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Full Length Research Paper

Antimicrobial efficacy of AH-Plus, adseal and endofill against *Enterococcus faecalis*- An *in vitro* study

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Antimicrobial activity is one of the desirable properties of an ideal sealer. The purpose of this study was to compare the antimicrobial effect of three different sealers (AH-Plus, adseal and endofill) on *Enterococcus faecalis.* The antimicrobial effect of three sealers was tested by the agar diffusion method. The freshly mixed sealers were placed in prepared wells of agar plates inoculated with *E. faecalis*. After incubation of the plates at 37°C for 7 days (168 h) under anaerobic condition, the diameter of zone of microbial growth inhibition produced around the wells was measured (in mm) after 3, 5 and 7 days. The data were statistically analyzed using Kruskal-Wallis and Friedman tests. In all determined intervals, the antibacterial activity of endofill was significantly higher than other test materials ($p<0.05$). AH-Plus had moderate effect on *E. faecalis*, while Adseal showed the lowest antibacterial activity on tested bacteria. The endoffill sealer showed the highest antimicrobial effect compared to AH-Plus and Adseal sealers. Furthermore, the antimicrobial activity of all sealers decreased with time.

Key words: Antimicrobial activity, agar diffusion method, endodontic sealers, *Enterococcus faecalis*.

INTRODUCTION

Successful root canal treatment requires proper cleaning and shaping of the root canal system, as well as hermetic sealing of the canal space with an inert, dimensionally stable and biologically compatible material (Scarparo et al., 2009). Therefore the search has continued for an endodontic sealer that fulfills the requirements for the ideal physicochemical and biological properties.

Microbes and their products are the main etiologic factors of root canal diseases (Bergenholtz, 1974). Therefore, the main objective of endodontic therapy is the elimination of microorganisms from the root canal system and the prevention of subsequent reinfection (Pizzo et al., 2006). However, mechanical root canal instrumentation, antibacterial irrigation, adequate filling of the root canal space, and use of inter-appointment antimicrobial dressing (for example, calcium hydroxide) do not result in complete disinfection of root canal space (Abdulkader et al., 1996). Grossman (1980) advocated that ideal root canal filling material should be bacteriostatic.

Therefore, antimicrobial activity of endodontic filling materials such as sealers play an important role in reducing or avoiding growth of these remaining microorganisms and therefore improve the success rate of endodontic treatment.

E. faecalis, has been used in numerous studies of the antibacterial properties of disinfecting agents because, this anaerobic bacteria is the most commonly recovered from the root canals of teeth with failed root treatment and has also been implicated in persistent root canal infections (Bystrom and Sundqvist, 1985; Gomes et al.,

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Table 1. Comparison of mean diameter of growth inhibition zones, by root canal sealers against *E. faecalis.*

Overall mean diameter of inhibition zones (mm)			
Sealer	**3rd day**	**5th day**	**7th day**
Endofill	14.80	14.762	14.691
AH-Plus	1.558	1.220	0.544
Adsel	1.00	0.132	0

Table 2. Results of P. value between 3 root canal sealers for each time period.

Sealer		P. value
3rd day	AD Seal Endofill AH Plus	0
5th day	AD Seal Endofill AH Plus	0
7th day	AD Seal Endofill AH Plus	0

2002).

The agar diffusion test (ADT) used to be the most commonly applied method to assess the antimicrobial activity of endodontic sealers (Abdulkader et al., 1996; Lai et al., 2001; Mickel et al., 2003).

The aim of this study was to evaluation the antimicrobial activities of three endodontic sealers (AH-Plus, adseal and endofill) against *E.faecalis* by measuring the diameter of zones of growth inhibition on the surface of agar plates at three different time intervals.

MATERIALS AND METHODS

The sealers used in this study were: AH-Plus (Dentsply De Trey, Konstanz, Germany), adseal (Metabiomed OK, Chungbuk, Korea) and endofill (Produits Dentaires SA, Vevey, Switzerland). For the agar diffusion test a base layer composed of 10.0 ml of brain heart infusion (BHI) agar (Oxide Ltd., Basingstoke, UK) with addition of 2% bacteriological agar (Difco) was poured into 20×100 mm sterile petri dishes. After solidification, the plates were taken to an incubator at 37°C for 24 h to check for sterility.

E. faecalis ATCC 29212 from frozen stock cultures was reactivated by transferences in brain heart infusion (BHI) broth followed by incubation at 37ºC for 24 h. For the inoculum, the culture in BHI broth incubated at 37ºC for a period of 15 to 18 h was used to standardize the final concentration of 1.5×10^8 cells/mL equivalent to the 0.5 standard of the McFarland scale using a 630 nm wavelength spectrophotometer (Pharmacia Biotech, São Paulo, SP, Brazil).

Immediately after removal from the incubator, the bacterial inocula were seeded with cotton sticks all over the dishes, based on the McFarland scale, except for negative control plate in which *E. faecalis* was not seeded.

After solidification of seed layer, each plate (75 plates) was divided evenly into 3 sections. In each section of each plate, a 6×4 mm well (6 mm in diameter) was created by removal of the agar at equidistant points using a sterile stainless steel cylinder. All sealers were mixed according to manufacture instructions, and an area of fixed size on the sidewall of the wells was coated with an equal amount of each material by using a cavity liner applicator. Positive control plates were streaked with bacteria, but no root canal sealer was used. All plates were incubated for 72 h at 37°C under aerobic condition, and zones of growth inhibition were measured at 3 (72 h), 5 (120 h) and 7 days (168 h) (Figures 1, 2 and 3). The inhibitory zone was considered to be the shortest distance (mm) from the outer margin of the sealer to the initial point of microbial growth. The diameter of the growth inhibition zones was analyzed by Kruskal-Wallis and Friedman test.

RESULTS

The results of antibacterial effects of the endodontic sealers have been summarized in Tables 1 and 2. Positive control plates showed bacterial growth in all cases. All 3 root canal sealers caused zone of inhibition after 72 and 120 h (3rd and 5th dayes). According to this study, endo-fill had the highest inhibitory zone and adseal, the least ($p<0.05$). In summary the sequence on antimicrobial activity of studied sealers was Endo-Fill>AH-Plus>Adseal. The results after 120 and 168 h with Friedman test showed that the effictiveness of the root canal sealers decreased slightly with time. Seven days after mixing, endofill showed the highest antibacterial activity than AH-Plus but 7-day-old adseal samples did not show antibacterial effect against *E.faecalis.*

DISCUSSION

The persistance of bacteria spacialy *E.faecalis* in root canal system often leads to failure of root canal treatment (Fabricius et al., 1982). So the use of an endodontic sealer with antibacterial properties may help to eliminate residual microorganisms, specially *E.faecalis*, that have survived the chemomechanical instrumentation and irrigation (Zhang et al., 2009).

E. faecalis, which is often associated with persistent

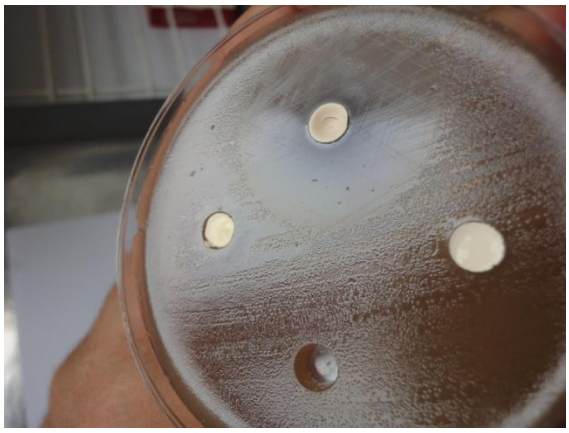

Figure 1. Zone of microbial growth inhibition for each substance tested against *E. faecalis* within a 72 h period (upper well Endofill- right well AH-Plus –left well adseal- lower well positive control group).

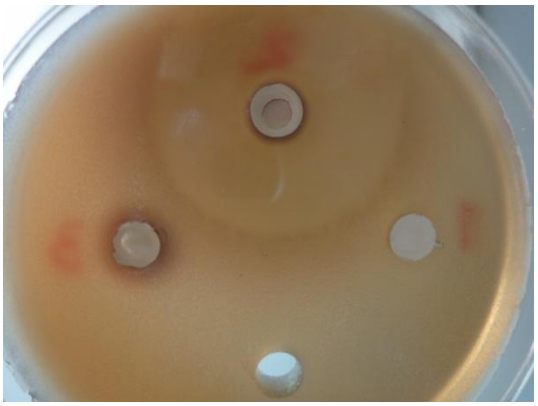

Figure 2. Zone of microbial growth inhibition for each substance tested against *E. faecalis* within a 120 h period.

Figure 3. Zone of microbial growth inhibition for each substance tested against *E. faecalis* within a 168 h period.

apical periodontitist, was chosen as the test organism for this study because it may be difficult to eliminate from root canals (Haapasalo and Orstavik 1987).

The agar diffusion test is one of the most frequently used methods for assessing the antimicrobial activity of root canal sealers (Chong et al., 1994). The advantage of this method is that it allows direct comparison of root canal sealers against the test microorganisms, indicating wich sealer has the potential to eliminate bacteria in the local microenvironment of the root canal system (Gomes et al., 2004). However; this prosedure does not depend only on the material toxicity to a given microorganism, but may also be influenced by the diffusion and affinity of the material in the culture medium. A material presenting easier diffusion will produce larger zone of inhibition of bacterial growth (al-Khatib et al.,1990; Abdulkader et al., 1996; Weiss et al., 1996; Sipert et al., 2005; Miyagak et al., 2006; Pizzo et al., 2006).

Today, numerous root canal sealer are available, based on various formulas, such as epoxy resin sealers, calcium hydroxide-based sealers and zinc oxide egenol (ZOE) cements (Lai et al., 2001). In present study, the antimicrobial effect of AH-Plus, adseal and endo-fill sealers were tested by using the ADT.

The AH-Plus and Adseal are an epoxy resin root canal sealer (Kaplan et al., 1999; Leonardo et al., 2000). Endofill, is a zinc oxide-eugenol-based sealer, and has shown good antibacterial activity when compared with calcium hydroxide-based sealers (Siqueira et al., 2000).

According to the results of this syudy the endo-fill had the greatest antimicrobial effect against E.faecalis. The eugenol is a potent antimicrobial agent, and therefore, the activity of ZOE- based sealers may be attributable to the free eugenol released from the set materials (Hume, 1986). The antibacterial effect of epoxy resin-based sealers might be related to the released of formaldehyde during the polymerization process (Leonardo et al., 1999). So this agent gives the AH-Plus and Adseal sealer their antibacterial properties. In this study, the resin-based sealers have lower antibacterial effect than ZOE-based sealer aginst E.faecalis, perhaps because only a small amount of formaldehyde was released over a brief period. Adseal showed less antibacterial activity than all other sealers in this study. The present study also showed that fresh sealers have antibacterial effect, whereas their antimicrobial activity decreased with time. When 7-day samples of adseal sealer were tested, in fact, no antibacterial activity was found. On the contrary, after 7 days from mixing, the endofill still exerted antibacterial activity, although to a lesser extent than 72-h samples. Several authors have studied the antibacterial properties of various root canal sealers against different microorganisms (Abdulkader et al., 1996; Bodrumlu and Semiz, 2006; Eldeniz et al., 2006). However, the results were controversial (Smadi et al., 2008; Zhang et al., 2009).

Our finding are consistent with studies that found large inhibitory zone produced by sealers similar to endofill such as Grossman's sealer (al-Khatib et al., 1990; Siqueira et al., 2000) and Procosol (Kaplan et al., 1999) against *E. faecalis* (Siqueira et al., 2000) which were also used in our study.

Pizzo et al. (2006) reported that only fresh AH-Plus possessed antibacterial activity, whereas 24 h and 7 day old samples did not show antibacterial effect against *E. faecalis.* Similar results were reported by Kayaoglu et al. (2005).

The result of the present study are consistent with the Gomes et al. (2004) study, in that the ZOE-based sealer such as endofill and endomethasone demonstrated the highest antimicrobial activity than epoxy resin-based sealer such as AH-Plus and sealer 26.

Conflicting results have been reported by Mohammadi and Yazdizadeh (2007) Who found AH-26 (epoxy resin sealer) had the largest inhibition zone in comparison with ZOE sealers. Difference in microorganism strains used may be the main reasons of these controversies (Sipert et al., 2005).

The present finding indicated that, due to long-lasting antibacterial activity of ZOE-based sealers, they may be effective in supplement chemomechanical preparation in disinfection of the root canal space and may also be of benefit in the treatment of persistent or recurrent infections. Additional studies, however; are needed to evaluate the antimicrobial effects within dentinal tubules and biocompatibility of these sealers. As endodontic sealers may come into direct contact with the periapical tissues, only those sealer should be used which have been proved to possess the least acceptable biocompatibility (Hauman and Love, 2003).

Conclusion

The present finding indicated that antibacterial activity of ZOE-based sealer is more than epoxy resin-based sealers, and Adseal was inferior in terms of its antibacterial activity to all sealers tested. Also all sealers exhibited bactericidal effect when freshly mixed, but the antibacterial activity of each sealer decreased with time. On the other hand, adseal lost antibacterial activity after 7 days.

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