Dental Research Journal

Original Article

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

Maryam Bidar[1], Mahboube Naderinasab[2], Ali Talati[3], Kiarash Ghazvini[2], Saeed Asgari[4], Behzad Hadizadeh[5], Maryam Gharechahi[5], Niloufar Attaran Mashadi[6]

[1]Dental Research Center, Dental School, [2]Department of Microbiology, Faculty of Medicine, [3]Oral and Maxillofacial Diseases Research Center, Dental School, [5]Department of Endodontics, Dental School, Mashhad University of Medical Sciences, Mashhad, [4]Dental Research Center, Dental School, Shahid Beheshti university of Medical Sciences, Tehran, [6]Dental School, Gilan University of Medical sciences (International branch), Rasht, Iran

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 *entrococcus faecalis, streptococcus muntas, 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

Received: July 2011
Accepted: May 2012

Address for correspondence:
Dr. Maryam Gharechahi,
Department of Endodontics,
Faculty of Dentistry,
Vakilabad Blvd, Mashhad,
P.O. Box: 91735-984, Iran.
E-mail: gharechahim@mums.ac.ir

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.[1] 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.[2] 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.[3]

Access this article online

Website: www.drj.ir

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.[4] In two separate studies, Asgary *et al*.[5] and Zarrabi *et al*.[6] found that CEM had significantly more antibacterial properties than MTA.

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

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*.[12] 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 1.5×10^8 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*.

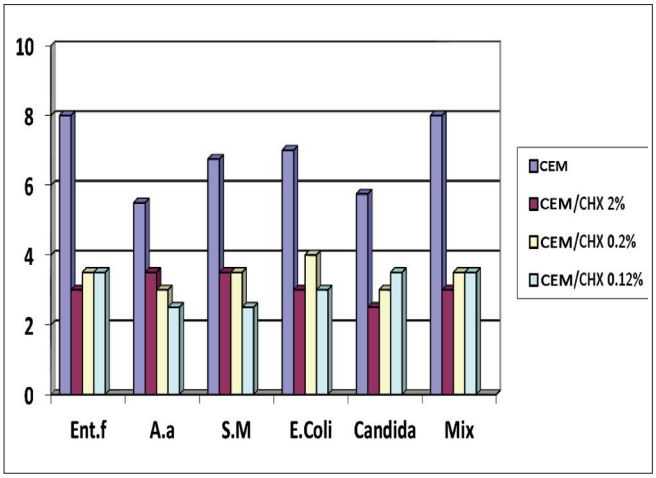

Figure 1: The bacterial growth in proximity of CEM cement in groups mixed with water and CHX

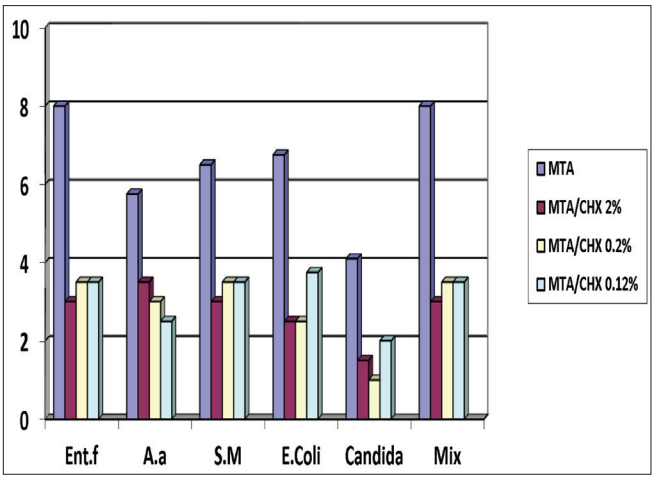

Figure 2: The bacterial growth in proximity of MTA cement in groups mixed with water and CHX

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.[2] 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.[13] *E. faecalis* and *Actinomyces* are robust microorganisms that may infect root canals[14,15]) and are more likely to be found in cases of failed endodontic therapy than in cases of primary infection.[16] *E. coli* is sometimes recovered from root canals and represents a standard organism used in antimicrobial testing.[17,18] *C. albicans* has the ability to form biofilms on different surfaces, and may be involved in cases of persistent and secondary infection.[19] *S. mutans* may have a major influence on both the initial pulpal lesion and subsequent pulpal pathology.[20] 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).[21] 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.[22] The DCT also helps us to determine whether the data gathered from a specific material reflect bactericidal or just bacteriostatic effects, regardless of the diffusion rates of the active agents.[21]

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.*[6] 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.*[5] 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.*[23] demonstrated that MTA had no bactericidal effect against *E. faecalis.* Eldeniz *et al.*[24] showed ineffectiveness of MTA against *E. faecalis.* Zarrabi *et al.*[6] found that MTA and CEM had no antibacterial effect against this microorganism. In contrast, Hezaimi *et al.*[25] stated that gray MTA were effective against *E. faecalis.* Asgary *et al.*[5] 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.[26]

The antifungal properties of MTA have been shown in several studies.[25,27-29] Only in one study (Zarrabi *et al*.'s),[6] 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*.[12] reported that the most antimicrobial activity of MTA was against *candida albicans*. Our results agree with those of Zarrabi *et al*.,[6] 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.[30] 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[31-38] but the mixture of CEM with this antimicrobial agent has not been studied. In 2004, Stow *et al*.[12] 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*.[31] 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.[39] 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[31] and Stow's findings.[12]

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*.[33] implanted a mixture of MTA and 0.12% CHX subcutaneously in rats and showed that it was biocompatible. Faria *et al*.[40] 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*.[41] 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*.[42] 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*.[34] 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.[43]

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.[44] 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.

ACKNOWLEDGMENTS

We would like to thank Dr. Habibollah Esmaili for his advice as a statistician in this study.

REFERENCES

1. Torabinejad M, Hong C, Pitt Ford T, Kettering D. Antibacterial effect of some root end filling materials. J Endod 1995;21:403-6.
2. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endod 1993;19:591-5.
3. Ferris DM, Baumgartner JC. Perforation repair comparing two type of mineral trioxide aggregate. J Endod 2004;30:422-4.
4. Asgari S, Shahabi S, Jafarzadeh T, Amini S. The properties of a new endodontic material. J Endod 2008;34:990-3.
5. Asgari S, Kamrani F, Taheri S. Evaluation of antimicrobial effect of MTA, calcium hydroxide, and CEM cement. Iran Endod J 2007;2:105-9.
6. Zarrabi MH, Javidi M, Naderinasab M, Gharechahi M. Comparative evaluation of antimicrobial activity of three cements: New endodontic cement(NEC), mineral trioxide aggregate(MTA) and Portland. J Oral Sci 2009;51:437-42.
7. Hirst RC. Chlorhexidine: A review of the literature. Periodontal Abstr 1972;20:52-8.
8. Birch RH, Melville TH. Preliminary sterilization of the endodontic field. Comparison of antiseptics. Br Dent J 1961;111:362-3.
9. Atkinson AM, Hampson EL. Sterilization of root canals. Br Dent J 1964;116:526-32.
10. Ayhan H, Sultan N, Cirak M, Ruhi MZ, Bodur H. Antimicrobial effects of various endodontic irrigants on selected microorganisms. Int Endod J 1999;32:99-102.
11. Gultz J, Do L, Boylan R, Kaim J, Scherer W. Antimicrobial activity of cavity disinfectants. Gen Dent 1999;47:187-90.
12. Stowe TJ, Sedgley CM, Stowe B, Fenno JC. The effects of Chlorhexidine Gluconate (0.12%) on the antimicrobial properties of tooth-colored ProRoot Mineral Trioxide Aggregate. J Endod 2004;30:429-31.
13. Baumgartner C, Siqueira J, Sedgley CM, Kishen A. Microbiology of Endodontic Disease. In: Ingle J, Bakland LK, Baumgartner C, editors. Endodontics. 6th ed. Hamilton: BC Decker Inc; 2008. p. 258.
14. Molander A, Reit C, Dahien G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31:1- 7.
15. Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative retreatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:86-93.
16. Rocas IN, Siqueira JR, Santos KR. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004;30:315-20.
17. Basrani B, Tjaderhane L, Santos JM. Efficacy of chlorhexidine- and calcium hydroxide-containing medicaments against Enterococcus faecalis *in vitro*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:618-24.
18. Heling I, Chandler NP. Antimicrobial effect of irrigant combinations within dentinal tubules. Int Endod J 1998;31:8-14.
19. Siqueira JF, Sen BH. Fungi in endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:632-41.
20. Hahn CL, Best AM, Tew JG. Cytokine induction by Streptococcus mutans and pulpal pathogenesis. Infect Immun 2000;68:6785-9.
21. Weiss EI, Shalhav M, Fuss Z. Assessment of antibacterial activity of endodontic sealers by a direct contact test. Endod Dent Traumatol 1996;12:179-84.
22. Fuss Z, Weiss EI, Shalhav M. Antibacterial activity of calcium hydroxide-containing endodontic sealers *in vitro*. Int Endod J 1997;30:397-402.
23. Estrela C, Bammann LL, Estrela CR, Silva RS, Pécora JD. Antimicrobial and chemical study of MTA, Portland cement, Calcium Hidroxide Paste, Sealapex and Dycal. Braz Dent J 2000;11:3-9.
24. Eldeniz A, Hadimli HH, Ataoglu H, Orstavik D. Antibacterial effect of selected root-end filling materials. J Endod 2006;32:345- 9.
25. Al-Hezaimi K, Al-Hamdan K, Naghshbandi J, Oglesby S, Simon JH, Rotstein I. Effect of white-colored mineral trioxide aggregate in different concentrations on *Candida albicans in vitro*. J Endod 2005;31:684-6.
26. Portenier L, Waltimo TM, Haapasalo M. Enterococcus faecalis the root canal Survivor and star in post treatment disease. Endod Topics 2003;6:135-59.
27. Al-Nazhan S, Al-Judai A. Evaluation of antifungal activity of MTA. J Endod 2003;29:826-7.
28. Mohammadi Z. The effect of bovine serum Albumin on the antifungal activity of MTA cement. J Dent Sci 2008;6:25-9.
29. Tanomaru F, Barros D, Watanabe E, Ito I. *In vitro* antimicrobial activity of endodontic sealers, MTA-based cements and Portland cement. J Oral Sci 2007;49:41-5.
30. Grossman L. Endodontic practice. 7th ed. Philadelphia: Lea and Febiger; 1970. p. 654-8.
31. Holt DM, Watts JD, Beeson TJ, Kirkpatrick TC, Rutledge RE. The anti-microbial effect against enterococcus faecalis and the compressive strength of two types of mineral trioxide aggregate mixed with sterile water or 2% chlorhexidine liquid. J Endod 2007;33:844-7.
32. Kogan P, He J, Glickman GN, Watanabe I. The effects of various additives on setting properties of MTA. J Endod 2006;32:569-72.
33. Sumer M, Muglali M, Bodrumlu E, Guvenc T. Reactions of connective tissue to amalgam, intermediate restorative material, mineral trioxide aggregate and mineral trioxide aggregate mixed with chlorhexidine. J Endod 2006;32:1094-6.
34. Hernandez EP, Botero TM, Mantellini MG, McDonald NJ, Nör JE. Effect of ProRoot MTA mixed with chlorhexidine on apoptosis and cell cycle of fibroblasts and macrophages *in vitro*. Int Endod J 2005;38:137-43.
35. Jeffcoat MK, Bray KS, Ciancio SG, Dentino AR, Fine DH, Gordon JM, *et al*. Adjunctive use of a subgingival controlled-release chlorhexidine chip reduces probing depth and improves attachment level compared with scaling and root planing alone. J Periodontol 1998;69:989-97.
36. Soskolne WA, Heasman PA, Stabholz A, Smart GJ, Palmer M, Flashner M, *et al*. Sustained local delivery of chlorhexidine in

the treatment of periodontitis: A multi-center study. J Periodontol 1997;68:32-8.
37. Yan P, Peng B, Fan B, Fan M, Bian Z. The effects of sodium hypochlorite (5.25%), Chlorhexidine (2%), and Glyde File Prep on the bond strength of MTA-dentin. J Endod 2006;32:58-60.
38. Bernardi F, Pincelli MR, Carloni S, Gatto MR, Montebugnoli L. Chlorhexidine with an Anti Discoloration System. A comparative study. Int J Dent Hyg 2004;2:122-6.
39. D'Arcangelo C, Varvara G, De Fazio P. An evaluation of the action of different root canal irrigants on facultative aerobic-anaerobic, obligate anaerobic, and microaerophilic bacteria. J Endod 1999;25:351-3.
40. Faria G, Celes MR, Rossi AD, Silva JS, Silva LA, Rossi MA. Evaluation of Chlorhexidine Toxicity Injected in the Paw of Mice and Added to Cultured L929 Fibroblasts. J Endod 2007;33:715-22.
41. Lessa FC, Aranha AM, Nogueira I, Giro EM, Hebling J, Costa CA. Toxicity of chlorhexidine on odontoblast-like cells. J Appl Oral Sci 2010;18:50-8.
42. Silva RA, Leonardo MR, Silva LA, Castro LM, Rosa AL, Oliveira PT. Effects of the association between a calcium hydroxide paste and 0.4% chlorhexidine on the development of the osteogenic phenotype *in vitro*. J Endod 2008;34:1485-9.
43. Gabler WL, Roberts D, Harold W. The effect of chlorhexidine on blood cells. J Periodontal Res 1987;22:150-5.
44. Spångberg L, Engstrom B, Langeland K. Biologic effects of dental materials. 3. Toxicity and antimicrobial effect of endodontic antiseptics *in vitro*. Oral Surg Oral Med Oral Pathol 1973;36:856-71.

How to cite this article: Bidar M, Naderinasab M, Talati A, Ghazvini K, Asgari S, Hadizadeh B, *et al*. The effects of different concentrations of chlorhexidine gluconate on the antimicrobial properties of mineral trioxide aggregate and calcium enrich mixture. Dent Res J 2012;9:466-71. **Source of Support:** This study was supported by the Vice Chanceller of Research and the Dental Research Center of Mashhad. **Conflict of Interest:** None declared. University of Medical Sciences