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ORIGINAL RESEARCH

In vitro Evaluation of the Minimum Bactericidal Concentrations of Different Root-End Filling Materials

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ABSTRACT

Aim: The aim of this study was to evaluate the minimum bactericidal concentrations (MBC) of root-end filling materials ProRoot MTA, MTA Angelus and IRM.

Materials and methods: Macrodilution broth method was used. Microorganisms used were: *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212) and *Streptococcus mutans*. Serial two-fold dilutions of root-end filling samples were prepared in macrodilution tubes with concentrations ranging from 1/2 to 1/512. The samples dilutions were incubated for 24 hours. After incubation, 0.1 ml of diluted culture was inoculated onto the surface of supplemented sheep blood agar (Merck, Germany) and all plates were incubated at 37°C in aerobic condition for 24 hours. The MBC was defined as the lowest concentration of root-end filling samples where no growth was recorded.

Results: MBC of both mineral trioxide aggregate (MTA) products against *S. aureus* were recorded as 15.62 mg/ml and for IRM 31.25 mg/ml MBC for both MTA groups against *E. faecalis* were recorded as 31.25 mg/ml and for IRM 62.5 mg/ml. MBC of all root-end filling samples against *S. mutans* were recorded as 62.5 mg/ml.

Conclusion: All tested root-end filling materials showed acceptable MBC against *S. aureus* and *E. faecalis*.

Clinical significance: All tested materials can be used safely for filling of a root-end cavity.

Keywords: Antimicrobial activity, Macrodilution broth method, Mineral trioxide aggregate, Minimum bactericidal concentration.

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INTRODUCTION

In most clinical situations, traditional endodontics is sufficient to obtain success and guarantee periapical tissue repair. However, even after an adequate root canal obturation infection may persist in root canal with high anatomic complexity with a great number of bacteria.[1] It is well known that periapical lesion occurs as a result of the presence of bacteria in periradicular tissues, which are resistant against the organic defence of the host and the chemical solutions used in the root canal treatment and may require further procedures, such as periapical surgery.[2] Surgical treatment usually involves the placement of a root-end filling material designed to seal the root canal contents from the periradicular tissues and repair defects.[3]

Although various materials have been used as root-end filling, recently mineral trioxide aggregate (MTA) gained popularity depending on superior characteristics. Recently, two commercial brands of MTA available in the markets are ProRoot MTA (Dentsply/Tulsa Dental, Tulsa; OK, USA) and MTA-Angelus (Angelus Indústria de Produtos Odontológicos Ltd, Londrina, PR, Brazil). Both products, that have similar chemical compositions, can be used as root-end filling material.

Yasuda et al[4] reported that MTA exhibited no antimicrobial activity against S*taphylococcus aureus*, *Enterococcus faecalis*, *Candida albicans*, *Streptococcus mutans* and *Streptococcus sanguis.* However, Tanomaru-Filho et al[5] investigated the antimicrobial activity of MTA-based cements with agar diffusion method. In contradistinction to Yasuda et al,[4] the results of the study showed that MTA-based cements possessed antimicrobial activity over *S. aureus*, *C. albicans* and *E. faecalis*. Estrela et al[6] showed that the inhibition zones to *S. aureus* and *E. faecalis* were identical for both materials. This indicates that, despite the slight difference in their compositions, these cements have similar antimicrobial properties.

The aim of this study was to evaluate the minimum bactericidal concentrations (MBC) of root-end filling materials–ProRoot MTA, MTA Angelus and IRM–against three different microorganism strains.

MATERIALS AND METHODS

Determination of MBCs of root-end filling materials—ProRoot MTA, MTA Angelus and IRM—and samples for the three reference strains of facultative bacteria was performed by macrodilution broth method as described by the CLSI.[7] Serial 2-fold dilutions of samples were prepared in macrodilution tubes with concentrations ranging from 1/2 to 1/512. A final inoculum of approximately 10^5 CFU in Mueller-Hinton broth (Merck, Germany) was inoculated into tubes of containing samples dilutions and incubated for 24 hours. After incubation, 0.1 ml of diluted culture was inoculated onto the surface of supplemented sheep blood agar (Merck, Germany) and all plates were incubated at 37°C in aerobic condition for 24 hours. MBC was taken as the concentration at which a 99.9% reduction in CFU of the original inoculum occurred and was considered the lowest concentration of samples which prevented growth and reduced the inoculum by a 99.9% within 24 hours. The experiments were performed in triplicate. For each test, as positive control strain *S. aureus* ATCC 25923 and control penicillin was included, and all results with this strain were within the published CLSI quality control ranges.

RESULTS

MBC (mg/ml) results of samples against reference strains were shown in Table 1.

Table 1: MBC (mg/ml) of samples against reference strains

Material	*S. aureus*	*E. faecalis*	*S. mutans*
IRM	31.25 mg/ml	62.5 mg/ml	62.5 mg/ml
ProRoot MTA	15.62 mg/ml	31.25 mg/ml	62.5 mg/ml
MTA-Angelus	15.62 mg/ml	31.25 mg/ml	62.5 mg/ml

Analysis of efficacy of the materials against *S. aureus* showed that MBC for both ProRoot MTA and MTA Angelus were recorded as 15.62 mg/ml and for IRM 31.25 mg/ml. MBC for both MTA groups against *E. faecalis* were recorded as 31.25 mg/ml and for IRM 62.5 mg/ml. MBC of all root-end filling samples against *S. mutans* were recorded as 62.5 mg/ml. The results showed that both ProRoot MTA and MTA angelus had similar values of MBC.

DISCUSSION

Although diffusion methods are simple procedure for the susceptibility testing of bacteria[8] according to Milici et al[9] the results obtained by the diffusion assay correlated well with those obtained by the dilution method. The agar diffusion method, which only indicates the medicament potential to eliminate bacteria within the root canal system, was commonly used to evaluate the antimicrobial activity of root-end filling materials.[5,10] While agar diffusion method does not exactly mirror the antimicrobial effect of the medicaments within dentine tubules, they do allow direct comparisons of the ability of various agents to diffuse through agar and inhibit the test organisms.[11] However, poorly diffusing materials will present very small zones of inhibition, even if they are potent antimicrobial agents.[12] The broth microdilution test was suggested method based on MBC, rather than agar diffusion methods, should be used to determine of resistance to microorganisms. While agar diffusion tests, although aqueous in nature, do not mirror exactly the antimicrobial effect of the medicaments within dentine tubules, they do allow direct comparisons of the ability of various agents to diffuse through agar and inhibit the test organisms.[11] In addition, agar diffusion tests only show inhibition of growth (i.e. bacteristatic), that may not be the same as bacterial death (i.e. bactericidal).[13] MBC is the minimal concentration of a material that kills the inoculum. MBC measurements have several theoretical limitations.[14,15] MBC determinations are normally performed against logarithmic growth phase cultures; in clinical infections organisms may be growing more slowly and in these conditions the bactericidal activity of some agents may be reduced or lost.[16] However, the reduction of bactericidal activity is important to evaluate the long-term antibacterial activity of materials such as MTA and IRM.

All tested bacteria were sensitive to the root-end filling materials used in the present study. Most endodontic infections are mixed and polymicrobial, with predominance of strict anaerobes, some facultative anaerobes and rarely aerobes. Facultative anaerobic microorganisms such as *E. faecalis* and *S. aureus* are considered to have the highest resistance in the oral cavity, with the potential to cause failure of root canal treatment.[17] Therefore this study was performed to compare the antimicrobial activity of root-end filling materials against such endodontic pathogens.

Recently, two compositions of MTA–gray and white–are available. In our study all tested MTA specimens were white color MTA. Al-Hezaimi et al[18] investigated the minimal inhibitory concentrations (MIC) of different compositions of MTA against *E. faecalis* and *S. sanguis*. Under the conditions of the study, gray MTA required lower concentrations than white MTA to exert the same antibacterial activity. To the best of our knowledge, this is the first study evaluating the MBCs of MTA and IRM. Previous studies reported antimicrobial activity of MTA against facultative bacteria.[5,19] Luczaj-Cepowicz et al[20] compared the antimicrobial activity of ProRoot MTA and MTA-Angelus and reported that both products demonstrated acceptable antibacterial action against the standard strains

of *S. mutans*, *S. sanguis* and *S. salivarius*. Antimicrobial activity of ProRoot MTA and MTA-Angelus was evaluated by Duarte et al[21] and antimicrobial activity of materials was associated with the presence of calcium oxide which induces an increase in the pH. In conclusion the results demonstrated that MTA-Angelus, manufactured in Brazil, is the equivalent product to ProRoot MTA. In our study, the MBC of both MTA commercial brands were compared and showed similar results against all tested bacteria. The similar antibacterial activity of both materials may be associated with the presence of calcium oxide which induces an increase in the pH.

In a similar *in vitro* study Eldeniz et al[22] evaluated the antibacterial activity of various root-end filling materials against *E. faecalis* and *S. aureus* with the direct contact test. IRM and ProRoot MTA were generally more potent inhibitors of bacterial growth than the other tested materials. Under the conditions of the present study, the MBC of MTA products were equivalent and were recorded as 15.62 mg/ml and 31.25 mg/ml against *S. aureus* and *E. faecalis*, respectively. Since MBC is not a commonly used method in dentistry, in order to clarify the effectiveness of MTA and IRM, the result may be evaluated mutually with the results of Turner et al.[23] Turner et al[23] investigated the MBC of nisin, which is a bacteriocin and has antimicrobial and bactericidal activity against a broad spectrum of bacteria,[24] and found that the MBC of nisin for *E. faecalis* was 70 mg/ml.

CONCLUSION AND CLINICAL SIGNIFICANCE

On the basis of methodology used, it may be concluded that ProRoot MTA, MTA-Angelus and IRM presented acceptable MBCs against *S. aureus* and *E. faecalis*. All tested materials can be used safely for filling of a root-end cavity.

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