ORIGINAL ARTICLE

Comparative Evaluation of Antifungal Efficacy of Five Root Canal Sealers against Clinical Isolates of *Candida albicans*: A Microbiological Study

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Abstract

Aim and objective: The aim of this study was to evaluate and compare the antifungal efficacy of MTA Fillapex, Metapex, zinc oxide eugenol cement, Endomethasone, and Endoflas against *Candida albicans*.

Materials and methods: Root canal exudates of 30 patients were tested against MTA Fillapex (Angelus), Metapex (BioMed), zinc oxide eugenol (Deepak Enterprise), Endomethasone (Septodont), Endoflas FS (Sanlor Laboratories), MTA (Angelus) (positive control), and glycerine (negative control). Children with failed endodontic cases were included in the study. Tube dilution and agar diffusion methods were used to check the antifungal efficacy of the root canal sealers. In tube dilution method, 24-well culture plates containing freshly mixed material along with *Candida albicans* were used. Wells containing MTA (Angelus) along with Sabouraud dextrose agar and *Candida albicans* served as positive control while glycerine along with Sabouraud dextrose agar and *Candida albicans* served as negative control. All plates were incubated at 37°C for 24 hours. Growth of the fungi was monitored after 24 hours by the presence of the turbidity. The samples were recultured to test the experimental material using agar well diffusion method, and the Petri plates were incubated for 24 hours and 72 hours. Zone of inhibition was measured after respective time period. Paired *t* test was used for the data analysis.

Results: It was seen in tube dilution method Endomethasone showed least turbidity while maximum was shown by Metapex; similar results were seen in case of agar well diffusion method in which largest zone of inhibition was shown by Endomethasone while smallest was by Metapex.

Conclusion: It was concluded that Endomethasone showed maximum efficacy against *Candida albicans* as compared to Metapex.

Keywords: Agar-well diffusion, *Candida albicans*, Endomethasone, Metapex, MTA Fillapex, Root canal sealers, Tube dilution test, Zinc oxide eugenol.

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Introduction

Microorganisms are the main reason for occurrence of pulpal/periapical diseases.[1] The microbial environment of an infected root canal is a habitat for various bacteria, spirochetes and fungi.[2] Reminance of these microorganisms after primary root canal treatment may lead to treatment failure. It has also been seen that fungus plays a major role in the occurrence of various endodontic diseases, *Candida albicans* being the most common fungal species found in oral environment.[1]

In most instances of primary root canal infection, the prevalence of the presence of candidal species is not routinely seen, as compared to secondary or persistent infection which has 20% existence in the canal.[1] Various *in vitro* studies have shown the presence of yeast cells and hyphae within the dentinal tubules leading to a possible pathway for *Candida albicans* strains into the tubules.[3] *Candida albicans* has the ability to penetrate the tubular structure of dentine and resides within the root canal system.[4]

In case of a failed endodontic procedure, specific fungal species grow as a result of intercommunication between certain bacteria due to changed intracanal oxygen pressure and environment.[1] It has also been shown that reduction in number of certain bacteria in root canal system may lead to excessive fungal growth with low nutrient requirement.[1]

Various intracanal therapeutic drugs have been recommended for use in pediatric dentistry, in order to control the infection.[5] However, most of the available root-end fillers may not provide a completely hermetic seal. Therefore, their antibacterial and antifungal properties play a significant role in the prevention of further periradicular contamination and in reduction of bacterial/fungal count.[1]

Antibacterial properties of root canal sealers help to prevent recolonization and multiplication of microbial flora in the root canal system along with neutralization of the toxic products.

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In the present study, a comparative evaluation of antifungal efficacy of zinc oxide eugenol and calcium hydroxide-based root canal sealers was undertaken against *Candida albicans*.

Materials and Methods

The various root canal sealers used in the present study are MTA Fillapex (Angelus), zinc oxide eugenol cement (Deepak Enterprise), Metapex (Meta BioMed), Endomethasone (Septodont) and Endoflas FS (Sanlor Laboratories), and MTA (Angelus).

The study was conducted on root canal exudates collected from 30 children with age ranging from 10 years to 13 years. Patients were selected from those attending the outpatient Department of Pedodontics and Preventive Dentistry at Santosh Dental College and Hospital, Pratap Vihar, Ghaziabad.

Inclusion Criteria

Children with persistent apical periodontitis in permanent molars.

Exclusion Criteria

Local Criteria

Children with fresh endodontic treatment.

Systemic Criteria

- History of prolonged antibiotic and steroid treatment.
- Children with chronic diseases such as diabetes and allergies.
- Children with any developmental abnormality.
- Children with mental retardation, cerebral palsy, and dyslexia.

Sample Collection

The visible surface of the tooth and the extraoral areas around the mouth, that is the lips and cheeks up to the zygoma were scrubbed with 5% povidone iodine solution to maintain a sterile working environment. Dentinal shaving was collected from root canal of a permanent molar with failed endodontic treatment. The files were stored in sterile microcentrifuge tube containing saline and sent to the lab to check the presence of *Candida albicans*.

The collected dentinal shavings in saline were spread on Sabouraud dextrose agar plate and incubated at 37°C for 24 hours. Identification of yeast cells was done on the basis of colony shape and color on Sabouraud dextrose agar plate. Colony was picked up from the culture plate and was mixed with 0.5 mL of animal serum followed by incubation at 37°C for 3 hours. The slide was prepared and checked under the microscope, confirming the presence of *Candida albicans* in the form of germ tube.

Susceptibility Test

Fungal sample was then re-streaked on Sabouraud dextrose agar plate and incubated at 37°C for 24 hours. A suspension of Sabouraud dextrose broth was made to achieve a final density of 10^4 CFU/mL (colony forming unit/milliliter).

Each material was tested against a sample obtained from 30 patients. Tissue culture test plates consisting of 24 wells were used for each concentration of 50 mg/mL of different test materials and divided into following experimental groups:

- Group I—Endomethasone N
- Group II—Zinc oxide eugenol
- Group III—Metapex
- Group IV—MTA Fillapex
- Group V—Endoflas FS
- Group VI—white MTA (positive control)
- Group VII—glycerine (negative control)

White MTA was taken as positive control due to established antifungal effect, while glycerin was taken as negative control to overcome the procedural error and to standardize the fungal growth.

Tissue culture plates were incubated at 37°C for 24 hours. Growth of the fungi was monitored after 24 hours by measuring the turbidity in the wells through spectrophotometer.

Maintaining the wells, suspension was recultured to obtain a final concentration of yeast cells ranging 0.5–2.5 × 10^4 CFU/mL followed by spreading of the yeast cells on Sabouraud dextrose agar plate. Two wells per plate were prepared of 4 mm diameter. In each well, 40 µL of drug with following concentrations—(1) zinc oxide 50 mg/mL in eugenol; (2) endomethasone 50 mg/mL in eugenol; (3) Endoflas 50 mg/mL in eugenol; (4) MTA 50 mg/mL in water (5) Metapex 50 mg/mL in ethanol; and (6) MTA Fillapex 50 mg/mL in ethanol—was added. Positive control and negative control were set for every experiment. Plates were kept in incubator at 37°C, and zone of inhibition was analysed after 24 hours and 72 hours.

Statistical Analysis

The software version used for the statistical analysis was SPSS (statistical package for social sciences version) 20.0. SPSS is a program for statistical analysis. Bivariate statistics were used consisting of mean and paired *t* test. The values were reported in number (*n*), percentage (%) and mean (*x*). The statistical tests used were paired *t* test for difference between the mean values. The level of significance was taken at 5% ($p \leq 0.05$).

Results

In the present study using tube dilution test, the mean optical density of different test materials in increasing order was Endomethasone (3.2083) < Endoflas (4.0310) < zinc oxide eugenol (4.1893) < MTA Fillapex (6.4867) < mineral trioxide aggregate (8.2133) < Metapex (8.2223) (Fig. 1).

On comparing the optical density of all the test materials, the optical density of Endomethasone (3.2083 OD) was minimum while maximum optical density mean (8.2223 OD) was shown by Metapex concluding that Endomethasone restricted the growth of

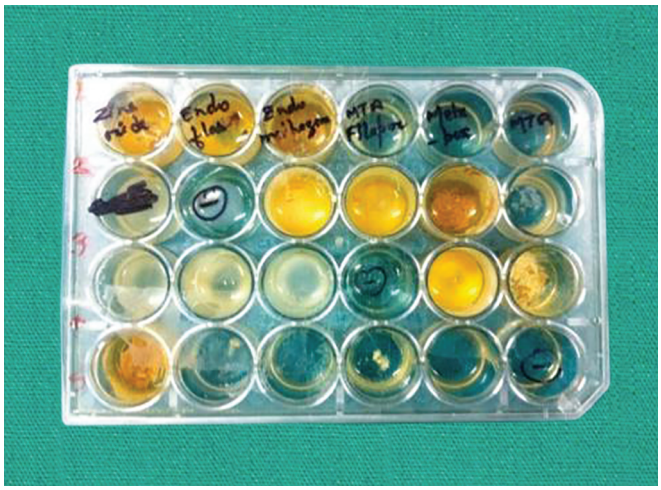
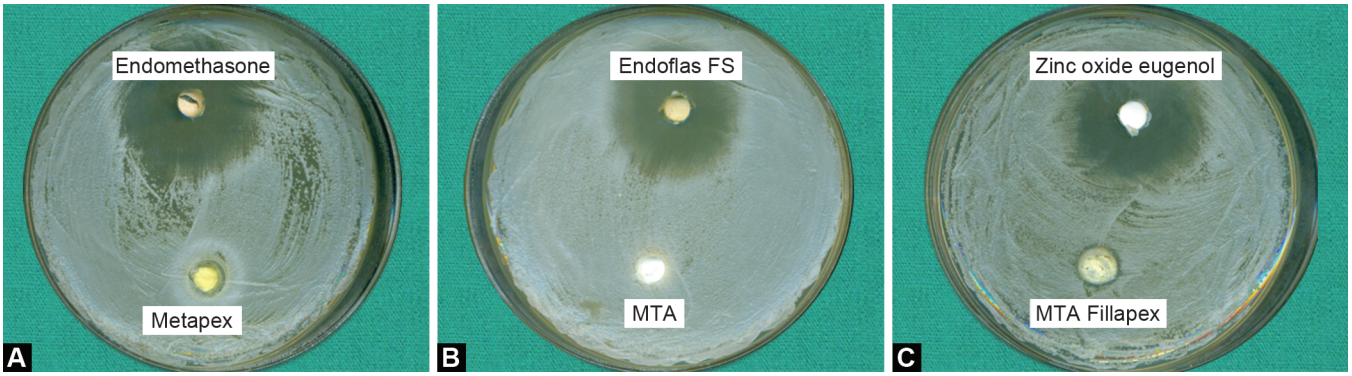


Fig. 1: Tube dilution test



Figs 2A to C: Agar well diffusion method: (A) Zone of inhibition from Endomethasone and Metapex; (B) Zone of inhibition is from Endoflas FS and MTA; (C) Zone of inhibition is zinc oxide eugenol MTA Fillapex

Table 1: Means of optical densities of the various root canal sealers used for the study

	n	*Minimum (OD)*	*Maximum (OD)*	*Mean*	*Std deviation*
Zinc oxide eugenol OD	30	3.56	4.86	4.1893	0.34426
Endoflas OD	30	3.15	4.81	4.0310	0.39367
Endomethsone OD	30	2.28	3.79	3.2083	0.39825
MTA OD (Positive)	30	7.45	9.45	8.2133	0.52549
MTAFillapex OD	30	5.84	7.43	6.4867	0.40441
Metapex OD	30	7.31	9.21	8.2223	0.46999
Glycerin OD (Negative)	30	12.36	14.58	13.6110	0.47946

Table 2: Means of zone of inhibition measured from the samples of the various root canal sealers used for the study

	n	*Minimum (mm)*	*Maximum (mm)*	*Mean*	*Std deviation*
Zinc oxide eugenol (mm)	30	13.00	19.50	15.3833	1.53513
Endoflas (mm)	30	14.00	19.50	16.4167	1.43289
Endomethsone (mm)	30	14.00	20.50	16.7500	1.75062
MTA (positive)	30	2.50	5.00	3.6833	0.62261
MTA Fillapex (mm)	30	4.50	8.50	6.3667	1.04166
Metapex (mm)	30	3.00	5.00	3.9667	0.69398
Glycerin (mm) (negative)	30	0	0	0	0

Candida albicans to the maximum level in Sabouraud agar broth by showing least turbidity in comparison with Metapex which showed maximum optical density (8.2223 OD) showing minimum inhibition of *Candida albicans* in Sabouraud agar broth by showing maximum turbidity.

In agar well diffusion method, the mean zone of inhibition for all the test materials in decreasing order was: Endomethasone (Fig. 2A) (16.7500 mm) > Endoflas (Fig. 2B) (16.4167 mm) > zinc oxide eugenol (Fig. 2C) (15.3833 mm) > MTA Fillapex (Fig. 2C) (6.3667 mm) > Metapex (Fig. 2A) (3.9667 mm) > MTA (Fig. 2B) (3.6833 mm). Mean maximum zone of inhibition was shown by Endomethasone (Tables 1 and 2, Figs 3 and 4).

Discussion

In case of failed endodontic procedures, bacterial persistence is too high, and species apart from bacteria that are isolated from the failed root canal are fungi.[6]

Fungi are found in small numbers in oral microflora, and the most commonly found fungal pathogens are of genera *Candida* and *Aspergillus*.[7] The fungal infections are endogenous in nature; they usually arise due to imbalance of microflora as a result of immunosuppression or disruption of protective barrier system. Host present with some predisposing factors are more prone to fungal infection.[7]

An endodontic sealer with good antimicrobial properties and sealing ability helps to eliminate microbial inhabitant from the canal.[8] The stabilization of infected root canal environment using certain root canal sealers helps to control candida species level.[9]

In this study, five different root canal sealers were used (Endomethasone, Endoflas, zinc oxide eugenol, MTA Fillapex, Metapex) against *Candida albicans*.

These root canal sealers were selected for the study as their antibacterial properties have been studied by various researchers, but no comparative antifungal properties of these materials had been discussed.

Thus, this study was taken up to evaluate and compare the antifungal property of those root canal sealers against *Candida albicans*.

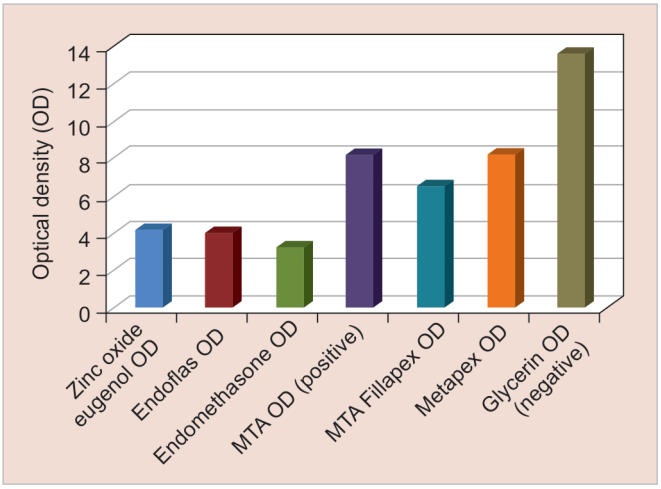


Fig. 3: Comparison of mean of optical density of experimental materials against *Candida albicans* to white MTA (positive control)

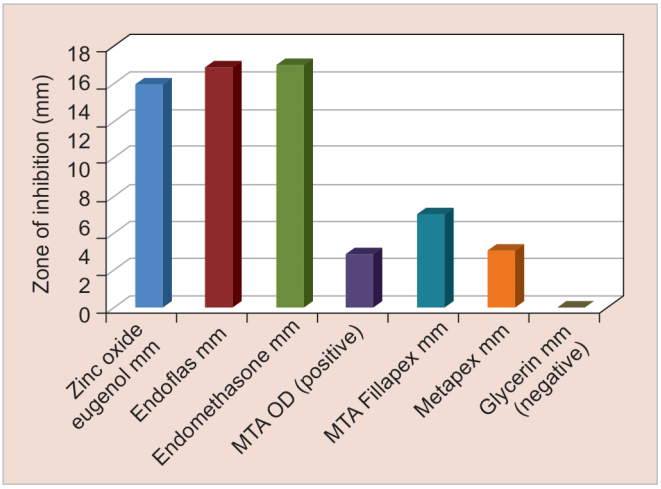


Fig. 4: Comparison of mean of zone of inhibition of experimental materials against *Candida albicans* to white MTA (positive control)

The methodologies that were followed were agar well diffusion method and tube dilution test.

Agar well diffusion is a widely accepted method to check the antimicrobial effect of dental materials since it allows direct comparison of materials against the microbe. It also gives an indication as to which material has the ability to show maximum antimicrobial activity. However, there are certain disadvantages of this method; as this methodology is diffusibility dependent, the materials which are more diffusible might show larger zone of inhibition and antimicrobial activity; apart from that, there are other drawbacks which can vary the results such as incubation time, lack of contact between experimental material and agar media.[10]

Tube dilution test is an effective method for checking antifungal efficacy as it provides direct contact between fungal cells and the materials. The medium used for the fungal growth was Sabouraud broth which has an acidic pH (5.6), favorable for fungal growth, and it inhibits the growth of most of other bacteria favoring direct interaction between materials and the fungal cells.[11]

Badr et al. did a comparative study in which Endofill, a zinc oxide eugenol-based sealer, showed maximum antifungal efficacy against *Candida albicans* with least efficacy shown by Apexit, a calcium hydroxide-based sealer.[12] The result of the test was similar to our study.

Many researchers like Leonardo (2000), Michel (2003) and Reddy (2007) have attributed antimicrobial effect of zinc oxide eugenol cement to eugenol.[13]

Kaiwar et al. carried out a study to check antimicrobial effect of Endoflas, Metapex, AH plus, AH 26 against *Enterococcus fecalis* and observed that Endoflas had maximum antimicrobial effect.[8]

Though the previous studies were on antibacterial properties of Endoflas FS, similar result was seen in the present study showing its antifungal activity against *Candida albicans*.

Endoflas contains eugenol and iodoform in its composition. Eugenol is a phenolic compound that helps to denature the protein content of the microorganism resulting in making its protein nonfunctional, while iodoform acts as oxidizing agent liberating iodine which inactivates the protein content of microorganism.[8]

Saha et al. performed a comparative evaluation of antimicrobial efficacy of three root canal sealers, Endomethasone, Apexit and AH 26 using agar well diffusion method. Endomethasone, a zinc oxide eugenol-based sealer showed maximum zone of inhibition affirming the highest antimicrobial activity.[14]

Endomethasone was found to be the most effective of all test materials used in present study too in both the methodologies, which could be due to combined effect of corticosteroid, formaldehyde and zinc oxide eugenol, thus increasing its antifungal efficacy.[15]

Reyhani et al. did a comparative study to check antimicrobial effect of Apexit plus, Ephiany, MTA Fillapex, and Dorifill against *Enterococcus feacalis* using agar well diffusion method at different time intervals of 3, 5, and 7 days and found that maximum antibacterial effect was shown by MTA Fillapex in comparison with Apexit plus, Ephiany, and Dorifill.[16]

MTA Fillapex combines the beneficial effects of MTA along with resins resulting in superior properties including adhesiveness, dimensional stability, working time radiopacity, flow and antibacterial effect, while disalicylate helps to reduce the inflammatory response.[17]

In the present study, MTA Fillapex had shown similar effect on *Candida albicans* as seen on their bacterial counterpart.

Harni Priya et al. did a comparative evaluation of antimicrobial potential of root canal filling materials such as zinc oxide eugenol, Vitapex, calcium hydroxide and Metapex against the microflora of infected nonvital primary teeth in which Metapex showed no inhibitory effect against *Candida albicans* while zinc oxide eugenol showed maximum antifungal activity.[18]

Bystrom et al. found that calcium hydroxide-based sealer, to be effective in its action, had to maintain pH more than 12.5 which usually falls to 9.5 on setting, resulting in decline in antimicrobial activity.[19] Similar results were seen in the present study; Metapex (calcium hydroxide-based sealer) showed minimum antifungal efficacy against *Candida albicans*.

The result of the study showed that the eugenol-based test materials had better antifungal effect in comparison with calcium hydroxide-based sealers.

In addition, the antifungal efficacy of root canal sealers diminished after 24 hours of incubation.

In the present study, the results obtained were contradictory to the results obtained regarding antifungal efficacy of MTA as work done by different researchers. This might be due to methodology

followed in the study where the dispersion of MTA was less as compared to rest of the eugenol-based materials.

Conclusion

The study design for the experiment was *in vitro*, where endomethasone showed maximum antifungal efficacy while Metapex showed minimum, and the data obtained might differ in case of *in vivo* study due to complex microbial environment of the oral cavity. Future research also needs to focus on the *in vitro* experimental methodologies so as to create similar oral environment to test the exact nature of sealers to know the way they act on fungal microbiota.

Clinical Significance

The experiment was performed to compare and evaluate the efficacy of different root canal sealers against clinical isolates of *Candida albicans* which could be helpful in treating cases of persistent endodontic infection by taking into consideration the potency of these root canal sealers along with other aseptic protocols.

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