In vitro antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate, Portland cement and EndoRez

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Abstract

Sipert CR, Hussne RP, Nishiyama CK, Torres SA. *In vitro* antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate, Portland cement and EndoRez. *International Endodontic Journal*, **38**, 539–543, 2005.

Aim To determine *in vitro* the antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate (MTA), Portland cement and EndoRez on various species of microorganisms.

Methodology The diffusion method on Müller–Hinton agar (MH) was employed. A base layer was made using MH agar and five wells were made by removing agar at equidistant points. Sealers were placed into the wells immediatelly after manipulation. The microorganisms *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 27853 and *Candida albicans* ATCC 10231 were seeded by pour plate. The plates were kept at room temperature for 2 h for prediffusion and then incubated at 37 °C for 24 h. Aliquots of 10 mL of 0.05% triphenyltetrazolium chloride gel were added for optimization and the zones of inhibition were measured.

Results Sealapex and Fill Canal demonstrated antimicrobial activity for all strains. For MTA and Portland cement, only *E. coli* was not inhibited. No antimicrobial activity was detected for EndoRez.

Conclusions In this laboratory study, Fill Canal, Sealapex, MTA and Portland cement presented antimicrobial activity whilst EndoRez did not.

Keywords: antimicrobial activity, endodontic sealers, Endorez, Mineral Trioxide Aggregate, Portland cement, Sealapex.

Received 8 June 2004; accepted 1 April 2005

Introduction

As microorganisms are the main aetiological factor in pulpitis and apical periodontitis, their elimination during root canal treatment by instrumentation, irrigation and intracanal medication is essential. However, even after these procedures, bacteria might still be found inside the root canal system (Byström & Sundqvist 1983) with the potential for disease to persist or emerge (Ørstavik 1981). Thus, root filling plays an essential role in the control of reinfection by entombing residual organisms through the antimicrobial activity of endodontic sealers (Grossman 1980).

A second factor involved in the outcome of root canal treatment is the healing potential of tissues damaged by pulp/periapical pathology and root canal treatment procedures. The stimulus for healing depends on the absence of irritating agents originating from bacterial metabolic products, or of chemical origin from sealing materials (Leonardo *et al.* 2003, Ørstavik *et al.* 2004). Thus, for the healing process to occur, the sealer employed should not damage periapical tissues, and it would be an advantage if it could stimulate the deposition of hard tissue, therefore promoting biological sealing (Leonardo *et al.* 2003, Ørstavik *et al.* 2004).

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One endodontic sealer that is associated with the healing of periapical tissues is Sealapex (Sybron-Kerr, Romulus, MI, USA), a calcium hydroxide-based sealer known to contain a higher concentration of Ca^{2+} than other calcium hydroxide-based sealers (da Silva *et al.* 1997). EndoRez (Ultradent Products Inc., South Jordan, UT, USA), a new sealer containing a biocompatible methacrylate, has been described as having potential to penetrate dentinal tubules because of its hydrophilic characteristics (Ahlberg & Tay 1998). It has been reported to be well-tolerated by connective tissues (Zmener 2004) and acceptable as a root canal sealer in a clinical study (Zmener & Pameijer 2004).

Mineral Trioxide Aggregate (MTA) Angelus (Odonto-Lógika Ind. Prod. Odontológicos Ltda., Londrina, Paraná, Brazil) is a biocompatible material developed for bone repair procedures that has been compared with Portland cement (Cia. de Cimento Portland Itaú, Itaú de Minas, MG, Brazil) because of similarities in their chemical composition and tissue response (Estrela *et al.* 2000, Holland *et al.* 2001a,b).

Fill Canal (Ligas Odontológicas Ltda., Catumbi, São Paulo, Brazil) is a zinc oxide (ZOE)-based sealer with known activity against facultative microorganisms (Leonardo *et al.* 2000) like other ZOE-containing sealers (Barkhordar 1989, Canalda & Pumarola 1989, Pumarola *et al.* 1992, Torabinejad *et al.* 1995, Fuss *et al.* 1997, Mickel & Wright 1999). Its antimicrobial action is probably because of the presence of eugenol (Leonardo *et al.* 2000).

The purpose of this study was to evaluate the antimicrobial activity of Fill Canal, Sealapex, MTA, Portland cement and EndoRez.

Materials and methods

The composition of cements MTA Angelus, Portland cement and the sealers Sealapex, EndoRez and Fill Canal is shown in Table 1. These materials were evaluated in triplicate for antimicrobial activity using an agar diffusion method. Fill Canal was used for a positive control of inhibition.

The study was conducted on double-layered plates, in which the base layer was made of 10.0 mL of sterilized Müller–Hinton agar (MH; Difco, Detroit, MI, USA) poured in 20 × 100 mm sterilized Petri plates. Five wells (one for each material) were made by removal of agar at equidistant points and then filled immediately by sealers/cements after being mixed according to the manufacturer's instructions. Fill Canal, Sealapex, MTA and Portland cement were poured inside the wells whilst EndoRez was injected into them.

Table 1 Composition of materials tested for antimicrobial activity

Material	Composition
Mineral Trioxide Aggregate	Tricalcium silicate, tricalcium aluminate, tricalcium oxidated, silicate oxide, bismuth
Portland cement	Tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, hydrated calcium sulphate, alkali oxide
EndoRez	Urethane dimethacrylate resin as a matrix, zinc oxide, barium sulphate, resins, pigments
Fill Canal	Powder: zinc protoxide, hydrogenated resin, barium sulphate, anhydrous sodium borate Liquid: eugenol, almond-oil
Sealapex	Calcium oxide, barium sulphate, silica, titanium dioxide, zinc stearate

The strains used for analysis were *Candida albicans* ATCC 10231, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228 and *Pseudomonas aeruginosa* ATCC 27853. After activation from stock culture, microorganisms were maintained in MH broth until used. Overnight cultures of the microorganisms were used.

All the microbial strains were grown at 37 °C for 24 h in MH broth and then seeded into 15.0 mL of the MH agar, to produce a turbidity of 0.5 on the Mc Farland scale, which corresponds to a concentration of 10^8 colony forming units mL^{-1}. This broth was used as the second layer. The seeded agar was added over the plates immediatelly after the insertion of the sealers/cements. The plates were maintained at room temperature for 2 h for prediffusion of the materials and then incubated at 37 °C for 24 h. The same procedure was conducted in a plate without the addition of bacterial seeding.

A total number of 22 plates were employed: each microorganism (seven) was tested in triplicate and one plate was used without strain seeding.

Aliquots of 10.0 mL of triphenyltetrazolium chloride (TTC) gel prepared with 1.0% MH agar and 0.05% of 2, 3, 5 TTC (Sigma, St Louis, MO, USA) were added and the plates were incubated again at 37 °C for 30 min according to procedures previously reported by Leonardo *et al.* (2000). This procedure is useful to differentiate areas of microbial growth (red areas)

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diffusion zones. The zones of inhibition around the wells were then measured with a millimetre ruler with accuracy of 0.5 mm.

Statistical analysis

Statistical analysis was not performed because of the difference of diffusibility in agar of various sealers/cements. As the size of zone of an inhibition does not necessarily reflect the strength of antimicrobial agent, the assessment of activity of each material was performed considering the presence or absence of the zone of inhibition as reported in earlier studies (Barkhordar 1989, Al-Khatib *et al.* 1990, Leonardo *et al.* 2000).

Results

Table 2 shows the antimicrobial activity of endodontic sealers determined by the means and standard deviation of zones of inhibition in millimetres. The reading at 24 h of incubation provided the following data: Fill Canal, Sealapex, MTA and Portland cement all showed evidence of inhibition. Conversely, EndoRez did not demonstrate any antimicrobial activity. The strain of *E. coli* was not inhibited by MTA and Portland cement whilst Fill Canal and Sealapex inhibited the growth of all tested strains.

Discussion

The antimicrobial activity of endodontic sealers can be evaluated *in vitro* by the agar diffusion method (Tobias 1988, Siqueira Jr & de Uzeda 1997, Leonardo *et al.* 2000). However, the selection of the agar medium and microorganisms, control and standardization of inoculation density, incubation and reading point of the zones of inhibition are factors that affect the results of diffusion tests in an agar medium. Indeed, many different media, different methods of inoculum preparation or both have been used.

One other factor that must be considered in results, when the agar diffusion method is employed for the evaluation of antimicrobial activity of sealers, is the differentiation between zones of diffusion and inhibition, a factor that was improved by the addition of TTC gel (1.0 agar and 0.05% TTC) as described by Leonardo *et al.* (2000). This was performed to indicate the presence of viable cells (microorganisms) that appear red in colour (Wexler 1991) improving measurement precision specially in areas with material diffusion.

Many studies have reported the inhibitory activity of zinc oxide-based sealers (Barkhordar 1989, Canalda & Pumarola 1989, Pumarola *et al.* 1992, Torabinejad *et al.* 1995, Fuss *et al.* 1997, Mickel & Wright 1999, Leonardo *et al.* 2000). Fill Canal was used in the present study as a control and the results revealed that all strains tested were inhibited. This finding is similar to that observed in other studies. This antibacterial action is probably related to the presence of eugenol (Leonardo *et al.* 2000). Eugenol is a bactericidal at relatively high concentrations (10^{-2}–10^{-3} mol L^{-1}) being able to induce cell death and inhibit cell growth and respiration, even in lower concentrations; however, it can inhibit white cell chemotaxis, synthesis of prostaglandins and nerve activity. Several biochemical mechanisms have been proposed to explain the cytotoxicity of eugenol and its utilization in restorations is targeted to prevent bacterial penetration (Markowitz *et al.* 1992).

Sealapex has been studied extensively because of its ability to induce biological apical sealing (Holland & de Souza 1985). The effectiveness of this sealer against facultative microorganisms has also been reported (Barkhordar 1989, Canalda & Pumarola 1989, Pumarola *et al.* 1992). Al-Khatib *et al.* (1990) reported a higher effectiveness against microorganisms for Sealapex when compared with other calcium hydroxide-based sealers. Abdulkader *et al.* (1996) found inhibitory activity when utilizing strains of *Capnocytophaga ochracea*, *Porphyromonas gingivalis* and *Peptostreptococcus micros*, whereas the same activity against

Table 2 Means and standard deviation of inhibition haloes into the well method (mm)

	Microorganisms						
Sealers	*C. albicans*	*E. coli*	*E. faecalis*	*M. luteus*	*P. aeruginosa*	*S. aureus*	*S. epidermidis*
Mineral Trioxide Aggregate	15.0 ± 1.73	0.0	13.00 ± 1.00	10.33 ± 0.57	9.00 ± 1.00	10.33 ± 1.52	10.66 ± 0.57
Portland cement	15.33 ± 1.15	0.0	8.33 ± 1.52	8.33 ± 0.57	8.00 ± 0.0	10.33 ± 0.57	9.66 ± 0.57
EndoRez	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fill Canal	19.33 ± 0.57	21.33 ± 1.52	9.00 ± 0.0	16.00 ± 1.00	46.66 ± 1.52	11.33 ± 2.08	13.00 ± 0.0
Sealapex	23.66 ± 0.57	22.00 ± 3.60	14.00 ± 1.73	15.00 ± 1.00	16.00 ± 1.00	7.33 ± 0.57	10.33 ± 0.57

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F. nucleatum, *P. gingivalis*, *P. intermedia* and *P. endodontalis* could not be found by Lai *et al.* (2001). Recent studies (Leonardo *et al.* 2000, Lai *et al.* 2001, Mickel *et al.* 2003) have confirmed the effectiveness of Sealapex against facultative strains, in accordance with the results of the present investigation. The antimicrobial activity of Sealapex might be associated with its ionization, which releases hydroxyl ions that increase the pH, thus rendering the environment unfavourable for the growth of microorganisms (da Silva *et al.* 1997, Leonardo *et al.* 2000).

The antimicrobial activity of MTA was evaluated by Torabinejad *et al.* (1995), who detected its efficiency against some facultative bacteria; however, no activity was found for *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis* and *E. coli* or against anaerobic bacteria. The study of Estrela *et al.* (2000) did not reveal any antimicrobial activity of MTA or Portland cement against *S. aureus*, *E. faecalis*, *P. aeruginosa*, *B. subtilis*, *C. albicans*, yet both materials were found to contain the same chemical elements, except for the presence of bismuth in MTA. These data are in accordance with studies that report a similar composition and behaviour between these materials (Estrela *et al.* 2000, Holland *et al.* 2001a,b, Saidon *et al.* 2003). The present investigation demonstrated that, from the seven tested strains, MTA and Portland cement did not inhibit the growth of *E. coli*, a result that is at variance with that of Estrela *et al.* (2000). The prediffusion period of 2 h before incubation of the plates is compatible with the results described by Leonardo *et al.* (2000) with Sealapex. The mechanism of action of MTA has also been reported to be similar to calcium hydroxide as both MTA and Portland cement contain calcium oxide, which in contact with water could form calcium hydroxide (Holland *et al.* 2001a,b).

In this study, no antimicrobial activity when tested against facultative microorganisms was observed for EndoRez, a new resin-based sealer. The possible action of EndoRez is described by its hydrophilic characteristics, which potentially improves its sealing properties because of penetration into dentinal tubules (Ahlberg & Tay 1998); however, no data are available on its antimicrobial activity.

Conclusion

The sealers Fill Canal and Sealapex displayed inhibitory activity against all facultative strains tested. Once again, MTA and Portland cement showed similarity in their behaviour, with inhibitory activity for six of the seven strains tested. Antimicrobial properties were not found for EndoRez.

Acknowledgements

The authors would like to express their gratitude to André Luis da Silva and Dalva Ribeiro de Oliveira for excellent technical assistance.

References

Abdulkader A, Duguid R, Saunders EM (1996) The antimicrobial activity of endodontic sealers to anaerobic bacteria. *International Endodontic Journal* **29**, 280–3.

Ahlberg KM, Tay WM (1998) A methacrylate-based cement used as a root canal sealer. *International Endodontic Journal* **31**, 15–21.

Al-Khatib ZZ, Baum RH, Morse DR, Yesilsoy C, Bhambhani S, Furst ML (1990) The antimicrobial effect of various endodontic sealers. *Oral Surgery, Oral Medicine, Oral Pathology* **70**, 784–90.

Barkhordar RA (1989) Evaluation of antimicrobial activiy in vitro of ten root canal sealers on *Streptococcus sanguis* and *Streptococcus mutans*. *Oral Surgery, Oral Medicine, Oral Pathology* **68**, 770–2.

Byström A, Sundqvist G (1983) Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. *Oral Surgery, Oral Medicine, Oral Pathology* **55**, 307–12.

Canalda C, Pumarola J (1989) Bacterial growth inhibition produced by root canal sealer cements with a calcium hydroxide base. *Oral Surgery, Oral Medicine, Oral Pathology* **68**, 99–102.

Estrela C, Bammann LL, Estrela CR, Silva RS, Pecora JD (2000) Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. *Brazilian Dental Journal* **11**, 3–9.

Fuss Z, Weiss EI, Shalhav M (1997) Antibacterial activiy of calcium hydroxide-containing endodontic sealers on *Enterococcus faecalis* in vitro. *International Endodontic Journal* **30**, 397–402.

Grossman L (1980) Antimicrobial effect of root canal cements. *Journal of Endodontics* **6**, 594–7.

Holland R, de Souza V (1985) Ability of a new calcium hydroxide root canal filling material to induce hard tissue formation. *Journal of Endodontics* **11**, 535–43.

Holland R, de Souza V, Nery MJ et al. (2001a) Reaction of rat connective tissue to implanted dentin tube filled with mineral trioxide aggregate, portland cement or calcium hydroxide. *Brazilian Dental Journal* **12**, 3–8.

Holland R, de Souza V, Murata SS, et al. (2001b) Healing process of dog dental pulp after pulpotomy and pulp covering with mineral trioxide aggregate or Portland cement. *Brazilian Dental Journal* **12**, 109–13.

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Lai CC, Huang FM, Yang HW, et al. (2001) Antimicrobial activity of four root canal sealers against endodontic pathogens. *Clinical Oral Investigation* **5**, 236–9.

Leonardo MR, da Silva LA, Tonomaru Filho M, Bonifácio KC, Ito IY (2000) In vitro evaluation of antimicrobial activity of sealers and pastes used in endodontics. *Journal of Endodontics* **26**, 391–4.

Leonardo MR, Salgado AA, da Silva LA, Tonomaru Filho M (2003) Apical and periapical repair of dogs' teeth with periapical lesions after endodontic treatment with different root canal sealers. *Pesquisa Odontológica Brasileira* **17**, 69–74.

Markowitz K, Moynihan M, Liu M, Kim S (1992) Biologic properties of eugenol and zinc oxide-eugenol. *Oral Surgery, Oral Medicine, Oral Pathology* **73**, 729–37.

Mickel AK, Wright ER (1999) Growth inhibition of *Streptococcus anginosus* (milleri) by three calcium hydroxide sealers and one zinc oxide-eugenol sealer. *Journal of Endodontics* **25**, 34–7.

Mickel AK, Nguyen TH, Chogle S (2003) Antimicrobial activity of endodontic sealers on *Enterococcus faecalis*. *Journal of Endodontics* **29**, 257–8.

Ørstavik D (1981) Antibacterial properties of root canal sealers, cements and pastes. *International Endodontic Journal* **14**, 125–33.

Ørstavik D, Qvist V, Stoltze K (2004) A multivariate analysis of the outcome of endodontic treatment. *European Journal of Oral Science* **112**, 224–30.

Pumarola J, Berastegui E, Brau E, Canalda C, Jimenez de Anta MT (1992) Antimicrobial activity of seven root canal sealers. Results of agar diffusion and agar dilution tests. *Oral Surgery, Oral Medicine, Oral Pathology* **74**, 216–20.

Saidon J, He J, Zhu Q, Safavi K, Spangberg LS (2003) Cell and tissue reactions to mineral trioxide aggregate and Portland cement. *Oral Surgery, Oral Medicine, Oral Pathology, Endodontics* **95**, 483–9.

da Silva LA, Leonardo MR, da Silva RS, Assed S, Guimarães LF (1997) Calcium hydroxide root canal sealers: evaluation of pH, calcium ion concentration and condutivity. *International Endodontic Journal* **30**, 205–9.

Siqueira JF Jr, de Uzeda M (1997) Intracanal medicaments: evaluation of the antibacterial effects of chlorhexidine, metronidazole, and calcium hydroxide associated with three vehicles. *Journal of Endodontics* **23**, 167–9.

Tobias RS (1988) Antibacterial properties of dental restorative materials: a review. *International Endodontic Journal* **21**, 155–60.

Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD (1995) Antibacterial effects of some root end filling materials. *Journal of Endodontics* **21**, 403–6.

Wexler HM (1991) Susceptibility testing of anaerobic bacteria: myth, magic, or method? *Clinical Microbiology Review* **4**, 470–84.

Zmener O (2004) Tissue response to a new methacrylate-based root canal sealer: preliminary observations in the subcutaneous connective tissue of rats. *Journal of Endodontics* **30**, 348–51.

Zmener O, Pameijer CH (2004) Clinical and radiographic evaluation of a resin-based root canal sealer. *American Journal of Dentistry* **17**, 19–22.

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