ANTIMICROBIAL ACTIVITY OF ENDODONTIC SEALERS BASED ON CALCIUM HYDROXIDE AND MTA

Juliane M. G. Tanomaru[1], Mário Tanomaru-Filho[1], Juliana Hotta[1], Evandro Watanabe[2], Izabel Y. Ito[2]

[1]Restorative Dentistry, Araraquara Dental School, São Paulo State University (UNESP), Araraquara, SP, Brazil.
[2]Microbiology, Ribeirão Preto Pharmaceutics School, University of São Paulo (USP), Ribeirão Preto, SP, Brazil.

ABSTRACT

The aim of this study was to evaluate the antimicrobial activity of a new root canal sealer containing calcium hydroxide (Acroseal) and the root canal sealer based on MTA (Endo CPM Sealer), in comparison with traditional sealers (Sealapex, Sealer 26 and Intrafill) and white MTA-Angelus, against five different microorganism strains. The materials and their components were evaluated after manipulation, employing the agar diffusion method. A base layer was made using Müller-Hinton agar (MH) and wells were made by removing agar. The materials were placed into the wells immediately after manipulation. The microorganisms used were: Micrococcus luteus (ATCC9341), Staphylococcus aureus (ATCC6538), Pseudomonas aeruginosa (ATCC27853), Candida albicans (ATCC 10231), and Enterococcus faecalis (ATCC 10541). The plates were kept at room temperature for 2 h for prediffusion and then incubated at 37°C for 24 h. The results showed that Sealapex and its base paste, Sealer 26 and its powder, Endo CPM Sealer and its powder, white MTA and its powder all presented antimicrobial activity against all strains. Intrafill and its liquid presented antimicrobial activity against all strains except P aeruginosa and Acroseal was effective only against M. luteus and S. aureus.

Key words: root canal filling material, antimicrobial activity, Mineral Trioxide Aggregate.

ATIVIDADE ANTIMICROBIANA DE CIMENTOS ENDODÔNTICOS À BASE DE HIDRÓXIDO DE CÁLCIO E MTA

RESUMO

O objetivo deste estudo foi avaliar a atividade antimicrobiana do cimento endodôntico contendo hidróxido de cálcio (Acroseal) e do cimento à base de MTA (Endo CPM Sealer), comparados com cimentos tradicionais (Sealapex, Sealer 26 e Intrafill) e com o MTA-Angelus branco, sobre 5 diferentes espécies de microrganismos. Os materiais foram avaliados logo após a manipulação, assim como seus componentes. O método utilizado foi o de difusão em agar. Uma camada base foi confeccionada usando agar Müller-Hinton (MH) e poços foram formados pela remoção do agar. Os materiais foram colocados nos poços logo após sua manipulação. Os microrganismos usados foram: Micrococcus luteus (ATCC 9341), Staphylococcus aureus (ATCC6538), Pseudomonas aeruginosa (ATCC27853), Candida albicans (ATCC 1023) e Enterococcus faecalis (ATCC 10541). As placas foram mantidas na temperatura ambiente por 2 horas para pré-difusão e então incubadas a 37°C por 24 h. Os resultados demonstraram que o Sealapex e sua pasta base, o Sealer 26 e seu pó, o Endo CPM Sealer e seu pó, o MTA branco e seu pó apresentaram atividade antimicrobiana sobre todas as cepas avaliadas. O Intrafill e seu líquido apresentaram ação antimicrobiana sobre todos os microrganismos exceto P aeruginosa e o Acroseal somente sobre M. luteus e S. aureus.

Palavras chave: material obturador de canal radicular, atividade antimcrobiana, Mineral Trióxido Agregado.

INTRODUCTION

The main goal of the root canal filling procedure is to achieve a complete seal of the root canal system. If this step fails, the results of the endodontic therapy may be compromised[1,2].

In search of materials with adequate biological properties, the inclusion of calcium hydroxide in the composition of endodontic sealer cements has been proposed[3-6].

The first calcium hydroxide-based endodontic cement released was Sealapex. Holland & Souza[3] demonstrated that this material presents excellent biocompatibility when tested in dogs and monkeys and was able to induce apical seal with mineralized tissue.

Table 1: Root canal filling materials used in the study.

Material	Manufacturer
Sealapex Sealapex Base Sealapex Catalizer	SybronEndo, Orange, CA, USA
Acroseal Acroseal paste A Acroseal paste B	Specialités-Septodont, Saint Maur-des-Fossés, Cedex, France
Sealer 26 Sealer 26 powder Sealer 26 resin	Dentsply, Rio de Janeiro, RJ, Brasil
CPM Sealer CPM Sealer powder CPM Sealer liquid	EGEO S.R.L. Bajo licencia MTM S.A., Buenos Aires, Argentina
MTA Branco (white) MTA Branco (white) powder MTA Branco (white) liquid	Ângelus, Londrina, Brasil
Intrafill Intrafill powder Intrafill liquid	SSWhite, Rio de Janeiro, RJ, Brasil

Table 2: Strains used as indicators of antimibrobial activity, their source, and morphotype.

Microrganisms	Source	Morphotype
Micrococcus luteus	ATCC 9341	cg+
Staphylococcus aureus	ATTC 6538	cg+
Pseudomonas aeruginosa	ATCC 27853	bg-
Candida albicans	ATCC 1023	le
Enterococcus faecalis	ATCC 10541	cg+

cg+: Gram-positive cocci; bg-: Gram-negative bacillus; le:yeast

Other calcium hydroxide-based endodontic cements were later developed. Sealer 26, which combines epoxy resin with calcium hydroxide, has demonstrated good sealing capacity[7].

Acroseal, a sealer with a composition similar to Sealer 26, was more recently released. Studies on pH and calcium release have demonstrated that Sealer 26 promotes less alkalinization of the medium and lower calcium release than other calcium hydroxide-containing cements, such as Sealapex[4,8]. Similar results were observed for Acroseal[9].

Among the newer materials, Mineral Trioxide Aggregate (MTA) has been widely advocated in endodontics for treatment of root perforations, pulp capping, pulpotomy and retrograde fillings. MTA has an alkaline pH[10] and, according to Holland et al.[11,12], its mechanism of action is similar to that of calcium hydroxide. Recently launched, Endo CPM Sealer is a new MTA-based endodontic cement indicated for root canal sealing.

The introduction of endodontic cements containing calcium hydroxide and MTA warrants studies on the antimicrobial effect of these materials and their components, in order to better understand the properties of the cements and to determine which component is responsible for each of the effects observed. The aim of this study was to evaluate the antimicrobial activity of a new root canal sealer containing calcium hydroxide (Acroseal) and the root canal sealer based on MTA (Endo CPM Sealer), in comparison with traditional sealers (Sealapex, Sealer 26 and Intrafill) and white MTA-Angelus, against five different microorganism strains.

MATERIALS AND METHODS

The source of the various root canal filling materials is presented in Table 1. The morphotype and source of the strains used as indicators of antimicrobial activity are presented in Table 2. The materials were evaluated in duplicate for antimicrobial activity using the agar diffusion method.

The well method was conducted on double-layered plates. The base layer was composed of 10.0 mL sterilized Müller-Hinton agar (MH; Difco, Detroit, MI, USA) poured in 20 X 100 mm sterilized Petri plates. After solidification, a 5.0 mL seed layer, obtained by the addition of the inoculum at a concentration of 10^6 colony forming units/mL to 5.0 mL of MH, was added. Thereafter, eight wells, 4 mm in diameter (one for each material), were made by removal of agar at equidistant points and then filled immediately with the materials to be evaluated.

The root canal sealers were manipulated according to the manufacturer's instructions. The plates were maintained at room temperature for 2 h for prediffusion of the materials, and then incubated at 37°C for 24 h. After solidification, they were incubated at 37°C for 30 min. The inhibition zones around the

Table 3: Means of the inhibition zones (in millimeters)*.

Microorganisms / Materials	*M. luteus*	*S. aureus*	*P. aeruginosa*	*C. albicans*	*E. faecalis*
Sealapex	12	12	10	14.5	10.5
Sealapex Base	16.5	16.5	12	17.5	10.5
Sealapex Catalizer	0	0	0	0	0
Acroseal	10	13.5	0	0	6
Acroseal paste A	0	0	0	0	0
Acroseal paste B	12.5	16	0	0	6.5
Sealer 26	17.5	18	12.5	9	10.5
Sealer 26 powder	14	11	10.5	11	14
Sealer 26 resin	0	0	0	0	0
CPM Sealer	20.5	19.5	15	16	12
CPM Sealer powder	14.5	10	16	8.5	9
CPM Sealer liquid	10	7.5	6.5	0	0
MTA (white)	17	16	14.5	14.5	15
MTA (white) powder	12	12.5	13	14	13.5
MTA (white) liquid	0	0	0	0	0
Intrafill	22.3	13.5	0	18.5	7
Intrafill powder	23	15	0	0	6
Intrafill liquid	23.5	18	0	25	11.5

*Means of the duplicate assays

wells were then measured with a millimeter ruler with accuracy of 0.5 mm.

RESULTS

Table 3 shows the antimicrobial activity of materials tested and the mean diameter of the inhibition zones in millimeters. The results showed that Sealapex and its base paste, Sealer 26 and its powder, Endo CPM Sealer and its powder, white MTA and its powder presented antimicrobial activity against all strains. Intrafill and its liquid presented antimicrobial activity against all strains except *P aeruginosa,* and Acroseal was effective only against *M. luteus* and *S. aureus*.

DISCUSSION

The agar diffusion method used in the present study is one of the most commonly employed techniques to evaluate antimicrobial activity[13,14]. The prediffusion period, which consists of maintaining the innoculated culture medium at room temperature for 2 hours, is an important step to demonstrate the antimicrobial activity of calcium hydroxide-based materials, such as Sealer 26, Sealapex, and of MTA-based cements. Our results were in accordance with those obtained by Leonardo et al.[15], who employed similar methodology.

Statistical analysis was not performed due to the different degrees of diffusion in agar of the various materials/cements. In this context, the area of the inhibition zone does not necessary reflect the strength of the antimicrobial agent. Thus, the antimicrobial activity of each material was assessed by the presence (or absence) of an inhibition zone, as reported in previous studies[15-17].

The microorganisms utilized in this study included facultative bacteria and a yeast. These microorganisms are predominant in persistent or refractory periapical lesions[18,19].

Our results showed that Sealapex and its base paste, Sealer 26 and its powder, Endo CPM Sealer and its powder, white MTA and its powder presented antimicrobial activity against all strains. The antimicrobial activity of calcium hydroxide-based materials such as Sealapex and Sealer 26 may be related to ionization with subsequent release of hydroxide ions and rise in pH levels, resulting in an unfavorable environment for microbial growth[4,15]. This effect may be confirmed by the antimicrobial action of both the base paste of Sealapex and the powder of Sealer 26, which include calcium oxide or calcium hydroxide in their composition. Leonardo et al.[15], in a study with a similar agar diffusion methodology, observed that Sealapex and calcium hydroxide-based pastes presented antimicrobial action.

The antimicrobial activity of MTA was evaluated by Torabinejad et al.[20], who detected its effectiveness against some facultative bacteria. MTA-based materials contain calcium oxide in their composition. When this substance is mixed with water, formation of calcium hydroxide occurs, inducing a rise in pH levels by dissociation of the calcium and hydroxide ions, as demonstrated by Duarte et al.[10]. Antimicrobial effect was also observed for MTA powder and Endo CPM Sealer, which contain calcium oxide in their formulation, confirming that

calcium oxide plays a role in the antimicrobial activity of these materials.

Therefore, the antimicrobial activity of MTA and Endo CPM Sealer may be associated to their elevated pH. Torabinejad et al.[20] observed an initial pH of 10.2 for MTA, rising to 12.5 in 3 h. It is known that pH levels in the order of 12.0 can inhibit most microorganisms, including resistant bacteria such as *Enterococcus faecalis*[21].

In this study, MTA and Endo CPM Sealer presented similar antimicrobial activity, suggesting that the changes in composition during the manufacturing of Endo CPM Sealer did not interfere with the antimicrobial action of MTA.

Intrafill and its liquid presented antimicrobial activity against all strains except *P. aeruginosa*, and Acroseal was effective only against *M. luteus* and *S. aureus*. The antimicrobial action of the zinc oxide and eugenol-based cements such as Intrafill is due to the presence of eugenol. Saleh et al.[22] evaluated the survival of *E. faecalis* in infected dentin tubules, after sealing the root canals with different endodontic cements. Sealing the canals with gutta-percha and AH Plus or Grossman cement (zinc oxide and eugenol-based) was more effective in eliminating *E. faecalis* than sealing with Roekoseal.

Using a similar protocol, Sipert et al.[16] found "in vitro" antimicrobial action for Sealapex, Fill Canal, Pro Root MTA, and Portland. Fill Canal has a similar composition to Intrafill, which did not show antimicrobial activity against *Pseudomonas aeruginosa* in the present study.

Tanomaru-Filho et al.[23] observed similar antimicrobial activity in MTA-based Portland cement, and other endodontic materials used in retrograde obturation, such as Sealapex with zinc oxide and Sealer 26. The results of this study revealed even lower antimicrobial action for Acroseal cement. This material, despite presenting a formulation comparable to Sealer 26, did not show the same antimicrobial acitivity. Its effect was restricted to inhibiting *M. luteus* and *S. aureus*.

CORRESPONDENCE
Mário Tanomaru-Filho
Rua Humaitá, 1901, apto. 182, Centro, 14801-385
Araraquara, SP, Brasil.
Telephone +55-16-3301-6390, Fax. +55-16-3301-6392
e-mail: tanomaru@uol.com.br

REFERENCES

1. Leonardo MR, Leal JM. Materiais obturadores de canais radiculares. In: Leonardo MR. Endodontia: tratamento de canais radiculares: princípios técnicos e biológicos. São Paulo: Artes Médicas; 2005. v. 2, p.1062-145.
2. Nair PN, Henry S, Cano V, Vera J. Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after "one-visit" endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005; 99: 231-52.
3. Holland R, Souza V. Ability of a new calcium hydroxide root canal filling material to induce hard tissue formation. J Endod. 1985; 11:535-43.
4. Silva LAB, Leonardo MR, Silva RS, Assed S, Guimaraes LFL. Calcium hydroxide root canal sealers: evaluation of pH, calcium ion concentration and conductivity. Int Endod J. 1997; 30:205-9.
5. Leonardo MR, Silva LAB, Utrilla LS, Asssed S, Ether SS. Calcium hydroxide root canal sealers – histopathologic evalution of apical and periapical repair after endodontic treatment. J Endod. 1997; 23:428-32.
6. Duarte MA, Demarchi ACO, Moraes IG. Determination of pH and calcium ion release provided by pure and calcium hydroxide-containing AH Plus. Int Endod J. 2004; 37:42-5.
7. Siqueira JF, Roças IN, Valois CR. Apical sealing ability of five endodontic sealers. Austr Endod J. 2001; 27:33-5.
8. Duarte MA, Demarchi ACO, Giaxa MH, Kuga MC, Fraga SC, SOuza LC. Evaluation of pH and calcium ion release of three root canal sealers. J Endod. 2000; 26:389-90.
9. Eldeniz AU, Erdemir A, Kurtoglu F, Esener T. Evaluation of pH and calcium ion relaese of Acroseal sealer in comparison with Apexit and Sealapex sealers. Oral Surg Oral Med Oral Pathol Oral Endod Radiol 2007; 103:e87-e91.
10. Duarte MA, Demarchi ACO, Yamashita JC, Kuga MC, Fraga SC. pH and calcium ion release of 2 root-end filling materials. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003; 95:345-7.
11. Holland R, Souza V, Nery MJ, Otoboni Filho JA, Bernabé PFE, Dezan Junior E. Reaction of rat connective tissue to implanted dentin tube filled with Mineral Trioxide Aggregate and Calcium Hydroxide. J Endod. 1999; 25:161-6.
12. Holland R, Souza, V, Nery, MJ, Faraco Junior, IM, BErnabé, PFE, Otoboni Filho, JA. Reaction of rat connective tissue to implanted dentin tube filled with Mineral Trioxide Aggregate, Portland Cement and Calciun Hydroxide. Braz Dent J. 2001; 12:3-8.
13. Georgopoulou M, Kontakiotis E, Nakou M. In vitro evaluation of the effectiveness of calcium hydroxide and

paramonochlorophenol on anaerobic bacteria from the root canal. Endod Dent Traumat. 1993; 9: 249-53.
14. Lui JN, Sae-Lim V, Song KP, Chen NN. In vitro antimicrobial effect of chlorhexidine-impregnated gutta percha points on *Enterococcus faecalis*. Int Endod J. 2004; 37:105-13.
15. Leonardo MR, Silva LAB, Tanomaru Filho M, Cortes KC, Ito IY. In vitro evaluation of antimicrobial activity of sealers and pastes used in endodontics. J Endod. 2000; 26: 391-4.
16. Sipert CR, Hussne RP, Nishiyama CK, Torres SA. In vitro antimicrobial activity of Fill Canal, Sealapex, Mineral Trioxide Aggregate, Portland cement and EndoRez. In Endod. J. 2005; 38:539-43.
17. Barkhordar. Evaluation of antimicrobial activity in vitro of ten root canal sealers on *Streptococcus sanguis* and *Streptococcus mutans*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1989; 68:770-2.
18. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1998; 85:86-93.
19. Bulacio MA, Cangemi R, Cecilia M, Raiden G. In vitro antibacterial effect of different irrigating solutions on *Enterococcus faecalis*. Acta Odontol Latinoam. 2006; 19:75-80.
20. Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root end filling materials. J Endod. 1995; 21:403-6.
21. McHugh CP, Zhang P, Michalek S, Eleazer PD. pH required to kill *Enterococcus faecalis* in vitro. J Endod. 204; 30:218-9.
22. Saleh IM, Ruyter IE, Haapasalo MP, Ørstavik D. Adhesion of Endodontic Sealers: Scanning Electron Microscopy and Energy Dispersive Spectroscopy. J Endod. 2003; 29: 595-601.
23. Tanomaru Filho, M, Tanomaru, JMG, Barros, DB, Watanabe E, Ito, IY. In vitro antimicrobial activity of endodontic sealers, MTA-based cements and Portland cement. J Oral Sci. 2007;49:41-5.