

## Original Article

# The effects of different concentrations of chlorhexidine gluconate on the antimicrobial properties of mineral trioxide aggregate and calcium enrich mixture

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## ABSTRACT

**Background:** The aim of this study was to evaluate the antimicrobial activity of Mineral Trioxide Aggregate (MTA) and Calcium Enrich Mixture (CEM) (mixed with different concentrations of chlorhexidine (CHX).

**Materials and Methods:** Cements used in this *in vitro* study included Gray proRoot MTA and CEM with the microorganisms being *Enterococcus faecalis*, *Streptococcus mutans*, *Candida albicans*, *Actinomyces*, *Escherichia coli*, and a mixture of these microorganisms. CHX was used in the form of liquid at 0.2%, 2%, and 0.12% concentrations. Contact dilution and colony count method was used to evaluate the antibacterial activity of these cements. After 0, 24, 48, 72, and 96-hour intervals, we cultured the samples on blood agar medium. Colonies were counted after incubation at 37°. Data were statistically analyzed by a Kruskal-Wallis test to compare the antimicrobial activity of MTA and CEM.

**Results:** All concentrations of CHX were mixed with MTA and the CEM had antibacterial activities on all microorganisms' strains except for the *Enterococcus faecalis* and the mixture group. MTA had better antibacterial activity than the CEM, but this difference was not significant ( $P = 0.13$ ). The mixing of MTA and the CEM with CHX significantly increased the antibacterial properties of both cements ( $P < 0.03$ ). There was no statistically significant difference between the different concentrations of CHX. The antibacterial activity of the materials increased through time.

**Conclusion:** The mixture of MTA and CEM with different concentration of CHX significantly increased the antibacterial activity.

**Key Words:** Antimicrobial properties, calcium enrich mixture, chlorhexidine gluconate, mineral trioxide aggregate

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## INTRODUCTION

An ideal root-end filling material should produce a complete apical seal and be nontoxic, well tolerated by the periradicular tissues, nonresorbable, dimensionally

stable, easy to manipulate, and radiopaque. In addition, it should be bactericidal or bacteriostatic.<sup>[1]</sup> Mineral trioxide aggregate (MTA) exhibits several properties of an ideal root-end filling material, and has rapidly gained popularity since its introduction in 1993 by Torabinejad.<sup>[2]</sup> ProRoot MTA is marketed as gray-and-white-colored preparations. MTA is a powder that consists of fine hydrophilic particles that, in the presence of water or moisture, forms a colloidal gel that solidifies to form hard cement within approximately 4 hours. The more esthetic white-color preparation lacks tetra calcium aluminoferrite.<sup>[3]</sup>

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Recently, calcium enrich mixture (CEM) has been developed consisting of different calcium compounds (e.g., calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate, calcium hydroxide, and calcium chloride). Its physical properties conform to ISO 6876:2001. The clinical applications of CEM are similar to those of MTA, and both cements have a similar working time, PH, and dimensional stability.<sup>[4]</sup> In two separate studies, Asgary *et al.*<sup>[5]</sup> and Zarabi *et al.*<sup>[6]</sup> found that CEM had significantly more antibacterial properties than MTA.

CHX was initially used as a general disinfectant because of its broad antibacterial action.<sup>[7]</sup> In the early 1960s, CHX was introduced as an endodontic irrigant<sup>[8,9]</sup> and has since been reported as effective *in vitro* against species found in infected root canals such as *E. faecalis*<sup>[10]</sup> and *Actinomyces viscosus*.<sup>[11]</sup>

Enhancing the antimicrobial properties of endodontic materials has been possible by having the improvement of treatment prognosis as its purpose. More uses for CHX in endodontics are being developed with the purpose of improving prognosis by enhancing the antimicrobial properties of endodontic materials. Because CHX has been incorporated into other dental products with some success, the authors wanted to test the hypothesis that the antimicrobial properties of ProRoot MTA would be improved with the addition of CHX. Stowe *et al.*<sup>[12]</sup> added CHX to MTA and found that antimicrobial properties of ProRoot MTA improved as CHX 0.12% was added. The purpose of this *in vitro* study was to evaluate antimicrobial activity of ProRoot MTA and CEM when mixed with sterile water or different concentrations of CHX.

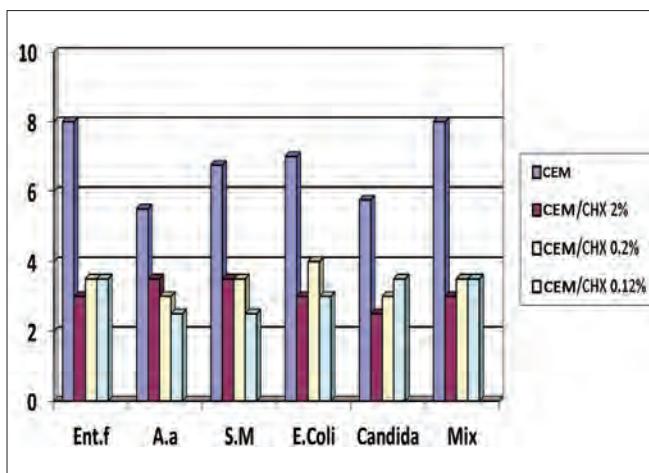
## MATERIALS AND METHODS

The test materials included gray ProRoot MTA (Dentsply, Tulsa dental, USA) and CEM (YektazystDandan, Tehran, Iran). The antimicrobial activity of the endodontic cements was evaluated by the contact dilution and colony count method against five reference strains: *Enterococcus faecalis* (ATCC 29212) *Escherichia coli* (ATCC 33780), *Streptococcus mutans* (ATCC25175), *Candida albicans* (ATCC 10231), and *Actinomyces viscosus* (ATCC 15987). The three concentrations of CHX (Natural Pharma, São Paulo, Brazil) used in this study were 0.12%, 0.2%, and 2%. Microbial strains were confirmed by both Gram staining and colony-forming, and growth

characteristics. Bacteria were diluted to obtain a suspension of  $1.5 \times 10^8$  colony-forming units/ml by standards (0.5 McFarland). Each type of cement was divided into four groups that each group had 30 samples. In the first group, the cement was mixed with sterile water. In groups 2-4, 0.12%, 0.2%, and 2% CHX were used to be mixed with the cement. In each group, 0.36 mg of the cement was mixed with 180 ml of liquid. Then, each group was divided into six subgroups and 180 ml of each five microbial mixture was added to microtubes. In the present study, the antimicrobial activity was also evaluated against the combination of five microorganisms. In addition to the experimental groups, positive and negative control groups were considered. Microbial suspension without cements used as positive control and cement without microbial suspension considered as negative control. Microtubes were incubated at 37°C and were evaluated at 0, 24, 48, 72, and 96 hours. The microbial culture prepared from each microtube on BHI blood agar and the colony count test was done by a microbiologist. Data were statistically analyzed by a Kruskal-Wallis test to compare the antimicrobial activity of MTA and CEM mixed with the different concentrations of CHX. The level of significance was set at 5% ( $P < 0.05$ ).

## RESULTS

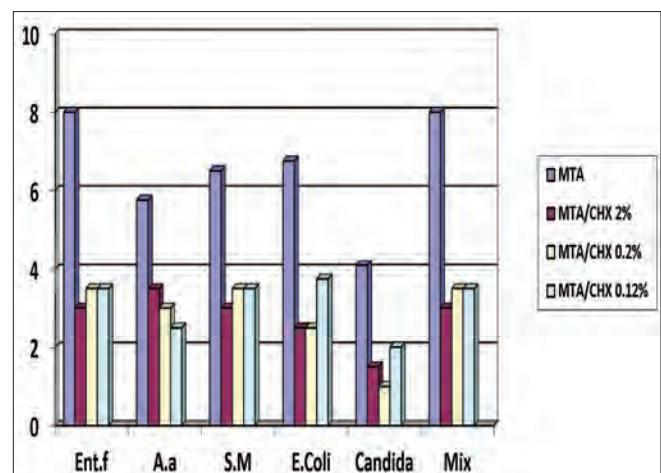
MTA had more antimicrobial activity at 0, 24, and 96 hours but the antimicrobial activity of CEM increased after 48 and 72 hours compared to MTA, although there was no statistically significant difference between them at any time intervals. The antimicrobial activity of both cements increased through time. MTA and CEM did not have any antimicrobial effects against *Enterococcus faecalis* and microbial combination. The most antimicrobial activities of MTA and CEM were against *candida albicans* and *Actinomyces viscosus*, respectively. The only significant difference between antimicrobial activity of MTA and CEM was against *candida albicans*. The greatest reduction of colony number was related to *candida albicans*. The antimicrobial activity of MTA and CEM increased significantly when mixed with CHX. There was no significant difference between antimicrobial properties of three CHX concentrations [Figures 1 and 2]. The mixture of all CHX concentrations with CEM and MTA resulted in antimicrobial effects against *Enterococcus faecalis*.



**Figure 1:** The bacterial growth in proximity of CEM cement in groups mixed with water and CHX

## DISCUSSION

The treatment outcome depends on successful elimination of the associated microorganisms and infected tissues as well as effective sealing of the root-end or perforation site to prevent future recontamination.<sup>[2]</sup> Several independent studies have shown that certain microorganisms are recovered from previously root-filled teeth that have become infected. These are chiefly *Enterococcus*, *Actinomyces*, *Propionibacterium*, yeasts, and *Streptococcus*, with occasional reports of other types.<sup>[13]</sup> *E. faecalis* and *Actinomyces* are robust microorganisms that may infect root canals<sup>[14,15]</sup> and are more likely to be found in cases of failed endodontic therapy than in cases of primary infection.<sup>[16]</sup> *E. coli* is sometimes recovered from root canals and represents a standard organism used in antimicrobial testing.<sup>[17,18]</sup> *C. albicans* has the ability to form biofilms on different surfaces, and may be involved in cases of persistent and secondary infection.<sup>[19]</sup> *S. mutans* may have a major influence on both the initial pulpal lesion and subsequent pulpal pathology.<sup>[20]</sup> In this study we investigated the antimicrobial activity of MTA and CEM and their mixture with different concentrations of CHX. For assessing antibacterial activity of root-end filling materials it may be important to use a test adapted to the activity of materials during setting or which are in a paste form, such as the direct contact test (DCT).<sup>[21]</sup> The DCT test is a quantitative and reproducible assay that allows testing of water-insoluble materials. It can also be used to test materials in various stages of setting.<sup>[22]</sup> The DCT also helps us to determine whether the data gathered from a specific material



**Figure 2:** The bacterial growth in proximity of MTA cement in groups mixed with water and CHX

reflect bactericidal or just bacteriostatic effects, regardless of the diffusion rates of the active agents.<sup>[21]</sup>

This was the first study that used contact dilution and colony count method for evaluation of antimicrobial activity of endodontic cements. In this method, cements were placed in close contact with microbial suspension which is similar to the clinical environment and then the antimicrobial results were reported qualitatively and quantitatively. Our results showed that there was no significant difference between the antimicrobial activity of MTA and CEM, and the antimicrobial activity of both cements were increased this way through time. Zarrabi *et al.*<sup>[6]</sup> found that the antimicrobial effect of MTA and CEM increased through time, and there was no significant difference between antimicrobial properties of these two cements. Asgary *et al.*<sup>[5]</sup> used the agar diffusion technique and found that CEM had a significantly more pronounced antibacterial effect than MTA which was in contrast with the results of this study. The difference can be due to the techniques used for evaluation.

In this study, MTA and CEM were ineffective against *E. faecalis*. The resilient characteristics of *E. faecalis* against endodontic cements were shown in different studies. Estrela *et al.*<sup>[23]</sup> demonstrated that MTA had no bactericidal effect against *E. faecalis*. Eldeniz *et al.*<sup>[24]</sup> showed ineffectiveness of MTA against *E. faecalis*. Zarrabi *et al.*<sup>[6]</sup> found that MTA and CEM had no antibacterial effect against this microorganism. In contrast, Hezaimi *et al.*<sup>[25]</sup> stated that gray MTA were effective against *E. faecalis*. Asgary *et al.*<sup>[5]</sup> demonstrated that both MTA and CEM

had antimicrobial activity against *E. faecalis*. The main reason of ineffectiveness of MTA and CEM on *E. faecalis* in most of studies is due to the capacity of this microorganism to survive under various stressful environmental conditions such as high PH of antimicrobial agents.<sup>[26]</sup>

The antifungal properties of MTA have been shown in several studies.<sup>[25,27-29]</sup> Only in one study (Zarrabi et al.'s),<sup>[6]</sup> the antifungal effect of CEM was investigated against *candida albicans*. In the present study, MTA and CEM showed antifungal activity against *candida albicans* and the most antimicrobial activity of MTA was found to be against *candida albicans*. Similar to our findings, Stow et al.<sup>[12]</sup> reported that the most antimicrobial activity of MTA was against *candida albicans*. Our results agree with those of Zarrabi et al.,<sup>[6]</sup> who reported that the most antimicrobial activity of CEM was against *Actinomyces* and in comparison with MTA, it showed greater antimicrobial properties. Optimal antimicrobial properties of a root-end filling material should be against combination of microorganisms.<sup>[30]</sup> Although the results are controversial in different studies about antimicrobial properties of MTA and CEM, they generally showed that these two cements do not have complete antimicrobial effect against different microorganisms or mixture of them. Several studies investigated different properties of mixture of MTA with CHX<sup>[31-38]</sup> but the mixture of CEM with this antimicrobial agent has not been studied. In 2004, Stow et al.<sup>[12]</sup> mixed MTA with 0.12% CHX for the first time and evaluated antimicrobial activity of MTA with agar diffusion test. They showed that MTA/CHX mixtures produced greater zones of inhibition than the MTA/water mixtures. Holt et al.<sup>[31]</sup> mixed MTA with 2% CHX, and demonstrated that the antimicrobial effect of MTA increased against *E. faecalis*. They also reported that MTA mixed with 2% CHX had lower compressive strength and could be used only in areas exposed to minimal compressive forces.

The antimicrobial effect of CHX on all the microorganisms used in this study has been shown previously.<sup>[39]</sup> In the present study, different concentrations of CHX (0.12%, 0.2%, and 2%) were used. There was no significant difference in antimicrobial effects of three concentrations of CHX. The antimicrobial activities of MTA and CEM were increased by adding CHX. Both MTA/CHX and CEM/CHX indicated antimicrobial effects against *E. faecalis*. Our results were in agreement with the results of Holt<sup>[31]</sup> and Stow's findings.<sup>[12]</sup>

One of the important properties of cements is their biocompatibility. In this study, adding CHX to MTA and CEM significantly increased their antimicrobial efficacy. However, if the addition of CHX compromises MTA and CEM biocompatibility, increased antimicrobial properties would not be beneficial. Sumer et al.<sup>[33]</sup> implanted a mixture of MTA and 0.12% CHX subcutaneously in rats and showed that it was biocompatible. Faria et al.<sup>[40]</sup> proved that 0.25% CHX could cause small foci of tissue necrosis while 0.125% CHX resulted in no necrosis at all; although moderate inflammatory infiltrate was seen in both concentrations. Lower concentrations of CHX induced apoptosis, while tissue necrosis was found at higher concentrations. They suggested that CHX may have an unfavorable effect on the resolution of apical periodontitis. Lessa et al.<sup>[41]</sup> reported that the higher the CHX concentration and the longer the contact time with the cells, the stronger its cytotoxic effects would be. Silva et al.<sup>[42]</sup> showed that the association between calcium hydroxide paste and 0.4% CHX had no effect on the development of the osteogenic phenotype. No significant variations were observed among control and calcium hydroxide CHX groups in terms of cell shape, cell viability, alkaline phosphatase activity, and the total amount of bone-like nodule formation. In contrast, Hernandez et al.<sup>[34]</sup> found in an *in vitro* study that MTA mixed with 0.12 CHX resulted in apoptosis of fibroblasts and macrophages. *In vitro* cytotoxic effects of CHX on various types of human cells were due to the direct contact of CHX with them. But the *in vivo* serum presence during the initial healing period seems to have provided significant protection against these cytotoxic effects.<sup>[43]</sup>

Long-term cytotoxic effects of CHX should also be investigated. Further studies are needed to be done to determine the other effects of CHX on MTA and CEM properties.

## CONCLUSIONS

It must be kept in mind that the most desirable endodontic antimicrobial medicament would be one that combines maximal antimicrobial effect with minimal toxicity.<sup>[44]</sup> In summary, the addition of 0.12, 0.2, or 2% CHX to MTA and CEM significantly enhanced the antimicrobial activity of these two cements. As the antimicrobial effect of 0.12% CHX did not differ from that of 2% CHX, with a lower toxicity, adding CHX at concentration of 0.12% to MTA and CEM is suggested.

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