

COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTION OF MTA, CALCIUM HYDROXIDE AND PORTLAND CEMENT

AVALIAÇÃO COMPARATIVA DA AÇÃO ANTIMICROBIANA DO MTA, HIDRÓXIDO DE CÁLCIO E CIMENTO PORTLAND

Caroline Sousa RIBEIRO¹, Fernanda Akemi KUTEKEN¹, Raphael HIRATA JÚNIOR², Miriam F. Zaccaro SCELZA³

1- DDS, Undergraduate student, Department of Endodontics, Fluminense Federal University, Niterói, RJ, Brazil.

2- DDS, MSc, PhD, Associate Professor, Department of Microbiology, State University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil.

3- DDS, MSc, PhD, Associate Professor, Department of Endodontics, Fluminense Federal University, Niterói, RJ, Brazil.

Corresponding address: Miriam Fátima Zaccaro Scelza - Av. 28 de Setembro 44/ 704 - Vila Isabel - 20551-031 - Rio de Janeiro, RJ, Brazil
Tel: (55) (21) 2234-0901 - e-mail: scelza@terra.com.br

Received: October 31, 2005 - **Modification:** March 09, 2006 - **Accepted:** June 07, 2006

ABSTRACT

The present study aimed to evaluate and compare the antimicrobial effect of MTA Dentsply, MTA Angelus, Calcium Hydroxide and Portland cement. Four reference bacterial strains were used: *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacteroides fragilis*, and *Enterococcus faecalis*. Plates containing Mueller-Hinton agar supplemented with 5% sheep blood, hemin, and menadione were inoculated with the bacterial suspensions. Subsequently, wells were prepared and immediately filled with materials and incubated at 37°C for 48 hours under anaerobic conditions, except *P. aeruginosa*. The diameters of inhibition zones were measured, and data analyzed using ANOVA and the Tukey test with 1% level of significance. MTA Dentsply, MTA Angelus and Portland cement inhibited the growth of *P. aeruginosa*. Calcium Hydroxide was effective against *P. aeruginosa* and *B. fragilis*. Under anaerobic conditions, which may hamper the formation of reactive oxygen species, the materials failed to inhibit *E. faecalis*, and *E. coli*.

Uniterms: Mineral trioxide aggregate; Calcium hydroxide; Portland cement; Antimicrobial activity.

RESUMO

Objetivo do presente trabalho foi avaliar e comparar o efeito antimicrobiano do MTA Dentsply, MTA Angelus, hidróxido de cálcio e cimento Portland sobre quatro cepas bacterianas: *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacteroides fragilis*, e *Enterococcus faecalis*. Placas contendo agar Muller-Hinton suplementadas com 5% de sangue de carneiro, hemina e menadiona foram inoculadas com as suspensões bacterianas. Poços foram confeccionados com auxílio de perfuradores e imediatamente preenchidos com os materiais, e incubados a 37°C por 48 horas em atmosfera de anaerobiose, exceto *P. aeruginosa*. O diâmetro dos halos de inibição foi medido e os dados analisados usando o teste estatístico ANOVA e o de Tukey com nível de significância de 1%. O MTA Dentsply, MTA Angelus e Cimento Portland inibiram o crescimento da *P. aeruginosa*. O hidróxido de cálcio foi efetivo contra *P. aeruginosa* e *B. fragilis*. Sob atmosfera de anaerobiose, condição que pode impedir a formação de espécies reativas do oxigênio, nenhum dos materiais foi capaz de exercer efeitos sobre *E. faecalis* e *E. coli*.

Unitermos: Agregado de trióxido mineral; Hidróxido de cálcio; Cimento Portland; Efeito antimicrobiano.

INTRODUCTION

Progress has been made in understanding the nature of root canal infection and periapical diseases. Advances in techniques and materials increase the success of surgical intervention in cases of failure of conventional root canal treatment or retreatment⁶.

During periradicular surgery, residual soft tissues surrounding the root apex are removed, and root end cavity is prepared subsequently to the apex resection. Dental materials used in root end fillings must achieve perfect isolation of root canal in order to prevent the leakage of fluids arising from periradicular tissues that might support the growth of viable microorganisms eventually present in endodontic microniches.

Ideal retrofilling materials must be present: 1- adhesion ability and three-dimensional sealing of the root canal system; 2- be non-toxic; 3-dimensional stability even in the present of humidity; 4-not be absorbed and 5-present radiopacity^{2,10}. In addition, antimicrobial, allied to biocompatibility, are properties that clearly contribute to the success of the surgical procedure^{4,12,15}.

Mineral Trioxide Aggregate consists of an aggregate mainly composed by tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminum ferrite, dehydrated calcium sulfate and bismuth oxide¹⁶. Due to its compatibility and sealer properties, MTA is indicated for retrofillings in periapical surgeries, in sealing root drilling, in the protection of pulp tissue, in apical sealing in cases of incomplete root formation, and in treatment of root resorption¹⁷. Although Portland cement shows similarities with MTA in its main components (tricalcium silicate, dicalcium silicate, tricalcium aluminate, and tetracalcium aluminum ferrite), few reports have dealt with biocompatibility or antimicrobial aspects of this cement. Recently, the antimicrobial property of Portland cement was investigated along with other endodontic filling materials against Gram-positive and Gram-negative bacteria. Nevertheless, Portland cement and MTA formulations was investigated with microorganisms grown aerobically, and did not include strictly anaerobic Gram-negative bacteria¹⁴. Thus, the aim of this study was to assess the antimicrobial properties of Portland cement and MTA against four bacterial species grown in anaerobic environment.

MATERIAL AND METHODS

Test materials: Mineral Trioxide Aggregate (MTA Dentsply, Tulsa dental - USA, and MTA Angelus, Londrina Pr, Brazil) was manipulated strictly following the manufacturer's instructions. Calcium Hydroxide (CHPP.A. Quimis, Mallinkrodt Inc., St Louis, MO, USA in sterile saline solution), and Portland cement (Votoran, Votorantim, São Paulo, Brazil) were manipulated as described elsewhere⁹.

Antimicrobial activity of endodontic materials were evaluated by agar diffusion method against four reference strains: *Enterococcus faecalis* (ATCC 29212), *Pseudomonas*

aeruginosa (ATCC 27853), *Bacteroides fragilis* (ATCC 23745), and *Escherichia coli* (ATCC 33780). *Bacteroides fragilis* strain was incubated under anaerobic conditions or cultivated in pre-reduced anaerobic sterilized Trypticase Soy Broth (TSB-PRAS, Difco – Detroit, USA). Facultative or aerobic strains were grown in TSB 37°/24h. Bacteria were diluted to obtain a suspension of approximately 5×10^8 colony forming units mL⁻¹ (0.5 in McFarland's nephelometer) in sterile TSB. Microbial strains were confirmed by Gram's stain and by colonial and growth characteristics. Bacterial suspensions were inoculated with sterile cotton swabs onto Muller-Hinton Agar Plates (MHA – Difco, Detroit, USA) supplemented with sheep blood (5% v/v), hemin (0.1 mg mL⁻¹), and menadione (0.001 mg mL⁻¹). Wells were prepared on MHA with a copper puncher of 3mm diameter and 4mm depth, and immediately filled with freshly manipulated test materials. Negative controls consisted of wells filled with liquefied MHA. After prediffusion of test materials for 2h at room temperature, agar plates were subsequently incubated at 37°C/48h. Controls of contamination of dental materials, and agar medium sterility were performed by incubation of agar plates without bacterial inoculation. All strains, except *P. aeruginosa* were incubated in anaerobic atmosphere (80% N₂, 10% CO₂, and 10% H₂) in a Gaspack jar. To assess the effect of aerobic atmosphere on antimicrobial activity of MTA formulations and Ca(OH)₂, facultative microorganisms: *E. coli* and *E. faecalis* were also incubated aerobically 37°C/24h.

Microbial inhibition zones were measured with a 0.5mm precision rule, and results were expressed as a mean (and standard deviation) of three independent experiments.

Data were submitted to statistical analysis by ANOVA and Tukey tests with level of significance to compare the differences among two MTA compositions, Portland cement and Calcium hydroxide.

RESULTS

Antimicrobial activities of mineral trioxide compositions and Portland cement are expressed in Table 1. MTA formulations of Angelus and Dentsply, Portland cement, and calcium hydroxide were showed to inhibit *P. aeruginosa*. Nevertheless, MTA formulations and Portland cement were incapable to inhibit *E. coli*, *E. faecalis* and *B. fragilis*. The calcium hydroxide also showed inhibitory activity, with larger inhibition zones against *P. aeruginosa* and *B. fragilis*. The Table 2 expresses the effect of aerobic atmosphere on antimicrobial activity of calcium hydroxide, MTA Angelus and MTA Pro-root against *E. coli* and *E. faecalis*. Aerobic atmosphere rendered *E. faecalis* susceptible to both MTA formulations and Ca(OH)₂. *E. coli* strains were only susceptible to Ca(OH)₂.

DISCUSSION

Antimicrobial activity of endodontic sealers has been evaluated *in vitro* by agar diffusion method. Although used by many authors^{1,8,10} differences in agar medium, diffusion capacity of inhibitory agents, bacterial strains and cellular density, as well as anaerobic atmosphere may interfere with formation of inhibition zones around materials used in antimicrobial testing⁹. However, there is not a consensus regarding to a gold standard test for the appraisal of antimicrobial testing of cements and other solutions used in dental therapy. The results of inhibitory activity of both Portland cement and MTA were similar to those observed in the literature related to *P. aeruginosa* and *E. coli* strains, which demonstrated sensitivity and resistance to both materials, respectively⁸. In contrast to the results presented in this investigation, Portland cement and MTA Angelus were recently shown to inhibit a diversity of aerobic and facultative microorganisms, including *E. faecalis* by agar diffusion method¹⁴. The incubation of the plates into anaerobic atmosphere aimed to reproduce the environment observed within the apical segment of infected root canal system. Differences in sensitivity among studies, in part, may be due to the anaerobic atmosphere during incubation procedure, since both MTA and Portland cement are rich in oxides. Subsequent to reaction with water on oxygen-rich environments, these compounds might generate reactive oxygen species (ROS), such as hydroxyl and hydroperoxyl radicals which exhibit antimicrobial activity³. Growth of anaerobes requires an appropriate environment to reduce the intracellular generation of ROS; favoring the growth of anaerobic microorganisms in anaerobic environments, the

formation of toxic oxygen radicals is likely to be reduced in intracellular location⁵. Moreover, antimicrobial activity of ROS is usually impaired by the presence of antioxidants and other reducing molecules such as quinones¹³. Variation on the concentration of reagents and/or molecules that may act as antioxidants present in culture medium might support the differences in results observed by many authors, who found sensitivity¹⁴ and resistance¹⁶ of *E. faecalis* strains to MTA. In this study *E. faecalis* strain became susceptible to both MTA formulations and Ca(OH)₂ after incubation in aerobic atmosphere, suggesting that oxygen-rich environments favor the antimicrobial activity of MTA. The *E. coli* strain remained resistant to MTA and susceptible to Ca(OH)₂ after incubation in aerobic atmosphere. Studies have demonstrated that *E. coli* wild strains are relatively resistant to ROS generated after incubation with metal ions such as Sn⁺, requiring the utilization of mutants deficient in SOS system repair for the observation of the lethality of chemical agents⁷. Calcium hydroxide usually increases pH and allowed an unfavorable microenvironment to the growth of *P. aeruginosa* and *B. fragilis* strains. However, the participation of ROS generation in antimicrobial activity of Ca(OH)₂ pastes may also be emphasized since *E. coli* and *E. faecalis* presented susceptibility after incubation in aerobic environments.

It is important to mention that the results presented in this study were obtained *in vitro* and performed with planktonic cells grown in culture medium. The materials tested could reveal different activities when used *in vivo* or *in vitro* with microorganisms grown in biofilms. Further studies are necessary to investigate the effect of these materials on bacteria commonly found in infected root canals.

TABLE 1- Antimicrobial activity of MTA-Angelus (MTA-A), MTA- Dentsply (MTA-D), Calcium hydroxide (Ca (OH)₂) and Portland cement

Bacterial strains	Inhibition zones (diameter in mm)			
	MTA-A	MTA-D	Ca(OH) ₂ *	Portland cement
<i>Pseudomonas aeruginosa</i>	21 (± 0.2887)	19 (± 0.5774)	22 (± 0.5774)	17 (± 0.2887)
<i>Escherichia coli</i>	0	0	0	0
<i>Enterococcus faecalis</i>	0	0	0	0
<i>Bacteroides fragilis</i>	0	0	13 (± 0.2887)	0

* Ca(OH)₂ showed larger inhibition zones against *P. aeruginosa* than *B. fragilis* ($p < 0.01$) by Tukey test.

TABLE 2- Effect of aerobic atmosphere on antimicrobial activity of MTA and calcium hydroxide against *E. coli* and *E. faecalis**

	<i>E. coli</i>			<i>E. faecalis</i>		
	MTA Angelus	MTA Pro-root	Ca(OH) ₂	MTA Angelus	MTA Pro-root	Ca(OH) ₂
	0	0	9,17 (±0,44)	5,67 (±0,17)	6,33 (±0,17)	6,67 (±0,67)

* Antimicrobial activity evaluated by agar diffusion method. Values represent the diameter (in mm) of zones of inhibition in mean of three experiments and standard deviation.

CONCLUSION

According to the results obtained it is possible to conclude that:

- MTA Dentsply, MTA Angelus and Portland cement showed similar antimicrobial properties against *P. aeruginosa*.
- Calcium Hydroxide paste showed an antimicrobial activity higher than MTA Dentsply, MTA Angelus and Portland cement.
- Aerobic environment interfered with antimicrobial activity of MTA formulations and Ca(OH)_2 against *E. faecalis* and of Ca(OH)_2 against *E. coli*.

ACKNOWLEDGMENTS

The authors thank Dr Ana Paula Colombo, Instituto de Microbiologia - UFRJ, for her suggestions and laboratory facilities during performance of the experiments and Prof. Licinio Esmeraldo da Silva, Departamento de Estatística, Instituto de Matemática, UFF. This work was supported by PIBIC-UFF-CNPq proc. No. 106560/2003-4.

REFERENCES

- 1- AL-Hezaimi K, Naghshshdandi J, Oglesby S, Simon JJ, Rotstein I. Effect of white-colored Mineral Trioxide Aggregate in different concentrations on *Candida albicans* in vitro. *J Endod.* 2005;31:684-6.
- 2- Andelin WE, Browning DF, Roland DD, Torabinejad M. Microleakage of resected MTA. *J Endod.* 2002;28:573-4.
- 3- Ascenzi JM, Favero MS. Disinfectants and antiseptics, modes of action, mechanisms of resistance, and testing. In: Lorian V, Ed. *Antibiotics in laboratory medicine*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 615-53.
- 4- Barbosa SV, Araki K, Spångberg LSW. Citotoxicity of some modified root canal sealers and their leachable components. *Oral Surg.* 1993;75:357-61.
- 5- Cabisco E, Tamarit J, Ros J. Oxidative stress in bacteria and protein damage by reactive oxygen species. *Int Microbiol.* 2000;3:3-8.
- 6- Chandler NP, Koshy S. The changing role of the apicectomy operation in dentistry. *J R Coll Surg Edinb.* 2002; 47:660-7.
- 7- Dantas FJS, Moraes MO, Carvalho EF, Valsa JO, Bernardo M Filho, Caldeira de Araújo A. Lethality induced by stannous chloride on *Escherichia coli* AB 1157: participation of reactive oxygen species. *Food Chem Toxicol.* 1996;34:959-62.
- 8- Duarte MAH, Weckwerth PH, Kuga MC, Simoes JRB. Evaluation of the contamination of MTA Angelus and Portland cement. *J Bras Clin Odontol Integr.* 2002;6:155-7.
- 9- Estrela C, Bammann LL, Estrela CR, Silva RS, Pécora JD. Antimicrobial and chemical study of MTA, Portland cement, Calcium Hydroxide Paste, Sealapex and Dycal. *Braz Dent J.* 2000;11:3-9.
- 10- Kayaoglu G, Erten H, Alçam T, Orstavik D. Short-term antibacterial activity of root canal sealers towards *Enterococcus faecalis*. *Int Endod J.* 2005;38:483-8.
- 11- Osorio RM, Hefti A, Vertucci FJ, Shawley AL. Citotoxicity of endodontic materials. *J Endod.* 1998;24:91-6.
- 12- Rafter, M. Apexification: a review. *Dent Traumatol.* 2005;21:1-8.
- 13- Rodriguez CE, Fukuto JM, Taguchi K, Froines J, Cho AK. The interactions of 9, 10-phenanthrenequinone with glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a potential site for toxic actions. *Chem Biol Interact.* 2005;155:97-110.
- 14- Sipert CR, Hussine RP, Nishiyama CK, Torres SA. In vitro antimicrobial activity of fill canal, Sealapex, Mineral Trioxide Aggregate, Portland cement and EndoRez. *Int Endod J.* 2005;38:539-43.
- 15- Tang HM, Torabinejad M, Kettering JD. Leakage evaluation of root end filling materials using endotoxin. *J Endod.* 2002;28:5-7.
- 16- Torabinejad M, Hong CU, Ford TRP, Kettering JD. Antibacterial effects of some root end filling materials. *J Endod.* 1995;21:403-6.
- 17- Torabinejad M. Sealing ability of MTA when used as root end filling material. *J Endod.* 1993;19:591-3.