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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

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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. fragillis*. 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

O 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 37ºC 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. fragillis*. 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 retreatment[6].

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 oxide[16]. 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 resorption[17]. 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 bacteria[14]. 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 (CHP P.A. Quimis, Mallinkrodt Inc., St Louis. MO, USA in sterile saline solution), and Portland cement (Votoran, Votorantim, São Paulo, Brazil) were manipulated as described elsewhere[9].

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 37º/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 37ºC/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% N_2, 10% CO_2, and 10%H_2) 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 37ºC/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 testing[9]. 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, respectively[8]. 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 method[14]. 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 activity[3]. 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 location[5]. Moreover, antimicrobial activity of ROS is usually impaired by the presence of antioxidants and other reducing molecules such as quinones[13]. 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 sensitivity[14] and resistance[16] 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 agents[7]. 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. facealis* 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
Pseudomonas aeruginosa	21 (± 0.2887)	19 (± 0.5774)	22 (± 0.5774)	17 (± 0.2887)
Escherichia coli	0	0	0	0
Enterococcus faecalis	0	0	0	0
Bacteroides fragilis	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**

E. coli			***E. faecalis***		
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*.

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