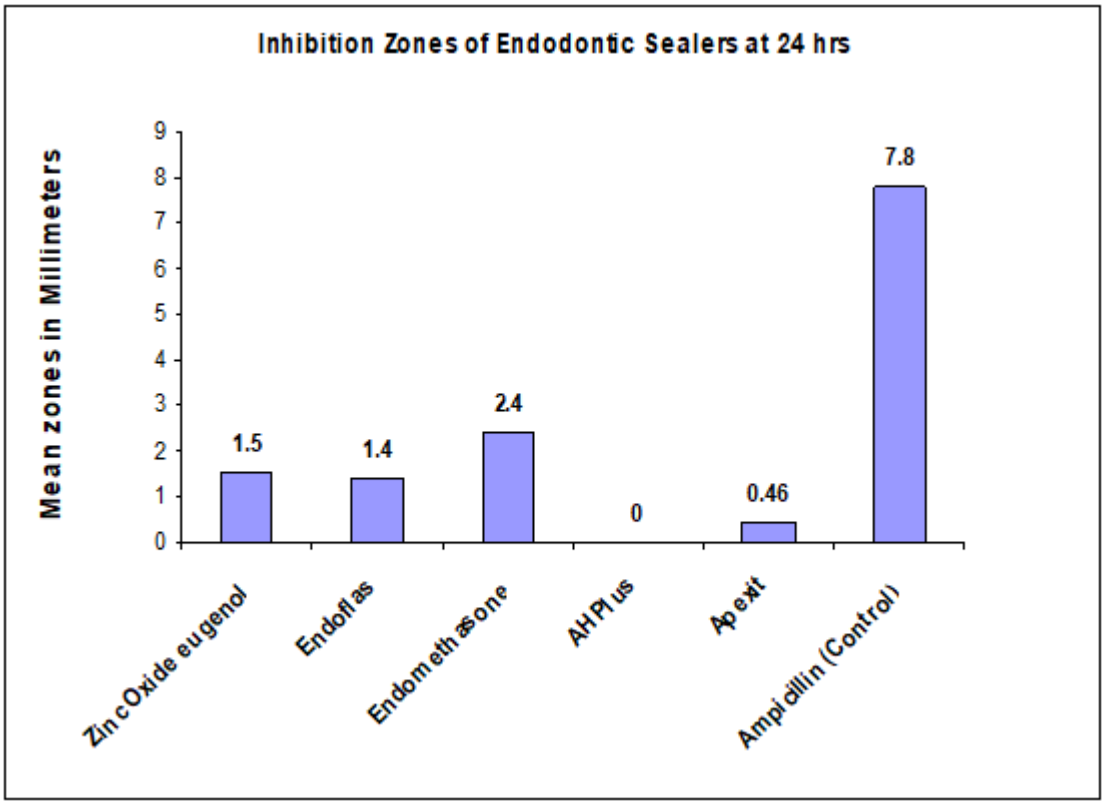
	Specimens									
Sealers	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Zinc Oxide eugenol	1	1	2	1	2	1	2	2	1	2
Endoflas	1	2	2	1	2	1	2	1	1	1
Endomethasone	2	2	2	3	3	2	2	2	3	3
AH plus	-	-	-	-	-	-	-	-	-	-
Apexit	1	0.5	0.5	0.5	0.5	0.5	0.5	0.2	0.2	0.2
Ampicillin (Control)	8	8	8	7	8	7	8	8	8	8

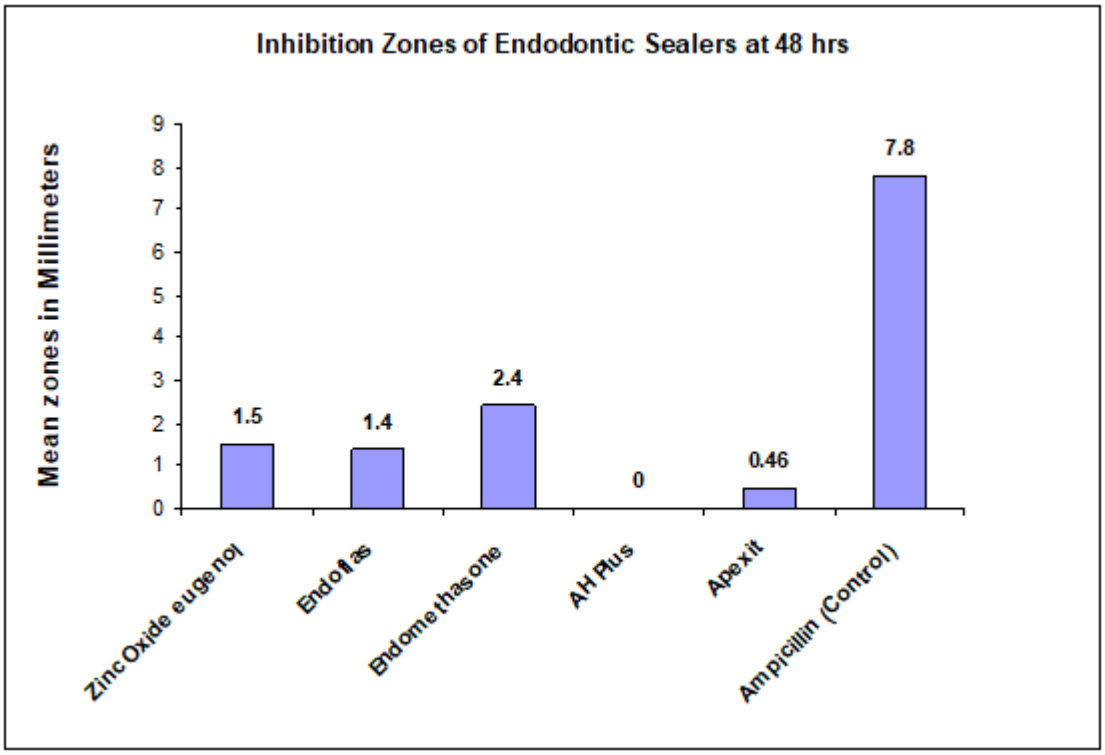
Inhibition zones in Millimetres at 48 Hours

	Specimens									
Sealers	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Zinc Oxide eugenol	1	1	2	1	2	1	2	2	1	2
Endoflas	1	2	2	1	2	1	2	1	1	1
Endomethasone	2	2	2	3	3	2	2	2	3	3
AH plus	-	-	-	-	-	-	-	-	-	-
Apexit	1	0.5	0.5	0.5	0.5	0.5	0.5	0.2	0.2	0.2
Ampicillin (Control)	8	8	8	7	8	7	8	8	8	8

Table: 1 Mean, standard deviation and test of significance of mean values between different study groups

Sealers	Mean ± S.D	P-value	Significant # groups at 5% level
Zinc Oxide eugenol	1.50 ± 0.53	<0.0001 (Sig)	I vs III (P=0.045) I vs IV (<0.0001) I vs V (P=0.03) I vs VI (P-0.0015) II vs III (P=0.03) II vs IV (P=0.0001 II vs V (P=0.003) II vs VI (P=0.0015) III vs III (P=0.03) III vs IV (P=0.0001) III vs V (P=0.0015) III vs VI (P=0.0015) IV vs III (P=0.0001) IV vs IV (P=0.0001 V vs V (P=0.0015)
Endoflas	1.40 ± 0.52		
Endomethasone	2.40 ± 0.52		
AH plus	0.00 ± 0.00		
Apexit	0.46 ± 0.24		
Ampicillin (Control)	`		





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 Apexit ($P<0.05$). Also, the mean value in Apexit 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. E. faecalis can survive with even scant amounts of substrate and without the support of other microorganisms, and grow to establish mono-infections that are difficult to eradicate using conventional root canal procedures. 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 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. Hence, E. faecalis was selected as the test microorganism in this study also.[8] Approximately one third of the canals of root filled teeth with persistent periapical lesions have shown high proportion of Enterococcus faecalis. Significant increase in E. faecalis contamination in retreatment cases has been shown due to the microorganism entering the canal during treatment, owing to an inadequate aseptic technique or because of a poor temporary seals between appointments.[9]

Enterococci are present more frequently in samples from vital cases and in teeth with extensive coronal destruction suggesting that they may enter the root canal from carious lesions and the gingival sulcus which appear to be normal oral habitats of these microorganisms. 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 Apexit 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 diffusing 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. 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.

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. A draw back of the agar diffusion test is that some materials diffuse more readily than others through an agar medium. As noted by Vibha H, a readily diffusing material would tend to show a greater inhibitory effect or larger zone of inhibition, this would not necessarily reflect its antibacterial effect. However, 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, the agar diffusion test has been recommended by Vibha H.[10]

The results obtained by Saha S, Samadi F, Jaiswal JN, Ghoshal U 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.[11]

In 2006, using the agar diffusion method, Aravind reported the inefficiency of calcium hydroxide based sealer in inhibiting Enterococcus faecalis and Candida albicans. In the present study, the antimicrobial activity of the calcium hydroxide - based material (Apexit) 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.[7]

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.[12]

In several studies, 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 and it is conceivable that it plays a major role within the activity of ZOE-based sealers.[11]

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 .

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.[13]

A gradual, continuous release of formaldehyde from the paraformaldehyde containing Endomethasone would account for the sustained antibacterial activity. Kaplan et al found the most effective antimicrobial sealers contain eugenol and formaldehyde. Without these agents, sealers were absolutely ineffective against E. faecalis.[11]

According to Peciuliene et al root canal filling materials, gradually lose their antibacterial activity in the root canal to an extent that allows survival and even growth of bacteria. These bacteria namely Peptostreptococcus, Fusobacterium nucleatum, Actinomyces, Lactobacillus, Enterococcus faecalis etc must be ecologically strong in such a way that they can survive in the environment of incompletely filled root canals where the availability of nutrients may often be limited, compared with primary apical periodontitis.[14]

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.

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.

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

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. Apexit was less effective against E .feacalis AH plus showed no antimicrobial activity on E. faecalis.

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