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Comparative Evaluation of the Antimicrobial Efficacy of Five Endodontic Root Canal Sealers Against Enterococcus faecalis and Candida albicans

An In vitro Agar Disc Diffusion Test

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ABSTRACT

The present in-vitro study was undertaken to evaluate the antimicrobial efficacy of a traditional zincoxide eugenol based sealer(Tubliseal) with a iodoform incorporated zincoxide eugenol based sealer (Endoflas FS), a calcium hydroxide based sealer (Apexit) and the epoxy resin based sealers (AH PLUS and RC Seal), against the micro organisms Enterococcus faecalis and Candida albicans. The method employed to test the antimicrobial efficacy was the Kirby-Bauer method (Agar Disc Diffusion). The sealers were mixed according to the manufacturer's instructions and 0.1 ml of each sealer was placed on the sterile paper discs. The diameter of the zones of inhibition was measured in millimeters with the help of an inhibition zone measuring scale and the values were recorded.

The antimicrobial efficacy of an iodoform incorporated zincoxide eugenol based sealer, Endoflas FS against Enterococcus faecalis and Candida albicans was statistically superior to the rest of the test groups. Endoflas FS performed far better than even the controls being employed (Amoxycillin and Nystatin) respectively. Tubliseal, a zincoxide eugenol based sealer also showed significant antimicrobial properties, but was statistically inferior to Endoflas FS Apexit, a calcium hydroxide based sealer did not show significant antimicrobial efficacy against both Enterococcus faecalis and Candida albicans. AH PLUS and RC seal, epoxy resin based sealers showed no antimicrobial properties whatsoever.

INTRODUCTION

The main objectives of endodontic therapy are to eliminate bacteria from the root canal and to prevent regrowth of residual microorganisms.

Root canal fillings consist of a core, usually guttapercha and a sealer. The sealer may be a zinc oxide eugenol based sealer, calcium hydroxide based sealer or resin based sealer. These sealers along with the use of guttapercha cones are one of the most reliable methods for filling the root canal space.

The overall success rate of endodontics can be improved by sealers that exhibit both excellent sealing ability as well as having antimicrobial properties. This will enable the sealer to cope better with persisting residual infection and bacteria reentering from the oral cavity.

Bacteria colonizing the root canal system may interact with the host tissue and cause periradicular endodontic disease. Disinfection of the root canal system, followed by filling with a tissue compatible material, usually allows periradicular healing to occur. The major function of sealers apart from holding gutta-percha points together should be to exhibit antimicrobial properties.

Endodontic sealers with antimicrobial properties can be beneficial to decrease or eliminate microorganisms

from the root canal space and thereby provide a better root canal therapy.

The zinc oxide eugenol based sealers exhibit their antimicrobial effects due to the eugenol content. Iodoform incorporated zinc oxide eugenol sealers exhibit enhanced antimicrobial effect due to the presence of iodoform a potent bactericidal agent and eugenol. Calcium hydroxide sealers are said to cause the antimicrobial effect due to the dissociation into calcium and hydroxyl ions and their high alkaline pH. The resin based sealers exhibit minimal antimicrobial effects mainly due to the absence of formaldehyde in most of the newer preparations. The microorganisms commonly isolated from failed root canal treated teeth are Enterococcus faecalis, Candida albicans and Actinomyces israelii.

It is important for us to use a sealer which has effective antimicrobial properties against these microorganisms in order to achieve a successful root canal therapy. This along with proper cleaning and shaping will help us in achieving the main goal of complete elimination of bacteria and the colonization of residual microorganisms.

Hence, the objective of this invitro study was to analyze the antimicrobial effect of a traditional zinc oxide eugenol based sealer (Tubliseal) with a iodoform incorporated zinc oxide eugenol based sealer (Endoflas FS), a calcium hydroxide based sealer (Apexit) and the epoxy resin based sealers (AH PLUS and R.C Seal), against the microorganisms Enterococcus faecalis and Candida albicans using a Agar Disc Diffusion Test.

MATERIAL AND METHODS

This study was done in the Department of Conservative Dentistry & Endodontics, Meenakshi Ammal Dental College & Hospital in association with the Department of Microbiology, Meenakshi Ammal Dental College & Hospital.

Five root canal sealers were selected for the study, of which Endoflas FS (Sanlor, Colombia) was a iodoform incorporated zincoxide eugenol sealer, Tubliseal (Kerr Dental Corporation, USA) was a zincoxide eugenol based sealer, AH plus (Dentsply Detrey, USA) and RC seal (Denfills, India), were resin based sealers and Apexit (Ivoclar Vivadent, Lichetenstein) was a calcium hydroxide based sealer.

The components of the different sealers are

Tubliseal

Base Paste	Catalyst Paste
Zincoxide	Eugenol
Oleo resins	Polymerized resin
Bismuth trioxide	
Thymoliodide	
Oil and Waxes	
Barium sulphate	

Endoflas FS

Powder	Liquid
Zincoxide	Eugenol
Iodoform	
Calcium hydroxide	
Barium sulphate	

Apexit

Base	Activator
Calcium hydroxide	Trimethyl hexadiol disalicylate
Zincoxide	Bismuth carbonate
Calcium oxide	Bismuth oxide
Silicon dioxide	Hydrogenised colophony
Zinc stearate	Tricalcium phosphate
Tricalcium phosphate	Zinc stearate
Poly dimethylsiloxane	1, 3 Butane diol disalicylate

AH Plus

Paste A	Paste B
Epoxy resins	Amines
Calcium tungstate	Calcium tungstate
Zirconium oxide	Zirconium oxide
Silica	Silica
Iron oxide pigments	Silicone oil

The strains of microorganisms used were standard strains of Enterococcus faecalis and Candida albicans obtained from the Dr. A. L Mudaliar Postgraduate Institute of Basic Medical Sciences, Taramani, Chennai. The four media used for Enterococcus faecalis were Brain Heart Infusion Broth, Mueller Hinton Agar, Blood Agar and MacConkey Agar and the two media used for Candida albicans are Brain Heart Infusion Broth and Sabouraud Dextrose Agar.

Brain Heart Infusion Broth is used for cultivation of the test microorganisms (Enterococcus faecalis and Candida albicans), Blood Agar is used as an enriched media for the growth of Enterococcus faecalis, Mueller Hinton Agar is the growth media for testing antibiotic susceptibility of Enterococcus faecalis, and Sabouraud Dextrose Agar is a selective medium for the growth of Candida albicans.

Amoxycillin discs (Span Diagnostics, India) were used as the control disc for Enterococcus feacalis and Nystatin discs (Span Diagnostics, India) were used as the control disc for Candida albicans.

Preparation of the specimens

The present study is carried out following the methods described by Kirby, Bauer, Sherris and Turck (1966)[6]. In the Kirby-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. Upon incubation at 37C for 16 to 18 hours the microorganisms grow on the surface of the plate except where the antibiotic concentration in the gradient around each disc is sufficiently high to inhibit growth.

Following incubation, the diameter of the zone of inhibition around each disc is measured in millimeters with the help of an inhibition zone measuring scale.

Preparation of the medium for Enterococcus faecalis

The prepared Mueller Hinton Agar medium was poured with the help of sterilized pipettes of 20ml capacity into sterilized petri dishes on a flat horizontal surface to a depth 4mm (20 ml). The poured petri dishes were stored at + 4C in a refrigerator and used within 1 week of preparation.

Four morphologically similar colonies of Enterococcus faecalis was touched with a sterile wire loop and the growth was transferred to a sterile test tube containing 1.5ml of sterile Brain Heart Infusion Broth and incubated at 37C in an incubator for about 2-4 hours to produce a bacterial suspension of moderate turbidity. The density of the bacterial suspension is standardized by comparing the broth at a density equivalent to the barium sulphate standard of 0.5 McFarland units, which is equivalent to 1.5×10^8 Colony Forming Units per milliliter (CFU/ml).

A sterile cotton swab was dipped into the bacterial suspension and the surplus removed by rotating the swab on to the side of the test tube used. The Mueller Hinton Agar medium was inoculated by even streaking of the cotton swab over the entire surface of the plate in three directions.

Preparation of the Medium for Candida albicans

The prepared Sabouraud Dextrose Agar was poured into sterilized pertri dishes with the help of sterile pipettes of 20ml capacity on the flat horizontal surface to a depth of 4mm (20mm). The poured petri dishes are stored at +4C in a refrigerator and used within 1 week of preparation.

Four morphologically similar colonies of Candida albicans was touched with a sterile wire loop and the growth was transferred to a sterile test tube containing 1.5ml of sterile Brain Heart Infusion Broth and incubated at 37C in an incubator for about 2-4 hours to produce a fungal suspension of moderate turbidity.

The density of the fungal suspension is standardized by comparing the Brain Heart Infusion Broth at a density equivalent to the barium sulphate standard of 0.5 McFarland units, which is equivalent to 1.5×10^8 Colony Forming Units per milliliter (CFU/ml).

A sterile cotton swab was dipped into the suspension and the surplus was removed by rotating the swab to the sides of the test tube used. The Sabouraud Dextrose Agar medium is incubated by even streaking of the swab over the entire surface of the plate in three directions.

Preparation of the discs:

After the inoculum was dried, five sterile discs (6mm in diameter) were applied with the help of sterile forceps and pressed gently to ensure even contact with the medium. The sealers are mixed according to manufacturer's instructions and 100 microliter (0.1ml) of each sealer is placed on the sterile paper disc with the help of micropipettes. The Amoxycillin discs are used as controls in the petri dishes inoculated with Enterococcus faecalis and Nystatin control discs was placed in the petri dishes inoculated with Candida albicans.

Interpretation of the results:

The petri dishes containing the sealer impregnated discs along with the microorganisms namely Enterococcus faecalis and Candida albicans was incubated for 18 hours at 37C in an incubator.

The diameter of the zone of inhibition of growth was measured in millimeters with the help of a 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.

The results were tabulated and statistically analyzed by Kruskal- Wallis one way ANOVA used to calculate the p- value and 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. The disc impregnated with the sealer, which exhibited the maximum zone of inhibition was considered as having the most efficient antimicrobial activity.

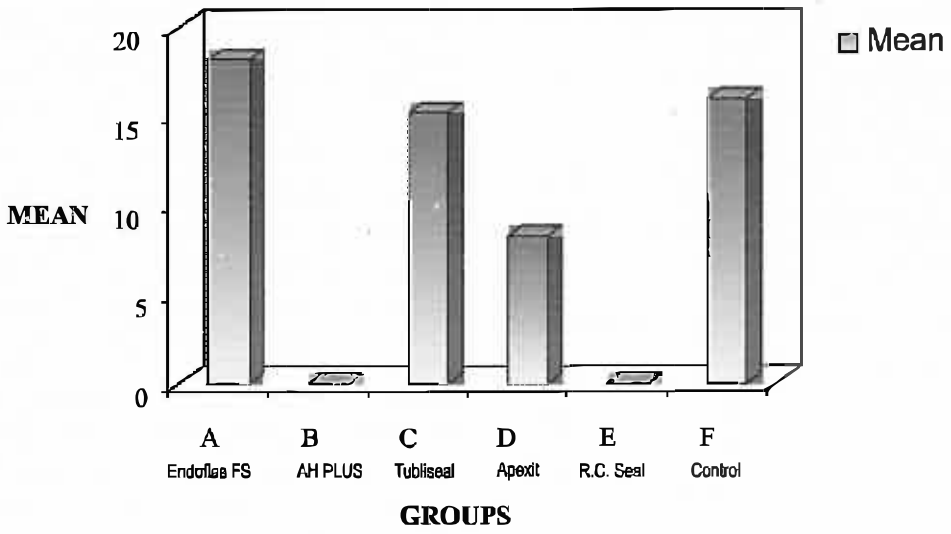
Table 1:

Mean Standard Deviation and Test of Significance of Mean Values between different study groups: Organism Tested - Enterococcus faecalis

Group	Mean ± S.D	p-value*	Significant# groups at 5% level
A (Endoflas FS))	18.2 ± 0.4		A Vs B,C,D,E,F
B (AH PLUS)	0.0 ± 0.0		B Vs C,D,F
C (Tubliseal)	15.2 ± 0.4		C Vs D,E,F
D (Apexit)	8.3 ± 0.3		D Vs E,F
E (R.C. Seal)	0.0 ± 0.0		E Vs F
F (Control)	16.0 ± 0.2	< 0.0001 (Sig)	

* Kruskal Wallis one-way ANOVA was used to calculate the p-value

Mann-Whitney U-test was used to identify the significant groups at 5% level after correcting the p-values for multiple comparisons by Bonferroni correction method



Inference:

Endoflas FS (Group A) showed the highest antimicrobial efficacy against Enterococcus faecalis, followed by the control (Group F), Tubliseal (Group C), Apexit (Group D), AH PLUS(Group B) and RC Seal (Group E) respectively.

The antimicrobial efficacy between all the groups tested was statistically significant except between AH PLUS and RC Seal which showed no antimicrobial efficacy.

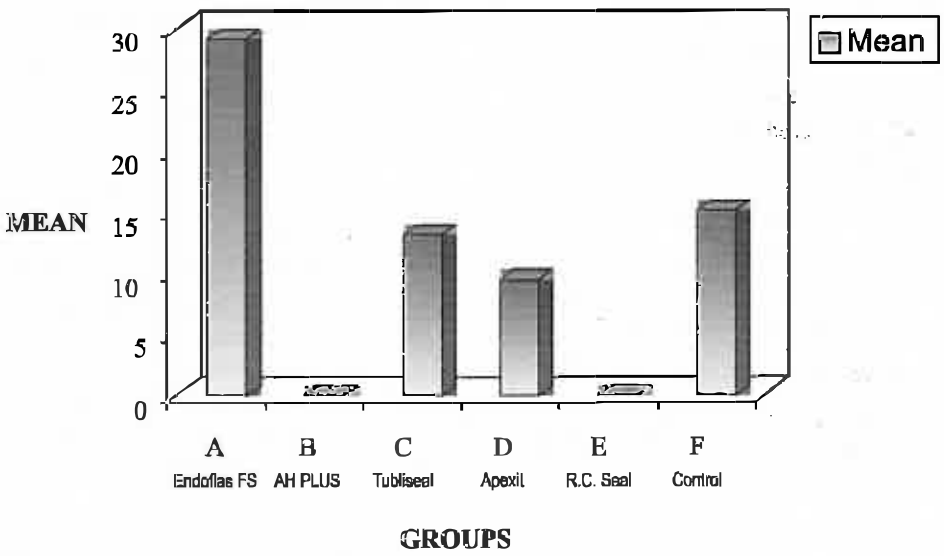
Table 2:

Mean Standard Deviation and Test of Significance of Mean Values between different study groups: Organism Tested - Candida albicans

Group	Mean ± S.D	p-value*	Significant# groups at 5% level
A (Endoflas FS)	29.2 ± 0.3		A Vs B,C,D,E,F
B (AH PLUS)	0.0 ± 0.0		B Vs C,D,F
C (Tubliseal)	13.2 ± 0.3		C Vs D,E,F
D (Apexit)	9.5 ± 0.4		D Vs E,F
E (R.C. Seal)	0.0 ± 0.0		E Vs F
F (Control)	15.1 ± 0.2	<0.0001 (Sig)	

* Kruskal Wallis one-way ANOVA was used to calculate the p-value

Mann-Whitney U-test was used to identify the significant groups at 5% level after correcting the p-values for multiple comparisons by Bonferroni correction method



Inference:

Endoflas FS (Group A) showed the highest antimicrobial efficacy against Candida albicans, followed by the control (Group F), Tubliseal (Group C), Apexit (Group D), AH PLUS(Group B) and RC Seal (Group E) respectively.

The antimicrobial efficacy between all the groups tested was statistically significant except between AH PLUS and RC Seal which showed no antimicrobial efficacy.

DISCUSSION

Chemo-mechanical preparation is undoubtedly 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[2]. 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[3]. 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 failure is the persistence of microorganisms in the apical part of root filled teeth[4]. However, most treatment failures are caused by very few bacteria and the yeast, Candida albicans. Sundqvist et al[5] recovered numerous species of anaerobic bacteria from failed root canal systems. Results of the study showed that 38% of failed root canal treated teeth were contaminated by the bacteria Enteroccocus faecalis and the yeast commonly isolated was Candida albicans.

Anaerobic bacteria thrive in an oxygen free environment that contains even very limited amount of nutrients[6]. Due to the low oxygen content of a closed root space, anaerobic bacteria, especially are given an ideal atmosphere to live and grow[7]. The ecological conditions (nutrients, oxygen tension, and bacterial relationships) in the root canal are favourable for the growth of anaerobic bacteria capable of fermenting amino acids and peptides, whereas bacteria that mainly obtain energy by fermenting carbohydrates are more restricted by the lack of such nutrients[8].

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[3]. Hence the ideal objective of the root canal treatment is not only the elimination of infection but also preventing reinfection of the treated root canal system.

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.

Zincoxide 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[9].

In order to improve the antimicrobial efficacy of zincoxide 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[9]. This has led to the introduction of several calcium hydroxide based sealers namely Calciobiotic root canal sealer, Sealapex and Apexit.

Epoxy resin based sealers (AH 26) were introduced because of its advantages such as high radiopacity, low solubility, slight shrinkage and antimicrobial efficacy[9]. The antimicrobial efficacy of AH 26 is attributed to the release of formaldehyde. However, formaldehyde is a known mutagenic and carcinogenic agent[10]. Hence, this sealant has been replaced by AH

PLUS an improved epoxy resin sealant. AH PLUS has retained the epoxy resin "glue" of AH 26 and also is free of formaldehyde release[6].

Hence, the objective of this invitro study was to analyze the antimicrobial effect of a traditional zincoxide eugenol based sealer (Tubliseal) with a iodoform incorporated zincoxide eugenol based sealer (Endoflas FS), a calcium hydroxide based sealer (Apexit) and the epoxy resin based sealers (AH PLUS and RC Seal), against the microorganisms Enterococcus faecalis and Candida albicans using a Agar Disc Diffusion Test.

The techniques employed to assess antimicrobial efficacy include Broth Dilution, Agar Disc Diffusion, Agar Disc Dilution, Spiral Gradient Test, E-Test and Automated Antimicrobial Testing Systems. Each of these techniques has their own inherent advantages and disadvantages[5].

Broth Dilution method includes both Microdilution and Macrodilution. The advantage of this method is that it evaluates both quantitatively and qualitatively, whereas the disadvantage being that chemical property of the material being tested can be altered and it is time consuming especially when the clinician wants to know the susceptibility of an organism to a number of antibiotics.

The Spiral Gradient Test, which is based on the agar dilution derivations, has the ability to test many organisms at a given time. Although, it is time saving, it is rather technique sensitive and needs special kits currently unavailable in India.

The E-Test, based on the agar diffusion derivations, offers the convenience of the disc diffusion procedure. It has the ability to generate minimum inhibitory concentration data, but is also technique sensitive like the previous test and needs special kits currently unavailable in India.

The Automated Antimicrobial Testing Systems, Vitek and Walkaway system, eliminates the need for overnight incubation and hence time saving. The limitations in these systems are that the chance of misinterpretation is high due to the turbid metric method of analysis and they are very expensive and currently unavailable in India.

Traditionally, Agar Diffusion method and Agar Dilution method are commonly employed for detecting antimicrobial susceptibility. In our study, Kirby-Bauer method (Agar Disc 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 homogenously dissolved and is a difficult and slow technique. Hence, we chose the Agar Disc Diffusion method, as in this method the chemical properties of the sealers are not changed[11] and the antimicrobial resistance can be detected by challenging bacterial isolates with antimicrobial discs[1]. Moreover, this is an easy and less technique sensitive method.

Amoxycillin was chosen as the control against Enterococcus faecalis as it is a potent bactericidal causing lysis of the bacterial cell wall[12]. Nystatin was chosen as the control against Candida albicans as it combines with the fungal cell membrane and interferes with vital cellular process thereby exhibiting both fungicidal and fungistatic activity[13].

Amongst the test groups, Endoflas FS showed statistically significant antimicrobial efficacy against both Enterococcus faecalis and Candida albicans when compared with rest of the groups tested. Its efficacy was even superior to the controls being employed namely Amoxycillin and Nystatin.

The superior antimicrobial efficacy of Endoflas FS (18.2 0.4mm against Enterococcus feacalis and 29.2 0.3mm against Candida albicans) could be attributed to the presence of eugenol and iodoform in its composition. In spite of the presence of eugenol in Tubliseal the antimicrobial efficacy was significantly inferior (15.20.4mm against Enterococcus feacalis and 13.20.3mm against Candida albicans) to Endoflas

FS. Thus, Endoflas FS probably produced larger zones of inhibition because of the added presence of iodoform.

Eugenol, a phenolic compound acts on microorganisms by protein denaturation whereby the protein becomes non-functional[14]. The antimicrobial effect of zincoxide eugenol sealers can be gauged by the results of the following studies. Andre Mickel et al[6] found that zincoxide eugenol based sealant Roth 811 showed larger zone of inhibition against Enterococcus feacalis when compared with calcium hydroxide based sealer Seal Apex and epoxy resin based sealer AH PLUS. Cox and coworkers[15] have shown that zinc oxide eugenol is also an effective bactericidal agent against bacterial species like Staphylococcus aureus and Streptococcus viridans. The results were apparently due to the eugenol content because zincoxide alone had no antimicrobial activity against microorganisms. Hume[16] has shown that in the dentin immediately beneath the zincoxide eugenol, the concentration of eugenol is sufficient to inhibit bacterial metabolism. In studies done by Fisher F.J[17] it was found that in carious dentine zincoxide eugenol was found to be a more effective antibacterial agent than calcium hydroxide. Eugenol being a phenolic compound is also effective against mycotic cells and vegetative forms[18].

Iodoform acts by the liberation of iodine, which is an oxidizing agent[19]. Oxidizing agents like iodine can irreversibly oxidize and thus inactivate essential metabolic compounds like protein, which has been accounted for the antimicrobial action[20].

The calcium hydroxide based sealer, Apexit showed zones of inhibition against Enterococcus faecalis (8.3 0.3mm) and Candida albicans (9.50.4 mm), which was statistically lesser than the zones of inhibition produced by both the zinc oxide eugenol based sealers namely Endoflas FS and Tubliseal.

Esterela et al[21], hypothesized that in calcium hydroxide the antimicrobial mechanism is influenced by its speed of dissociation into calcium ions and hydroxyl ions. The antibacterial effect of Apexit is also based on its dissociative ability into calcium and hydroxyl ions[18]. This dissociation into hydroxyl ions creates a high pH environment, which inhibits enzymatic activities that are essential for microbial metabolism, growth and cellular division. Brystrom and Sundqvist[22] found that for calcium hydroxide sealers to be an efficient antimicrobial agent, it should maintain a pH level greater than 12.5. As the calcium hydroxide sealers set the pH declines to 9.14, causing it to lose its effectiveness as Enterococcus faecalis can survive at a pH below 11.5. Apexit was ineffective against Candida albicans as seen by the minimal zones of inhibition. The absence of any significant effect on Candida albicans could lead to the conclusion that the release of hydroxyl ions is not sufficient to inhibit this yeast whose optimum growth pH is 5[24].

The resin based sealers AH Plus and RC seal showed no zones of inhibition against both Enterococcus faecalis and Candida albicans. 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[6] who found AH PLUS to be ineffective against Enterococcus faecalis and Kapalan et al[18] who found AH PLUS to be ineffective against Candida albicans.

Although sterilization of the root canals and periapex is ideal, in clinical practice it is not completely possible. Regardless, all efforts should be made to reduce endodontic microbes to a minimum. When a root canal is completely instrumented and irrigated, and the patient has no clinical manifestation, there are most likely few remaining microbes within the root canal, its lateral branches, the dentine or periapex. The routinely used intracanal medications have been shown to the ineffective in killing micro organisms like Enterococcus faecalis present in the root canals. As part of the obturation process, the use of an antimicrobial endodontic sealer can be another helpful adjunct in the destruction of endodontic microorganisms.

From the present study, it is evident that the zincoxide eugenol based sealers Endoflas FS followed by Tubliseal demonstrated superior antibacterial effects against both Enterococcus faecalis and Candida albicans, compared to the calcium hydroxide based sealer Apexit. AH Plus and RC seal, epoxy resin based sealers proved to have no antimicrobial properties.

CONCLUSION

1. The antimicrobial efficacy of an iodoform incorporated zincoxide eugenol based sealer, Endoflas FS against Enterococcus faecalis and Candida albicans was statistically superior to the rest of the test groups.
2. Endoflas FS performed far better than even the controls being employed (Amoxycillin and Nystatin) respectively.
3. Tubliseal, a zincoxide eugenol based sealer also showed significant antimicrobial properties, but was statistically inferior to Endoflas FS.
4. Apexit, a calcium hydorxide based sealer did not show significant antimicrobial efficacy against both Enterococcus faecalis and Candida albicans.
5. AH PLUS and RC seal, epoxy resin based sealers showed no antimicrobial properties whatsoever.
6. The antimicrobial property of Endoflas FS can be attributed to a combination of eugenol and iodoform, while the antimicrobial efficacy of Tubliseal can be attributed to eugenol alone.

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