Short-term antibacterial activity of root canal sealers towards *Enterococcus faecalis*

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Abstract

Kayaoglu G, Erten H, Alaçam T, Ørstavik D. Short-term antibacterial activity of root canal sealers towards *Enterococcus faecalis*. *International Endodontic Journal*, **38**, 483–488, 2005.

Aim To investigate the antimicrobial activity of root canal sealers on *Enterococcus faecalis*, either allowing or avoiding direct contact between sealers and bacteria.

Methodology Filter paper discs were immersed in standardized *E. faecalis* suspensions and exposed to freshly mixed sealers (MCS, AH Plus, Grossman's sealer, Sealapex, Apexit) in teflon wells for 30 min, with or without a filter membrane placed between filter paper discs and sealers (membrane-restricted contact test and direct contact test, respectively). After exposure, the filter paper discs were transferred to vials containing phosphate-buffered saline (PBS) and glass beads, and vigorously vortexed. PBS with resuspended bacterial cells was serially diluted and 25 μL droplets were seeded on TSA plates. The plates were incubated in air at 37 °C for 24 h and colony-forming units were counted. Using $\alpha = 0.05$ as level for statistical significance, the data obtained were analysed using Student's *t*-test.

Results In the direct contact test, MCS and AH Plus killed the bacteria to a level below the detection limit. They were followed in decreasing order of efficacy by Grossman's sealer, Sealapex and Apexit. In the membrane-restricted contact test, the sealers ranked: MCS, AH Plus, Grossman's sealer, Apexit and Sealapex, in descending order of antibacterial potency. MCS, AH Plus and Grossman's sealer significantly reduced the number of viable bacteria in both tests. Sealapex and Apexit were not statistically different from control.

Conclusions MCS, AH Plus and Grossman's sealer were effective in reducing the number of cultivable cells of *E. faecalis*. Calcium hydroxide-based sealers, Sealapex and Apexit were ineffective in this short-term experiment.

Keywords: antimicrobial activity, *Enterococcus faecalis*, filter membrane, filter paper, root canal sealers.

Received 21 September 2004; accepted 1 April 2005

Introduction

The success of endodontic treatment depends mainly on elimination of infecting microorganisms. This is achieved through chemo-mechanical preparation of root canals and leaving antimicrobial dressings in the root canal between appointments. However, microorganisms might still survive these challenges (Ørstavik *et al.* 1991, Molander *et al.* 1999). Therefore, root canal sealers with good sealing ability and antimicrobial activity are desired to entomb and kill the surviving microorganisms. Root canal sealers in current use contain many different antimicrobial agents, and several studies have investigated their antimicrobial activity (Pumarola *et al.* 1992, Abdulkader *et al.* 1996, Heling & Chandler 1996, Saleh *et al.* 2004).

Microorganisms infecting the root canal dentine might adhere superficially to the dentinal wall or penetrate deeper into the dentinal tubules (Ando & Hoshino 1990, Peters *et al.* 2001). Superficially adhering bacteria might be expected to be killed easier than those shielded in the depths of dentinal tubules, but

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microorganisms inside the dentinal tubules might also be challenged by antimicrobial components leaching from the sealer. Therefore, antimicrobial testing of sealers should take into consideration these two effects based on the contact of the sealer and the microorganism.

Enterococcus faecalis is a resilient bacterium frequently recovered from obturated root canals with signs of apical periodontitis (Sundqvist *et al.* 1998). When established in the dentinal tubules, it is difficult to eliminate this species through root canal medication. Therefore, it might be advantageous if the sealer exerts some antimicrobial activity as the last element in the treatment regimen.

The aim of this study was to investigate the antimicrobial activity of root canal sealers on *E. faecalis* either allowing or avoiding direct contact between the sealers and the bacterium.

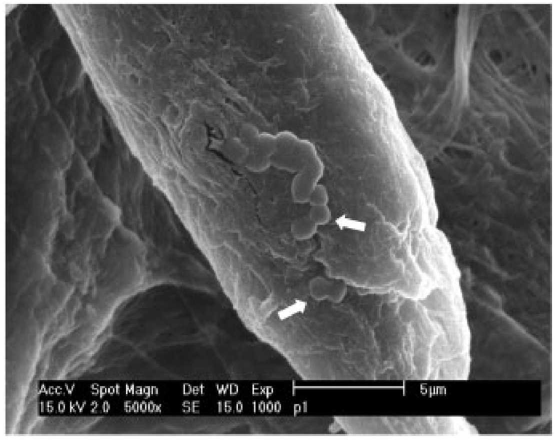

Figure 1 Scanning electron microscopy showing the bacterial cells (indicated with arrows) to be adhered on fibres on the surface of filter paper disc.

Materials and methods

Sealers tested in this experiment were Sealapex (Sybron Kerr, Romulus, MI, USA), Apexit (Vivadent, Schaan, Liechtenstein), Grossman's sealer (by prescription, NIOM laboratory, Haslum, Norway), AH Plus (Dentsply DeTrey, Konstanz, Germany), and MCS Canal Sealer (Lone Star Technologies, Westport, CT, USA). *Enterococcus faecalis* A197A, a root canal isolate from a persistent endodontic infection (Sirén *et al.* 1997) was used as the test microorganism. The bacteria were maintained on tryptone soy agar (TSA, Oxoid Ltd, Basingstoke, UK) at 4 °C. A few colonies were picked from TSA, and inoculated in tryptone soy broth (TSB, Oxoid Ltd). Bacterial growth was in air at 37 °C overnight. Bacterial numbers were standardized spectrophotometrically to $OD_{540} = 0.4$. Filter paper discs (Whatman no. 3; 4 mm in diameter) were immersed in the bacterial suspension for 10 min. The filter paper discs were removed from the suspension and drained of excess liquid. Scanning electron microscopy showed the bacterial cells to be adhered on fibres on the surface of filter paper discs (Fig. 1). The discs were placed on the bottom of teflon wells having a diameter of 5 mm and a depth of 3 mm. Approximately 15 µL of sealer, freshly mixed according to the manufacturer's instructions, was placed onto the discs with bacteria. In one series of experiments, a filter membrane (Whatman, Maidstone, UK, 0.22 µm pore size) was placed over the disc with bacteria before placement of the sealer. A teflon disc, instead of a sealer, was used for control. Four filter paper discs were used for the testing of each sealer and control in either series of tests. Exposure was for 30 min at 37 °C inside a sealed plastic box; the bottom was filled with water. After exposure, the bulk of the sealer was wiped off from the surface of paper discs in the direct contact test. In some cases, minor remnants remained on the surface. The filter paper discs were transferred to vials containing 2 mL of phosphate-buffered saline and glass beads, and vigorously vortexed. Serial dilutions were carried out and two droplets of 25 µL were seeded on TSA plates. The time from sampling to incubation did not exceed 10 min. The plates were incubated in air at 37 °C for 24 h and colony-forming units (CFU) were counted. The detection limit of the method was 40 CFU mL^{-1}. The data obtained were analysed using Student's *t*-test, using $\alpha = 0.05$ as the level of statistical significance.

Results

Bacterial numbers were reduced significantly with MCS, AH Plus and the Grossman's sealer in both tests. The insertion of a filter membrane dramatically impaired the activity of the sealers.

The experimental results are shown in Figs 2 and 3.

Discussion

The main finding in this study was that the iodoform-containing root canal sealer (MCS), the epoxy resin-based sealer (AH Plus) and the zinc oxide-eugenol-based sealer (Grossman's sealer) exerted strong antimicrobial activity on *E. faecalis*. Calcium hydroxide-based sealers

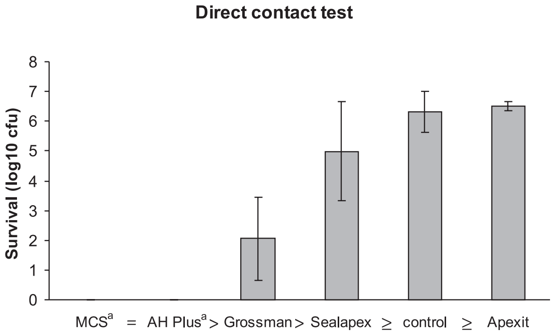

Figure 2 Mean $\log_{10}$ of the number of colony-forming units (CFU) after direct contact antibacterial test and the potency of the tested sealers and control in descending order. Bars represent the standard deviations for four paper discs. The sign '>' indicates statistical significance, the sign '≥' indicates stronger activity but no statistical significance, sealers in the left side of the sign '>' are significantly stronger than those remaining in the right side. [a]Below the detection limit (<40 CFU mL^{-1}).

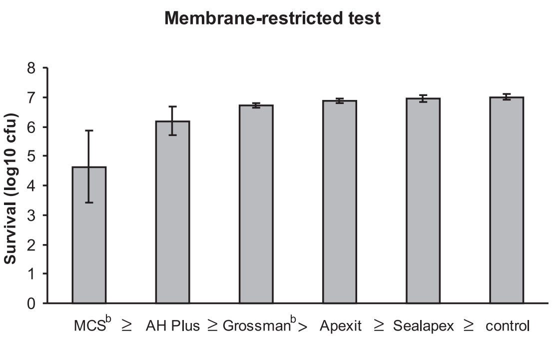

Figure 3 Mean $\log_{10}$ of the number of colony-forming units after membrane-restricted contact antibacterial test and the potency of the tested sealers and control in descending order. Bars represent the standard deviations for four paper discs. The sign '>' indicates statistical significance, the sign '≥' indicates stronger activity but no statistical significance, sealers in the left side of the sign '>' are significantly stronger than those remaining in the right side. [b]Note that significant difference exists between these sealers.

were not statistically different from the control in either test.

The antimicrobial activity of root canal sealers has been tested previously using various methods. These include agar diffusion tests (Al-Khatib *et al.* 1990, Abdulkader *et al.* 1996, Mickel & Wright 1999, Mickel *et al.* 2003), direct contact tests (Fuss *et al.* 1997, Çobankara *et al.* 2004) and dentine block models (Heling & Chandler 1996, Saleh *et al.* 2004). The method used in this study was a modification of a previously described method, by which the antimicrobial activity of root canal irrigation solutions was tested on fungi (Waltimo *et al.* 1999). It is designed especially for the study of antimicrobial effects of freshly mixed or nonsetting materials and pastes, and is therefore restricted to testing of the initial activity of the materials, not the long-term effects. It has been shown that cells of *E. faecalis* exposed initially to sublethal doses of antibacterial agents for some period become less susceptible to the lethal doses at a later time (Flahaut *et al.* 1996). Therefore, it is advantageous that an antibacterial agent acts rapidly on microorganisms. Consequently, knowledge of the early and the long-term antibacterial efficacy of root canal sealers is important.

Root canal sealers have limited and variable dentinal tubule penetration ability (Saleh *et al.* 2003); and sealer penetration might further decrease because of practical difficulties such as failure to remove smear layer or previous dressings (Kouvas *et al.* 1998, Çalt & Serper 1999). Then direct contact between sealer and microorganisms is not always possible clinically. This applies also to the microorganisms embedded under smear layer formed as a result of instrumentation of infected root canal walls. Therefore, this study employed, in addition to a direct contact test, a noncontact antibacterial test where contact between sealers and bacteria was limited by a filter membrane. Filter membrane, dentine powder or dentine slices have been previously used as barriers between the test material and the cells in cytotoxicity tests of dental materials (Meryon 1984, Yeşilsoy & Feigal 1985, Schmalz *et al.* 1996). A filter membrane test as described in this study has not been previously used as a barrier while testing the antimicrobial effectiveness of a root canal sealer.

Grossman's sealer is a zinc oxide-eugenol-based sealer. The eugenol in the formulation is a potent antimicrobial agent (Hume 1986). Zinc oxide-eugenol sealers possess strong antimicrobial activity (Al-Khatib *et al.* 1990, Abdulkader *et al.* 1996, Fuss *et al.* 1997, Mickel & Wright 1999, Saleh *et al.* 2004). The results of this study are in line with previous studies in that the number of viable bacteria was reduced significantly with the use of a zinc oxide-eugenol sealer.

Iodoform is a mild antiseptic because of the slow liberation of iodine when in contact with body fluids. It has bactericidal and fungicidal effects through its iodine content (Walton *et al.* 1989). Iodoform has been used as a principal constituent in the nonsetting, absorbable formulation, Kri I paste (Pharmachemie, Zurich, Switzerland), and in combination with calcium hydroxide, such as in Vitapex (Neo Dental Chemical Products Co., Tokyo, Japan), for filling of primary teeth (Tchaou *et al.* 1995, Pabla *et al.* 1997). It is added to the formulation of a zinc oxide-eugenol-based, setting sealer, Mynol C-T (Mynol, Broomall, PA, USA), as an antimicrobial component. However, there is a limited number of studies regarding the antimicrobial potency of iodoform-containing root canal filling materials. Kri I paste has antibacterial activity, and iodoform-zinc oxide-eugenol sealer has been shown to be more antibacterially effective than the Kri I paste on aerobic and anaerobic bacteria (Saggar *et al.* 1991, Tchaou *et al.* 1995, Pabla *et al.* 1997). MCS contains zinc oxide-eugenol in addition to iodoform. The eugenol in MCS might also be responsible for some of the antibacterial effect.

AH Plus is an epoxy-resin-based root canal sealer. Unlike its predecessor AH 26, very little formaldehyde is released by AH Plus (Cohen *et al.* 1998). Therefore, its antimicrobial activity is probably as a result of other components in the formulation. Among these, unset epoxy resin has been thought to be toxic (Schweikl *et al.* 1995, Schweikl & Schmalz 2000). In one study, extracts of paste A (containing epoxy resin), paste B (containing amines) and of the mixed sealer all reduced cell viability (Schweikl & Schmalz 2000). This suggests that amines, besides epoxy resin, can also be toxic and that unpolymerized residues in the mixture might maintain the toxic effect. Antimicrobial testing of AH Plus has also been carried out. While a study employing the agar diffusion test indicated no antimicrobial activity for AH Plus on *E. faecalis* (Mickel *et al.* 2003), direct contact tests and dentine block models, as more relevant methods, indicated efficacy of AH Plus on the same species (Saleh *et al.* 2004, Çobankara *et al.* 2004). The result of the present study is in agreement with the latter studies.

Calcium hydroxide is a strong antimicrobial with a pH of 12.5. In agar diffusion tests, Sealapex and Apexit have been found to exert slight antimicrobial activity on various bacteria (Al-Khatib *et al.* 1990, Abdulkader *et al.* 1996, Mickel & Wright 1999). Dentine block models and direct contact tests indicate that the antimicrobial effect of calcium hydroxide based sealers increase with time (Heling & Chandler 1996, Fuss *et al.* 1997, Saleh *et al.* 2004), probably the result of disintegration of the sealer and an increase in the available amount of hydroxyl ions over time. The finding in this study that no significant antimicrobial effect was observed with the freshly mixed calcium hydroxide-based sealers might be explained by a too slow release of hydroxyl ions during the relatively short duration of contact. Furthermore, *E. faecalis* is known to be resistant to the antibacterial effect of calcium hydroxide (Byström *et al.* 1985, Haapasalo & Ørstavik 1987), probably partly because of its proton pump mechanism which maintains ideal cytoplasmic pH levels in a lethally alkaline environment (Evans *et al.* 2002).

Antimicrobial activity of a material generally is accompanied by cytotoxicity. Root canal sealers with strong antibacterial activity have been found to be cytotoxic and even mutagenic (Geurtsen & Leyhausen 1997). Among the sealers that exerted strong antibacterial activity in this study, AH Plus and the zinc oxide-eugenol-based sealer have previously been shown to possess pronounced cytotoxicity in the freshly mixed state which disappeared after 24 h of setting (Araki *et al.* 1994, Schwarze *et al.* 2002). No report regarding the cytotoxicity of MCS is found in the literature. However, another formulation based on iodoform (Kri I) was more cytotoxic than zinc oxide-eugenol: while zinc oxide-eugenol cytotoxicity decreased to control levels after 1 day of setting, it took more than 7 days for the iodoform paste (Wright *et al.* 1994). However, high clinical success rates have been reported for the iodoform paste (Holan & Fuks 1993).

In this paper, the antimicrobial activity of various root canal sealers was investigated *in vitro* and found that MCS, AH Plus and Grossman's sealer effectively reduced the CFUs of *E. faecalis*. However, it should be taken into consideration that *in vivo* conditions, such as the presence of dentine or serum, might modify these effects (Meryon 1984, Portenier *et al.* 2001). In addition to antimicrobial activity, other properties such as biocompatibility, stability and sealability must also be considered when selecting a root canal sealer.

Conclusions

MCS, AH Plus and Grossman's sealer were effective in reducing the CFUs of *E. faecalis* either in direct contact or when restricted by a membrane. Calcium hydroxide-based sealers, Sealapex and Apexit were ineffective in this short-term experiment.

Acknowledgements

The financial support of The Research Council of Norway and NIOM-Scandinavian Institute of Dental Materials is gratefully acknowledged.

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