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# Antimicrobial Activity of MTA Fillapex, Real Seal SE, Acroseal and Zinc oxide Eugenol Sealers against Enterococcus Faecalis and Candida Albicans

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***Abstract:***
***Aim:****The aim of the present study was to evaluate the antimicrobial activity of different root canal sealers (MTA Fillapex, Real Seal SE, Acroseal and Zinc Oxide Eugenol) against Enterococcus fecalis and Candida albicans.*
***Materials and method*** *: The antimicrobial effect of different root canal sealers was evaluated by the direct contact with agar diffusion method. The freshly prepared mixed sealers were placed in prepared wells of agar plates inoculated with E. faecalis and C.albicans separately. All plates were incubated for 7 days at 37°C under aerobic conditions, and zones of inhibition were measured at 24, 48, 72 hours and 7 days. All statistical analyses were performed with the SPSS (PC version 10 software, IBM, NY, USA) statistical software.*
***Result :*** *The sealer MTA fillapex showed highest antimicrobial activity against C. albicans (16.0 ± 0.20 mm) followed by Real Seal SE (15.2 ± 0.20 mm). Amongst the tested sealers only MTA Fillapex showed antimicrobial activity against E. faecalis (12.40± 0.20 mm). No antimicrobial activity was observed with zinc oxide eugenol and Acroseal.*
***Conclusion :*** *Amongst the tested sealers MTA Fillapex and Real Seal SE both showed antimicrobial activity against C.albicans whereas only MTA Fillapex showed antibacterial activity against E. faecalis. Acroseal and Zinc Oxide Eugenol did not show any antimicrobial activity.*
***Keywords:*** *Agar Diffusion Test, E.faecalis, C.albicans, Root canal sealers*

## I. Introduction

The main purpose of endodontic therapy is to provide an inert atmosphere and to prevent microbes from infecting or re-infecting the root canal or the periapical tissues. [1] Antimicrobial activity has been long talked about and in present scenario, a major part is played by the property and the capacity of the sealer to do the same. Hence a good root canal sealer with significant antibacterial properties would be beneficial for further reduction of residual microorganism. Candida albicans and Enterococcus faecalis are the two microbes that have been the major reason for the failure of endodontic treatment. Maximum number of retreatment cases are reported because of these two. [2,3]

The success of obturation depends upon elimination of microorganisms through mechanical cleaning and shaping, supplemented by antibacterial irrigants, adequate filling of the empty space, and use of antimicrobial dressings between appointments, if necessary. [4,5] However, these procedures do not result in complete sterility of the root canal space. [6] One of the ideal requirement of root canal sealer is antimicrobial activity. Therefore, antimicrobial agents are added to root canal sealers to improve their antibacterial effect. [7,8]

MTA fillapex (Angelus Solucxoes Odontologicas, Londrina, Brazil) is mineral trioxide aggregate (MTA) based sealer, it consists of resins, silica, and MTA. It has high radioopacity, low solubility, low expansion during setting, promotes deposition of hard tissue and known for its antibacterial effect. [9]

Real Seal SE (SybronEndo, Orange, CA) is fourth generation methacrylate resin based sealer, in that they have further eliminated the separate etching/ bonding step. [10]

Acroseal (Septodont, St. Maurdes-Fosses Cedex, France) is calcium hydroxide-based sealer, it appears to have lower solubility than other calcium hydroxide sealers, probably because of its epoxy resin component. [11]

The agar diffusion test (ADT) has been widely used to test the antimicrobial activity of root canal sealers. This method allows direct comparisons between materials and also indicates which sealers are more likely to have antimicrobial activity within the root canal system. Besides that, its results are highly influenced by the diffusibility of the material across the medium. [6,12,13] Therefore, aim of the present study is to evaluate the antimicrobial activity of different root canal sealers against E.faecalis and C.albicans using agar diffusion method.

## II. Materials And Method

The materials evaluated for antimicrobial activity were MTA Fillapex, Real Seal SE, Acroseal, Zinc Oxide Eugenol cement (SS White, Rio de Janeiro, RJ, Brazil) against E. faecalis (ATCC 24212) and C. albicans (ATCC 24433) strains.

The bacterial strains were cultivated in a brain heart infusion broth (Difco; BD Diagnostic Systems, Sparks, MD, USA). The fungal strains were cultivated in Sabouraud's dextrose agar broth (Difco; BD Diagnostic Systems) in 48 hours. The broth culture suspensions were prepared and adjusted to No. 1 McFarland standard (approximately 3 × 108 cells/mL). Aliquots of the suspension containing E. faecalis and C. albicans were spread on four different 140-mm diameter Petri dishes containing Mueller-Hinton Agar medium (Merck, Darmstadt, Germany) for each microbial strain. Excess inoculum was removed with a pipette and the inoculated plates were dried for 15 minutes at 37°C. Each plate was divided evenly into 4 sections. In each section of each plate, a well 4 mm in diameter was created with a sterile stainless steel cylinder. All the tested sealers were mixed according to the manufacturer's instructions. A sample of each freshly mixed dental material was placed into wells in each section of the plates.

All plates were incubated for 7 days at 37°C under aerobic conditions, and zones of growth inhibition were measured at 24, 48, 72 hours and 7 days using a plastic ruler and was recorded for each material. The results thus obtained were statistically analyzed using the two-way analysis of vari¬ance (ANOVA). The effects of each factor were analyzed by one-way ANOVA, and the inhibition zones associated with each sealer and each duration of incuba¬tion were compared by Tukey's post hoc test. The value of $p<0.5$ is considered significant. All statistical analyses were performed with the SPSS (PC version 10 software, IBM, NY, USA) statistical software package.

## III. Results

The present study comparatively evaluate the antimicrobial activity of different endodontic sealers against E. Fecalis and C. Albicans. Total 16 samples for each species were taken and randomized equally in four groups (4 samples/group) and treated with MTA-Fillapex (MTA), Real seal SE (RS), Acroseal (AC) and Zinc oxide eugenol (ZOE). The outcome measure of the study was zone of inhibition (mm) assessed at 24, 48, 72 hrs and 7 days post treatment.

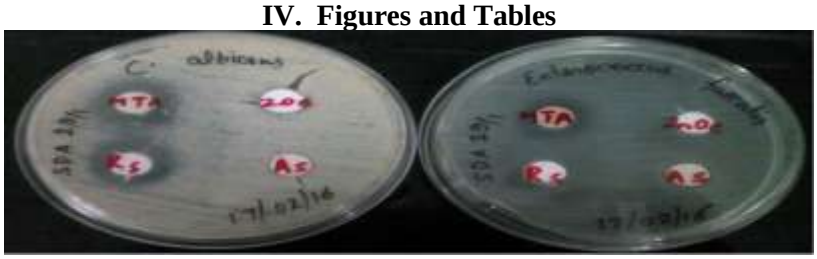
**Candida Albicans**

The post treatment C. Albicans zone of inhibition of four groups (MTA, RS, AC and ZOE) is summarized in Table 1. After 24 hrs, the C. Albicans mean zone of inhibition was highest in MTA (21.0±0.15 mm) followed by RS (17.2±0.20 mm). Further, in these groups, it decrease gradually after 24 hrs till 7th day and the decrease was evident similar among the groups. However, in ZOE and AC, it was found absent at all periods, thus not comparable with other groups (Fig 1).

**Enterococcus Faecalis**

The post treatment E. Faecalis zone of inhibition of four groups (MTA, RS, AC and ZOE) is summarized in Table 2. After 24 hrs, the E.faecalis mean zone of inhibition was found maximum around MTA (16.40±0.15 mm) and decreases till 7th day (12.40±0.20 mm). No other group showed any antimicrobial activity against E.faecalis at any time interval (Fig 1).

## IV. Figures and Tables




**zig 1 showing zones of inhibition against C.albicans and E.faecalis**

**Table 1:** Post treatment C. Albicans zone of inhibition (Mean ± SD, n=4) of four groups (in mm) and their effect on fungal strains**.**

| Periods | 24 hrs | 48hr | 72hr | 7day | Effect |
|---|---|---|---|---|---|
| MTA | 21.0 ± 0.15 | 19.0 ± 0.15 | 18.0 ± 0.15 | 16.0 ± 0.20 | Sensitive |
| RS | 17.2 ± 0.20 | 16.1 ± 0.20 | 15.2± 0.20 | 15.2 ± 0.20 | Sensitive |
| AC | 00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | Resistant |
| ZOE | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | Resistant |

**Table 2:** Post treatment E. faecalis zone of inhibition (Mean ± SD, n=4) of four groups (in mm) and their effect on bacterial strains**.**

| Group | 24hr | 48hr | 72hr | 7 Day | Effect |
|---|---|---|---|---|---|
| Mta | 16.40± 0.15 | 15.40 ± 0.20 | 14.20 ± 0.15 | 12.40± 0.20 | Sensitive |
| Rs | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | Resistant |
| Ac | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | Resistant |
| Zoe | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | 0.00 ± 0.00 | Resistant |

## V. Discussion

The selected microbes for this study was arbitrary to represent bacteria and fungus that have been commonly isolated from necrotic canals. E.faecalis is one of the most commonly isolated microorganisms from refractory periapical periodontitis[14] and it is one of the most drug resistant bacteria and has ability to survive up to 12 months even under nutrient deprived conditions in the root canal after routine root canal therapy. [15,16]

C.albicans is more often isolated from infected root canals, and is one of the common microorganisms that survive chemo-mechanical procedures and the application of root canal medicaments. [17]

In this study, the agar diffusion test was used, which is the most widely used in vitro method for the evaluation of antimicrobial activity. [18] This method allows direct comparisons between materials and also indicates which sealers are more likely to have antimicrobial activity within the root canal system. [19] Besides that, its results are highly influenced by the diffusibility of the material across the medium. [4] Most endodontic sealers possess antimicrobial components that need to be released from the sealer matrix to be effective. [19]

MTA Fillapex is resin salicylate and calcium silicate based root canal sealer. In the present study, MTA Fillapex was found to be most effective at 24 hr against C.albicans and E.faecalis, after that its antimicrobial activity decreases at 48hr, 72hr and 7th day respectively. On the contrary to our results Morgental et al stated that MTA Fillapex was effective before setting but not effective 7 days after setting. Such discrepancies are probably due to methodology. [9] The antimicrobial activity of MTA was reported by Torabinejad et al., who detected its efficiency against some facultative bacteria; however, no activity was found against E. faecalis, Staphylococcus aureus, Bacillus subtilis, and Escherichia coli or against anaerobic bacteria. [20] However, Stowe et al assessed the antimicrobial properties of MTA and found that it inhibited the growth of both E. faecalis and Streptococcus sanguis. [21] MTA contains calcium oxide, which forms calcium hydroxide on contact with water, which gives antibacterial property to MTA. [20,22,23] MTA Fillapex contains calcium silicate cement and with the moisture from dentin the hydration reactions of calcium silicates begins and calcium silicate hydrogel and calcium hydroxide exists which gives the high PH and antibacterial property to MTA Fillapex. [24] In present study antimicrobial property of MTA Fillapex could be related to calcium silicate, MTA and resin ingredient.

The results of present study showed that the most resistant microorganism to the antimicrobial activity of all sealers tested was Enterococcus faecalis except MTA Fillapex. This is one of the most resistant species usually found in root canal infection. The sealer MTA fillapex showed highest antimicrobial activity against C.albicans followed by Real Seal. No antimicrobial activity was observed with zinc oxide eugenol and Acroseal. In other study conducted by Filho et al showed different results from this study, where ZnO sealer demonstrated better antimicrobial activity than MTA-based cements. [25] The reason why ZnO sealer had better antimicrobial activity than MTA was not mentioned in the article. Eugenol content of ZnO sealer is generally known for its antimicrobial activity.

Although, Acroseal is a calcium hydroxide based sealer but it has shown no antimicrobial activity in this study, this result is in accordance with Tanomaru et al [26] but not with Pinheiro et al [27] where Acroseal was effective against E.faecalis.

Real Seal SE is a fourth generation resin based sealer which showed no antibacterial activity but little antifungal activity against C.albicans. This result is partially in accordance with Keshtkar and Riaz where Real Seal SE was ineffective against E.faecalis in ADT. [28] However, the results of present study was in vitro only, therefore more clinical trials are needed to confirm the effectiveness of tested sealers.

## VI. Conclusion

Amongst the tested sealers MTA Fillapex and Real Seal SE both showed antifungal activity whereas only MTA Fillapex was effective against E.faecalis, rest of the materials did not depict any antimicrobial activity in vitro.The introduction of the paper should explain the nature of the problem, previous work, purpose, and the contribution of the paper. The contents of each section may be provided to understand easily about the paper.

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