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Research Article

ANTIMICROBIAL EFFICACY OF ROOT CANAL SEALERS AGAINST *PEPTOSTREPTOCOCCUS MICROS*

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ABSTRACT

Microbes are considered as the primary etiologic agents in endodontic diseases. The ways of reducing these agents are: root canal debridement, antimicrobial irritants and antibacterial filling materials. But the complexity of the pulp canal system presents a problem for chemo mechanical preparation. One of the factors determining the success of endodontic treatment is the sealing material with a potent bactericidal effect. The aim of the present study was to assess the antimicrobial activity of endodontic sealers of different bases in-vitro against *Streptococcus micros*. Four root canal sealers were used in this study - an epoxy resin based sealer, **AH plus** (Dentsply International Inc., York, PA), Polymethacrylate resin based sealer, **Endorez** (Ultradent, South Jordan, UT), Calcium hyaronide hades sealer, **Metaplex** and Glycerol based sealer, **Gross mans** sealer. *Streptococcus micros* ATCC 29212 was used as a test organism, which was grown overnight at 37°C on Tryptic Soy Agar. In the present study all sealers were prepared in strict compliance with the manufacturer's instructions.The possibility of carryover of the sealers antibacterial activity was assessed by culturing 10 fold serial dilutions onto TSA plates and by comparing the survival of added bacteria in the 2 carryover controls (with and without sealer). Colonies on the plates were counted after incubation for 24 hours at 37ºC and CFU/mL was calculated.

Keywords: Endodontic diseases, Root canal Sealers, Tryptic Soy Agar, *Streptococcus micros*.

INTRODUCTION

Microorganisms and their by products are considered to be the primary etiologic agents in endodontic diseases[1]. Failure, during and after endodontic treatment are linked to the presence of bacteria in the root canal[2]. This result hence emphasises the importance of completely eliminating the bacteria from the root canal system[3] The most effective ways to achieve this aim are by means of instrumentation and irrigation. However, no less important than the biomechanics is an adequate filling of the root canal[4]. But the irregularity in shape (lateral canals, anastomosis, bifurcations and curvatures), solid or semisolid root canal filling material alone cannot provide an exact fit[5].

Currently used root canal materials do not form long-lasting perfect seal with the root canal wall. Leakage in micro amounts is a major clinical problem and one of the possible causes for failure of endodontic therapy[6]. Sealers which posse's antibacterial properties when used may be advantageous in clinical situations when there are persistent or recurrent infections [7]. It has been shown that the endodontic sealers offer the best antimicrobial activity following spatulation, and a gradual loss in the antimicrobial activity over time [8].

Agar diffusion methodology is the most commonly used technique to check the antibacterial activity of sealers.Activity mainly depends on the diffusion property and also the physical properties of the sealant material which may have limitations. A new method of direct contact was experimented [9].Measurement of kinetics of growth of bacteria can be used to check the antibacterial activity of the endodontic sealers[10].

Therefore, root canal sealers with good sealing ability and antimicrobial activity are desired tokill and eliminate residual microorganisms[11]. The aim of the present study was to assess the antimicrobial activity of endodontic sealers of different bases in-vitro against *Streptococcus micros* and also to study the efficacy of these sealers in preventing re-colonization after certain time interval and also the activity of residual bacteria in the oral root canal.

MATERIALS AND METHODS

Sealers:

Root canal sealers used in the present study involved, an epoxy resin based sealer, AH plus (Dentsply International Inc, York, PA), Polymethacrylate resin based sealer-Endorez (Ultradent, South Jordan, UT), Zinc oxide based-Gross mans sealer, Calcium hydroxide based sealer- Metaplex, which were prepared as per the manufacturer's instructions.

Microorganism:

Streptococcus micros ATCC 29212 was used as a test organism in the present study. It was grown overnight at 37ºC on Tryptic Soy Agar (BD chemicals USA) plates for the experiments. After checking for the purity, *Streptococcus micros* was suspended in sterile water and adjusted to a density of 1×10^6 colony forming units (CFU)/mL by using UV- Visiblespectrophotometer at 600nm using Mac Farland's as standard.

Modified DCT method:

Earlier studies have described the use of DCT to access the antimicrobial effect of the endodontic sealers. In the present study all sealers were prepared in strict compliance with the manufacturer's instructions. A 96 well micro titre plate (BIOFIL, USA) was held vertically and the side wall of the wells was coated with an equal amount of each material by using a cavity liner applicator. The sealers were tested 20 minutes after mixing as fresh specimens, and the other specimens were allowed to set for 1,3 and 7 days in a humid atmosphere at 37ºC before testing.

A 10µl of bacterial suspension (1×10^6 CFU/mL) was carefully placed on the surface of each sealer. Bacterial suspensions placed on the wall of uncoated wells were used as control. After incubation in 100% humidity at 37ºC for 2, 5, 20 and 60 minutes, 240µl of TSB (BD chemicals USA) was added to each well and mixed gently with a pipette for one minute. The bacterial suspension from each well was transferred and serially diluted in TSB. The survival of the bacteria was assessed by culturing aliquots of 20µl onto TSA plates after 10 fold serial dilutions. The colonies on the plate were counted after incubation at 37ºC for 24 hours and the CFU/mL was calculated. All experiments were performed in triplicates.

Controls for Carryover Effect:

To monitor the carryover effect of the sealers, the side wall of the wells was coated with the same amount of sealer as for DCT and mixed well for 20 minutes. 10µl of sterile water was placed in direct contact with each specimen and kept for incubation at 37ºC for 1 hour in 100% humidity. After incubation 240µL of TSB was added to each well. After gentle mixing for one minute, 10µl of the broth was transferred into 970µl TSB. A 20µl of bacterial suspension (1×10^6 bacteria) was added at the same time to the first dilution tube. In another carryover control no sealer was used, but the same amount of sterile water (10µl) was placed on the wall of the uncoated wells and processed further as described above. The possibility of carryover of the sealers antibacterial activity was assessed by culturing 10 fold serial dilutions onto TSA plates and by comparing the survival of added bacteria in the 2 carryover controls (with and without sealer). Colonies on the plates were counted after incubation for 24 hours at 37ºC and CFU/mL was calculated.

RESULTS

The results for the antimicrobial activity against ***Peptostreptococcus micros*** by the endodonticsealers by the modified DCT method are as follows. Each of the sealers used like **Zinc oxide, AH plus, Endorez and Metaplex** showed different activity for different incubation time.

Results for fresh specimen ie sealers used after 20 minutes of mixing showed varying results for each of the sealers, zinc oxide showed poor activity for the 2 minutes and 5 minutes contact time compared to the other sealers and with a one tenth reduction of the test organism in 20 minutes and a near to inhibition of the growth after 60 mins, where as AH plus and Metapex showed similar results for the initial 2 minutes with more than one tenth reduction in the count and complete eradication after 5 minutes, where endorez showed the maximum activity with no growth and complete reduction from initial contact of 2 minutes after.

After 24 hrs incubation the activity of zinc oxide remained the same as in 20 minutes after mixing results, showing maximum activity after 5 minutes. AH Plus and Metapex showed similar activity with least growth at 2 minutes exposure and complete killing of the organisms after 5 minutes.Endorez showed the maximum activity of complete eradication of all the organisms from 2 minutes exposure onwards, showing its effectiveness. Zinc Oxide showed the least activity of the 4 sealers.

The 3rd day incubation test result was similar to 24 hrs with complete inhibition of the bacteria after 2 minutes of incubation with Endorez showing the maximum inhibition followed by Metapex with complete inhibition after minutes. There was decrease in activity with Zinc oxide with no eradication throughout the incubation time and AH plus showing complete killing after 5 minutes of incubation.

Seven days test revealed a decrease in the activity of all the sealers. Endorez and Metapex showed similar activity for 20 minutes onwards with complete inhibition with a decrease in the intial 2 and 5 minutes. Zinc oxide and AHplus showed similar activity with no inhibition with all the incubation times indicating loss of efficacy of its antibacterial activity.

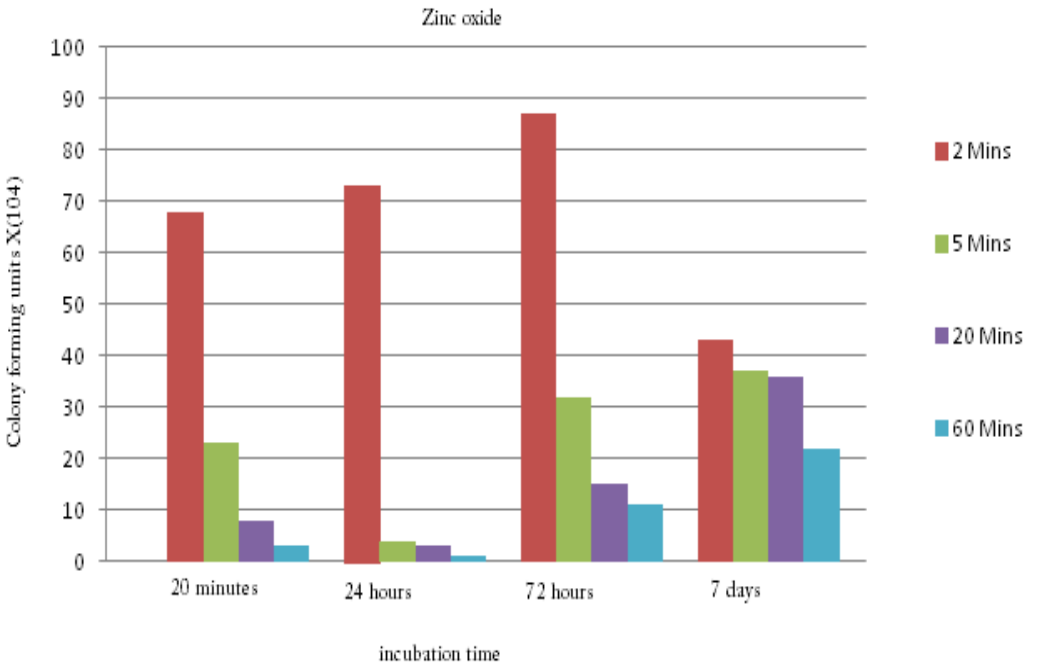

Figure 1: Antimicrobial Efficacy of Zinc Oxide Sealer against *Peptostreptococcus micros*

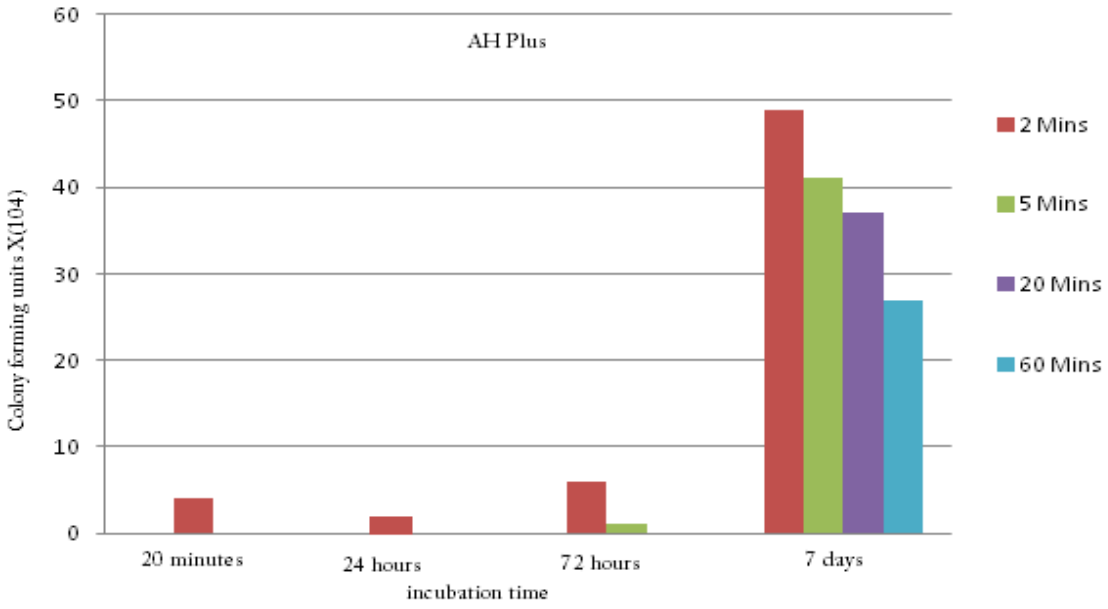

Figure 2: Antimicrobial Efficacy of AH plus Sealer against *Peptostreptococcus micros*

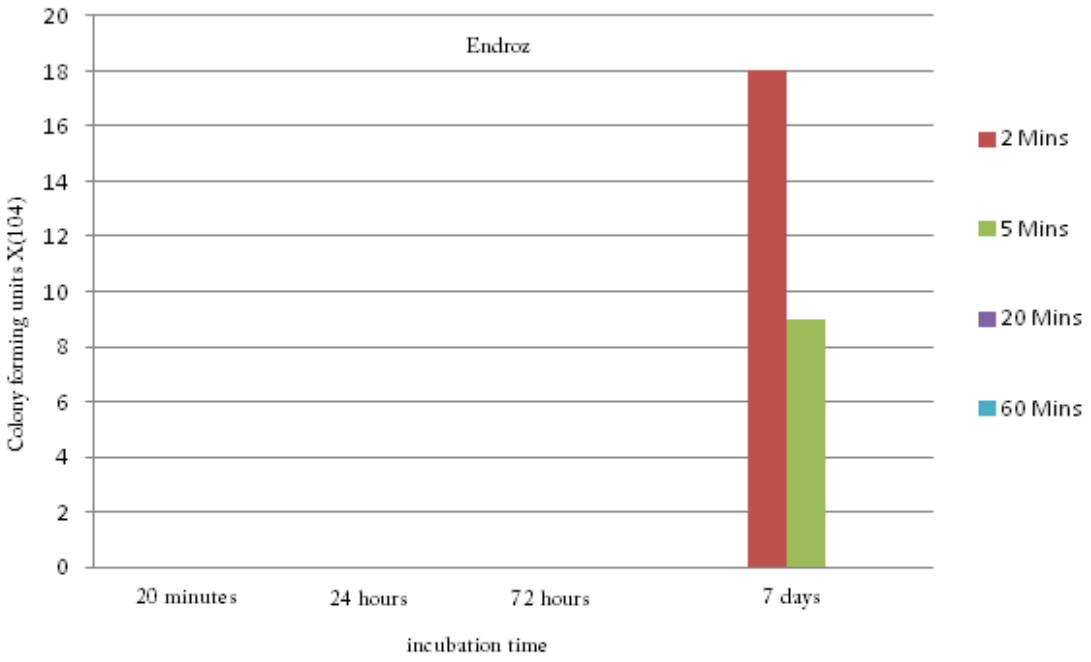

Figure 3: Antimicrobial Efficacy of Endorez Sealer against *Peptostreptococcus micros*

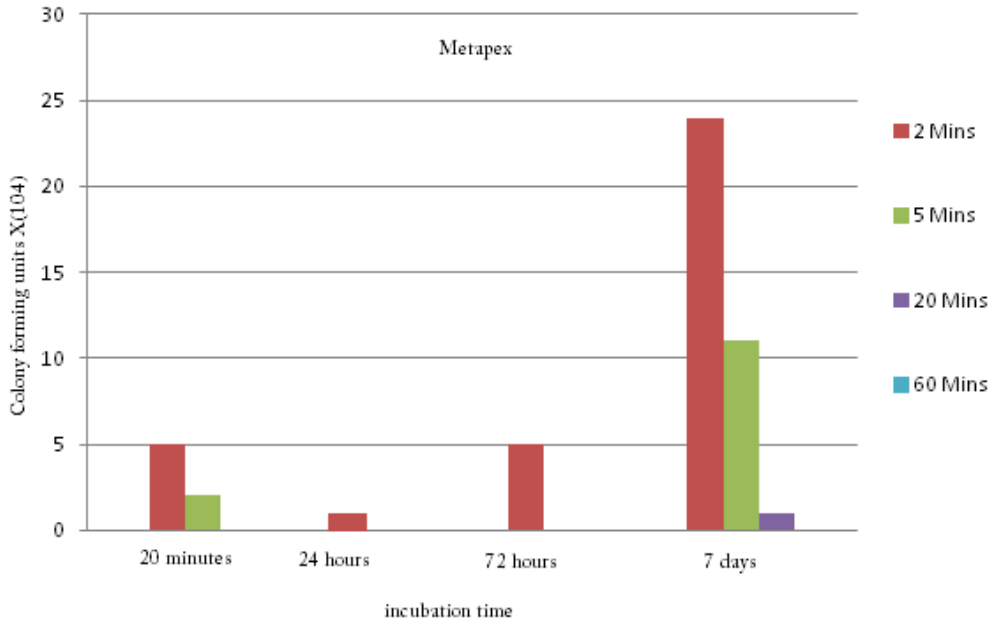

Figure 4: Antimicrobial Efficacy of Metapex Sealer against *Peptostreptococcus micros*

CONCLUSION

Microorganisms and their by products are considered to be the primary etiologic agents in endodontic diseases. The antimicrobial activity of four root canal sealers were used in this study, an epoxy resin based sealer **AH plus** (Dentsply International Inc., York, PA), Polymethacrylate resin based sealer **Endorez** (Ultradent, South Jordan, UT), Calcium hyaronide hades sealer **Metaplex** and Glycerol based sealer **Gross mans** sealer. Failure, during and after endodontic treatment are linked to the presence of bacteria in the root canal. This result hence emphasises the importance of completely eliminating the bacteria from the root canal system. The most effective ways to achieve this aim are by means of instrumentation and irrigation. However, no less important than the biomechanics is an adequate filling of the root canal. But the irregularity in shape (lateral canals, anastomosis, bifurcations and curvatures), solid or semisolid root canal filling material alone cannot provide an exact fit. Therefore, root canal sealers with good sealing ability and antimicrobial activity are desired to kill and eliminate residual microorganisms. Hence, the present study has been taken up to test the antimicrobial activity of currently used endodontic sealers, against microbes found in the tooth with a vital inflamed pulp or papal necrosis.

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