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In-vitro antimicrobial evaluation of Endodontic cavity sealers against Enterococcus faecalis

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Abstract: The aim of the present investigation was to evaluate the ability of four root canal sealers against the more resistive *Enterococcus faecalis*. *E faecalis* is a common pathogen which is a causative agent of oral cavities. The bacteriostatic effect was studied against the test organism with AH plus, Endorez, Metaplex and Grossmans sealer in 96 well titre plate. After different settling time (20 minutes, 1 day, 3days, and 7 days), the sealers were mixed with *E.faecalis* and incubated at humid environment. After incubation the mixer was diluted with 10-fold of tryptic soy broth and plated onto tryptic soy agar (TSA). The total number of colony-forming units permilliliter (CFU/mL) was determined. The maximum inhibition was observed with Grossman's sealers after 20 minutes of settling time followed by AH plus sealer after 1 day settling time period. The Endorez and Metapex have showed the maximum growth inhibition after 1 day and 7 days settling period. Thus these results conclude that Metapex and Endorez is effective against *E. faecalis*, despite its slower action compared with the Grossman and AH plus sealers

Keywords: *Enterococcus faecalis,* Cavity Sealers, AH plus, Endorez, Metaplex, Bacteriostatic, Antimicrobial activity.

I. INTRODUCTION

Bacteria are mainly responsible for the development of pulp/periapical diseases [1]. Therefore, elimination of bacteria during root canal treatment by instrumentation, irrigation and intracanal medication has always been an important part of successful endodontic treatment [2].

Grossman [3] advocated that the ideal root canal filling material should be bacteriostatic. Sundqvist 1998[4] recovered numerous species of anaerobic bacteria such as *Enterococcus faecalis, Streptococcus anginosus, Bacteroides gracilisand Fusobacterium nucleatum* after "failed" root canal therapy. Although Enterococcus *sp*. usually constitute a small proportion of the initial flora in the untreated root canal [5], it has been considered one of the most resistant species in the oral cavity and a possible cause of failure of root canal treatment [6, 7]. It is therefore necessary that root canal filling materials used in primary teeth treatment have antimicrobial activity [8, 9] and the broad spectrum of antimicrobial action against all these bacterial pathogens.

The main purpose of this study is to find out the ability of four different root canal sealers in the prevention of re-colonization, recontamination and re-growth of residual bacteria in the root canal system.

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II. MATERIALS AND METHODS

A. Sealers

Four root canal sealers were used in this study,(i) an epoxy resin based sealer, AH plus (Dentsply International Inc, York, PA), (ii) Polymethacrylate resin based sealer, Endorez (Ultradent, South Jordan, UT), (iii) Calcium hydroxide based sealer, Metaplex and (iv) Zinc oxide based sealer, Gross mans sealer.

B. Microorganism

Enterococcus faecalis ATCC 29212 was used as a test organism. It was grown overnight at 37ºC on Tryptic Soy Agar (BD chemicals USA) plates for the experiments. After checking the purity *E.faecalis* was suspended in sterile water and adjusted to a density of 1×10^6 colony forming units CFU/ml by using UV- Visible spectrophotometer at 600nm by considering standard Mcfarland's as reference.

C. Antibacterial activity

In the present study all sealers were prepared in strict compliance with the manufacturer's instructions. A 96 well microtiter plate (BIOFIL, USA) was held vertically and the side wall of the wells were coated with each sealer by using a cavity liner applicator. The sealers were allowed to settle for 20 minutes, 1 day, 3 and 7 days in a humid atmosphere at 37ºC. After settling a 10µl of bacterial suspension of *E.feacalis* (1×10^6 CFU/ml) was carefully placed on the surface of each sealer and an uncoated well as positive control. The sterile water was used as negative control. After incubation in 100% humidity at 37ºC for 2, 5, 20 and 60 minutes, 990µl of Tryptic Soy broth (TSB) was added to each well and mixed gently with a pipette for one minute. The bacterial suspension from each well was transferred and diluted with 10ml of Tryptic Soy TSB. The survival of the bacteria was assessed by culturing 20µl of the diluted fractions onto TSA plates. The CFU on the plate were counted after incubation at 37ºC for 24 hours. All experiments were performed in triplicates.

III. RESULTS

To examine the antimicrobial activities of the four sealers/cement (AH plus, Endorez, Metaplex and Gross mans sealer) were used against endodontic pathogen *Enterococcus feacalis.* The inhibitory action of four sealers was found out according to the Mcfarland's standards. The positive control showed maximum bacterial growth, whereas the negative control showed no bacterial growth. Bacterial strain *E.fecalis* was inhibited by all test materials as shown in the Figure 1-4.

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Fig 1: Showing the effect of Grossman's sealer against *Enterococcus feacalis*

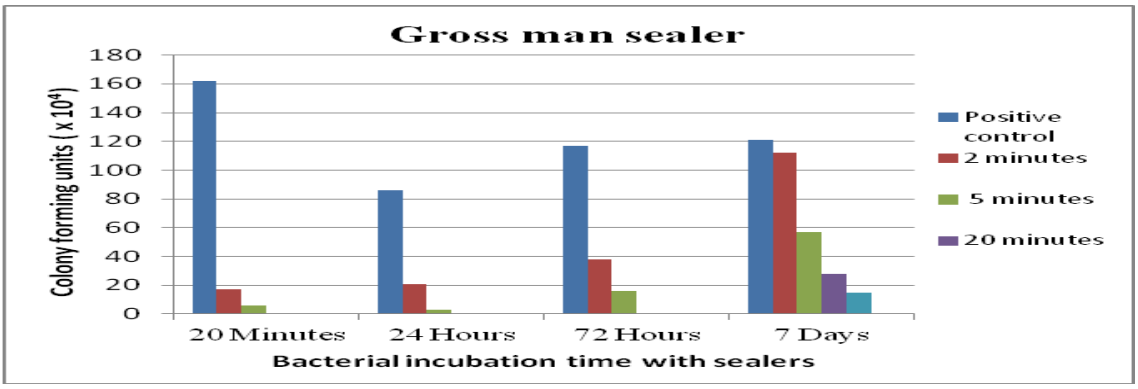

Fig 2: Showing the effect of AH sealer against Enterococcus feacalis

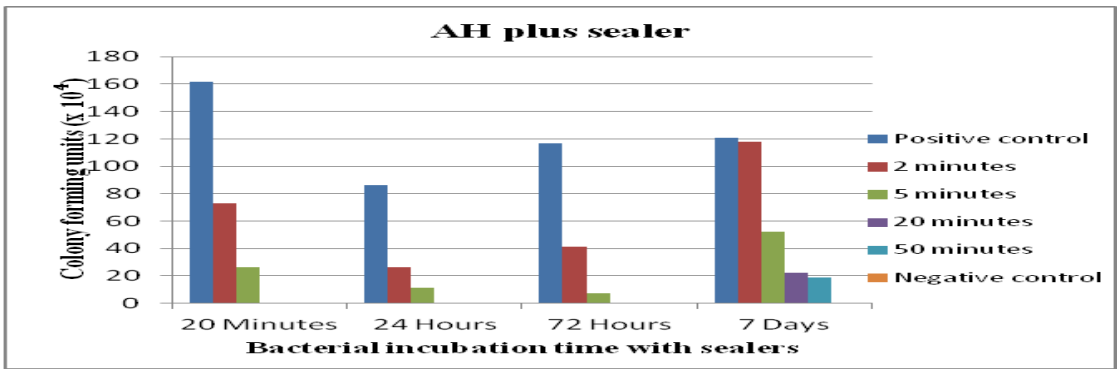

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Fig 3: Showing the effect of Endorez sealer against *Enterococcus feacalis*

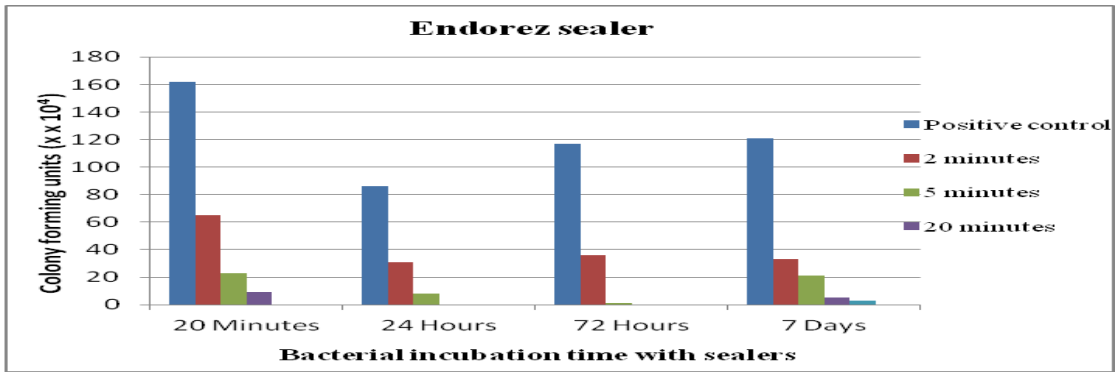
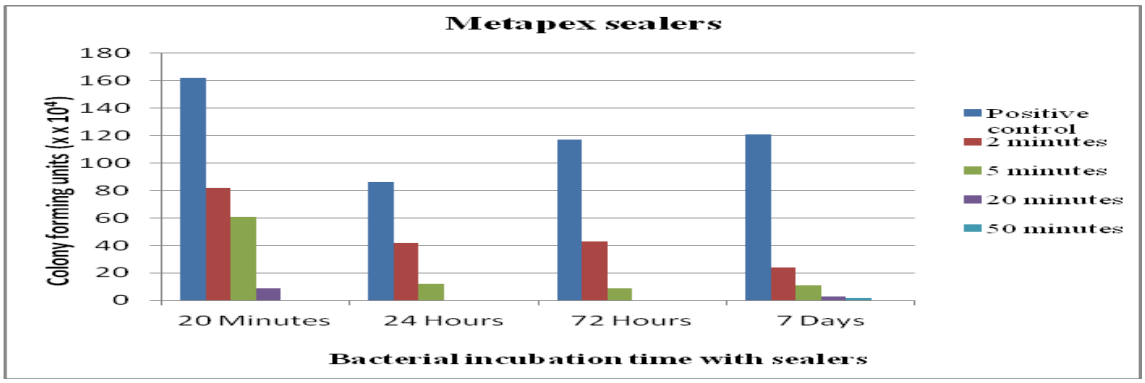


Fig 4: Showing the effect of Metapex sealer against *Enterococcus feacalis*



IV. DISCUSSION

The use of an endodontic sealer with antibacterial properties may be advantageous, particularly when pulpal or periapical infections are present. Antimicrobial activity of root canal sealers may help to eliminate residual microorganisms unaffected by chemomechanical preparation of the root canal system [10].

This study demonstrates the in-vitro antibacterial activity of four endodontic sealers. All the sealers have showed different antibacterial activity at different incubation time.

The maximum inhibition was observed with the Grossman's sealer used after 20 minutes of settling. Grossman's has found to inhibit one tenth of the test organism in 2 minutes followed by complete inhibition after 20 minutes. Highest inhibitory activity on the Enterococcus faecalis by Grossman's sealer is suggesting that release of zinc oxide have been involved in the effect [11]. Grossman sealers which used after the settling time 1 day, 3 and 7 days have been observed with the linear

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increase in the number of the bacterial colony growth (CFU/ml). This could be result of inefficient release of zinc oxide ions after 24 hours of settling.

Polymethacrylate resin based sealer, Endorez has found to be with 60 percent inhibition of test organisms after 20minutes of settling. The sealer endorez was observed to be effective in the inhibition even at 1st day, 3 day and at the 7th day of settling. These bacteriostatic actions were similar as the study of Zhang et al. [12] that reported EndoRez was bacteriostatic at the 1st, 7th, and 30th days.

The AH plus sealer with 20 minutes settling time has showed 55 percent of inhibition after 2 minutes and complete inhibition 20 minutes. The percentage of inhibition was decreased with the increasing in the settling time. The initial antimicrobial activity was because of the components of the epoxy resin and due to the discreet liberation of formaldehyde [13].

The calcium hydroxide based Metapex with 20 minutes settling time showed the least activity at 2 minutes and five minutes incubation. The maximum growth inhibition of about 80% was observed at after 7 days of settling. This antibacterial activity of Calcium hydroxide was due to the slow liberation of ions and increases in pH which is allowed an unfavorable microenvironment to the growth [14].
Long term studies are needed to study the effect of these sealers against various pathogens associated with dental decay.

V. CONCLUSION

According to the results obtained from this work it is possible to conclude that the sealers Endorez and Metapex can be used for the endotonic treatment for the long time bacteriostatic activity. These sealers can also reduce the load of the microorganisms during the root canal treatment and also during cavity filling and reduce the incidence of infection of the pathogen.

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