

## Full Length Research Paper

## Effect of adding chlorhexidine to calcium enriched mixture (CEM) on its antimicrobial activity

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In recent years, a new endodontic cement, calcium enriched mixture (CEM) has been introduced; with clinical applications similar to those of mineral trioxide aggregate (MTA). It has been shown that CEM has higher antibacterial activity than MTA. On the other hand, use of chlorhexidine (CHX) to promote the antibacterial activity of different dental materials is increasing. The aim of the present study was to evaluate the effect of adding CHX to CEM on its antibacterial activity. The antibacterial activities of the materials under study [(CEM cement + CEM solution + 2%CHX) and (CEM cement + CEM solution)] against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Escherichia coli* were evaluated using agar diffusion technique, followed by determination of the diameter of microbial zone of inhibition around the materials by three independent observers after 72 h. Data were analyzed by Mann-Whitney U test. Statistical significance was defined at  $P < 0.05$ . The mean diameters of zones of inhibition in the CEM + CEM liquid and CEM + CEM liquid + CHX groups against *P. aeruginosa*, *E. faecalis*, *S. aureus* and *E. coli* were (13.2 and 9), (21.10 and 6), (20.2 and 9) and (17 and 9.75) ml, respectively, with larger diameters in the CEM + CEM solution + CHX group as compared to CEM + CEM solution group with all the microorganisms ( $P < 0.05$ ). Incorporation of CHX with CEM resulted in an increase in antimicrobial activity of CEM.

**Key words:** CEM cement, chlorhexidine, antibacterial.

### INTRODUCTION

Mechanical pulp exposure and exposures due to caries in teeth with immature apices, without the symptoms and signs of irreversible pulpitis, should be sealed in order to preserve pulp vitality and prevent pathologic changes in

periradicular tissues. In addition, communication pathways between the root canal and the periodontium, including perforations, should be sealed with restorative materials to prevent bacterial leakage. Since these

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materials are at close contact with vital tissues they should be biocompatible and induce regeneration of the affected tissues and restore the conditions before their involvement (Torabinejad and Walton, 2009). Mineral trioxide aggregate (MTA) is the most commonly used material to this end in the field of endodontics. The main ingredients of MTA are calcium oxide, sulfur tricalcium, phosphate oxide, silicon oxide, aluminum oxide, sodium oxide, manganese oxide and chlorine, which are mixed with a water-based liquid to yield bioactive calcium and phosphate (Asgary et al., 2008). Calcium enriched mixture (CEM) cement is a new dental material with applications similar to those of MTA. The results of a recent study showed that CEM cement releases calcium and phosphate ions (Asgary et al., 2008). CEM cement has a pH value similar to that of MTA; however, it has higher fluidity as compared to MTA with shorter working time and less film thickness (Asgary et al., 2008). A study by Asgary et al. (2008) showed that CEM has antimicrobial activity against pathogens, similar to that of calcium hydroxide and better than that of MTA (Asgary and Kamrani, 2008; Zarrabi et al., 2009). Antifungal effects of MTA and CEM against *Candida albicans* have been compared and it has been shown that both materials completely destroy the fungus in 24 h.

Chlorhexidine has been initially introduced as an irrigation solution due to its broad-spectrum antibacterial activity (Barrios et al., 2013). Studies have shown that CHX is effective against bacterial species that are isolated from the infected root canals; these microorganisms include *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus salivarius*, *Escherichia coli* and *Candida albicans* (Ayhan et al., 1991; Nowicki and Sem, 2011).

Use of CHX to promote the antimicrobial properties of dental materials with the aim of improving prognosis is increasing. Studies have shown that adding 0.12% CHX to MTA increases its antibacterial activity (Stowe et al., 2004; Holt et al., 2007).

The aim of the present study was to evaluate the antimicrobial activity of CEM on *P. aeruginosa*, *E. faecalis*, *S. aureus* and *E. coli* using agar diffusion technique.

## MATERIALS AND METHODS

The antimicrobial activities of the materials under study were evaluated against *P. aeruginosa*, *E. faecalis*, *S. aureus* and *E. coli* using the agar diffusion technique. Standard microbial strains were provided by the Department of Microbiology, Faculty of Medicine, Tabriz University of Medical Sciences. All the bacterial strains were grown in Mueller-Hinton Broth (MHB) for 24 h at 37°C. Then a suspension was prepared from each bacterial strain at a concentration of  $1.5 \times 10^8$  CFU/ml (turbidity equal to McFarland's 0.5 standard solutions). Each suspension was used to culture bacterial species on MHA using a sterile swab.

The materials under study were placed on the basal layer in each plate in a well. The plates were incubated at 37°C for 24 h. A total of eight plates were used for each bacterial strain, that is, on the whole

34 plates were used, which were randomly divided into four groups and two plates were used as positive and negative controls, containing solutions with and without microorganisms, respectively. Evaluations were carried out in sterile MHA culture media measuring 4 mm in depth in plates measuring 2 × 10 cm. A sterile punch was used to produce two identical holes measuring 4 mm in diameter at least 3 mm apart from each other in the basal layer of each plate. Each hole was filled separately with the materials under study, which consisted of the following: a mixture of 1 g of CEM cement powder + 0.36 mL of CEM cement solution, and a mixture of 1 g of CEM cement powder + 0.18 mL of CEM cement solution + 0.18 mL of 2% CHX (Consepsis, Ultradent Products, South Jordan, Utah, USA). Finally, the diameter of zone of inhibition around each test material was measured using a ruler accurate to 0.5 mm, after 72 h.

Data were analyzed using descriptive statistics (mean ± standard deviation) and Mann-Whitney U test was used to compare means with SPSS 17. Statistical significance was set at  $P < 0.05$ . Kolmogorov-Smirnov test was used to evaluate normal distribution of data.

## RESULTS

With all the microorganisms under study, the mean diameters of zones of inhibition in the CEM + CHX group were significantly greater than those in the CEM group ( $P < 0.05$ ). Table 1 shows the mean diameters of zones of inhibition in the study groups.

## DISCUSSION

In the present study, the antibacterial activity of CEM mixed with chlorhexidine was evaluated against *P. aeruginosa*, *E. faecalis*, *S. aureus* and *E. coli*. The results showed the positive effect of adding CHX to CEM on its antimicrobial activity.

Treatment of teeth with immature apices and repair of perforations are two important procedures in the field of endodontics and MTA is the most commonly used material to this end. CEM was introduced by Asgari et al. (2008) in recent years. The main constituents of CEM are calcium oxide, sulfur tricalcium, calcium phosphate, calcium carbonate, calcium silicate, calcium hydroxide and calcium chloride. CEM has dental applications similar to those of MTA (Asgary et al., 2008).

Studies comparing these two materials have shown that they have comparable sealing ability; however, the antibacterial activity of CEM is higher than that of MTA (Asgary et al., 2008).

Since microorganisms are the main factors involved in the failure of endodontic treatment, the antimicrobial activity of materials used in endodontic treatment has always been of great significance. The bacterial species included in the present study are real endodontic pathogens, which are related to cases resistant to treatment (Sundqvist, 1992). Although aerobic bacteria or the related microorganisms do not have a great role in initiating primary infections, they are found with a high frequency in root canal treatment failure cases (Siren et

**Table 1.** The mean diameters of zones of inhibition (mm) in the study groups.

Bacterial species	Mean diameter of zones of inhibition	
	CEM + CHX	CEM
<i>E. Faecalis</i>	21.10±0.86	6±0.43
<i>P. aeruginosa</i>	13.20±0.47	9±0.76
<i>S. aureus</i>	20.20±0.09	9±0.8
<i>E. coli</i>	17±1.61	9.75±0.6

al., 1997). These bacteria can enter the root canal system before treatment, during treatment and after treatment to cause secondary infection (Saunders and Saunders, 1994).

In the present study, agar diffusion technique was used, which is the most commonly used method to evaluate antibacterial activity (Torabinejad et al., 1995) and has been used by many researchers in a large number of studies (Kayaoglu et al., 2005).

Use of CHX is on the rise to increase the antibacterial activity of dental materials to improve prognosis. CHX is a synthetic cationic bis-guanide, which consists of 2 similar circles of 4-chlorophenyl and two bi-guanide groups, which are connected to each other with a central chain of hexa-methylene (Greenstein et al., 1986; Barrios et al., 2013). CHX is a lipophilic and hydrophobic positively-charged molecule, which reacts with bacterial cell membrane phospholipids and lipopolysaccharides and enters the cell through a number of active and passive transport mechanisms (Athanasiadis et al., 2007). Its action is attributed to the reaction of its positive charge with the negative charge of phosphate groups on the cell membrane (Gomes et al., 2003). Therefore, the osmotic balance of the cell is disrupted, increasing cellular permeability and allowing CHX to enter the bacterial cell. CHX is a base and is stable like a salt. The most commonly used oral form of CHX is its gluconate form, which is soluble in water and at physiologic pH releases positively charged CHX (Greenstein et al., 1986; Balla et al., 2013). At low concentration of 0.2%, low-molecular-weight components such as potassium and phosphorus exit the cell. On the other hand, at concentrations higher than 2%, CHX results in cell death (Gomes et al., 2003).

The results of the present study showed that adding 2% CHX to CEM solution results in a significant increase in its antibacterial activity, consistent with the results of a study carried out by Bidar et al. (2012) in which direct contact method and bacterial species other than those used in the present study were used. The antibacterial effect of CHX against all the microorganisms in the present study has already been shown (D'Arcangelo et al., 1991). Studies have shown that CHX is effective against bacterial species found in infected root canals, including *S. aureus*, *E. faecalis*, *S. salivarius*, *E. coli* and *C. albicans* (Ayhan et al., 1999).

Of course it should be kept in mind that adding CHX to WMTA results in cell death (Hernandez et al., 2005) and decreases its compressive strength (Holt et al., 2008). In addition, a mixture of MTA and CHX gel did not set for the last seven days (Kogan et al., 2006). On the other hand, the solution and gel forms of CHX exert different effects on the setting time of MTA. Kogan et al. (2006) mixed MTA powder with CHX gel in order to evaluate the compressive strength of this mixture; however, since the mixture did not set up to seven days after mixing, it was not possible to measure its compressive strength. Therefore, it is suggested that further studies should be carried out on CEM to evaluate the effect of adding CHX on its physical properties, such as compressive strength, setting time and sealing ability.

### Conflict of Interests

The author(s) have not declared any conflict of interests.

### REFERENCES

- Asgary S, Eghbal MJ, Parirokh M (2008). Sealing ability of a novel endodontic cement as a root-end filling material. *J. Biomed. Mater. Res.* 87:706-709.
- Asgary S, Shahabi S, Jafarzadeh T, Amini S, Kheirieh S (2008). Properties of a new endodontic material. *J. Endod.* 34:990-993.
- Asgary S, Kamrani F (2008). Antibacterial effects of five different root canal sealing materials. *J. Endod.* 50:469-474.
- Athanassiadis B, Abbott PV, Walsh LJ (2007). The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. *Australiam. Dental. J.* 52:64-82.
- Ayhan H, Sultan N, Cirak M, Ruhi MZ, Bodur H (1999). Antimicrobial effects of various endodontic irrigants on selected microorganisms. *Int. Endod. J.* 32:99-102.
- Ballal NV, Moorkoth S, Mala K, Bhat KS, Hussein SS, Pathak S (2011). Evaluation of chemical interactions of maleic acid with sodium hypochlorite and chlorhexidine gluconate. *J. Endod.* 10:1402-1405.
- Barrios R, MFLLuque, Arias-Moliz MT, Ruiz-Linares M, Bravo M, Baca P (2013). Antimicrobial substantivity of alexidine and chlorhexidine in dentin. *J. Endod.* 11:1413-1415.
- Bidar M, Naderinasab M, Talati A, Ghazvini K, Asgari S, Hadizadeh B, Gharechahi M, Mashadi NA (2012). The effects of different concentrations of chlorhexidine gluconate on the antimicrobial properties of mineral trioxide aggregate and calcium enrich mixture. *Dent. Res. J. (Isfahan).* 9:446-471.
- D'Arcangelo C, Varvara G, De Fazio P (1999). An evaluation of the action of different root canal irrigants on facultative aerobic-anaerobic, obligate anaerobic, and microaerophilic bacteria. *J. Endod.* 25:351-353.
- Gomes BPFA, Souza SFC, Ferraz CCR (2003). Effectiveness of 2% chlorhexidine gel and calcium hydroxide against *Enterococcus faecalis* in bovine root dentine in vitro. *Int. Endod. J.* 36:267-75.
- Greenstein G, Berman C, Jaffin R (1986). Chlorhexidine: an adjunct to periodontal therapy. *J. Periodontol.* 57:370-376.
- Hernandez EP, Botero TM, Mantellini MG, McDonald NJ, Nör JE (2005). Effect of ProRoot MTA mixed with chlorhexidine on apoptosis and cell cycle of fibroblasts and macrophages in vitro. *Int. Endod. J.* 38:137-143.
- Holt DM, Watts JD, Beeson TJ, Kirkpatrick TC, Rutledge RE (2007). 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.* 33:844-7.
- Kayaoglu G, Erten H, Alacam T, Orstavik D (2005). Short-term antibacterial activity of root canal sealers towards *Enterococcus faecalis*. *Int. Endod. J.* 38:483-488.

- Kogan P, He J, Glickman GN, Watanabe I (2006). The effects of various additives on setting properties of MTA. J.Endod. 32:569-572.
- Nowicki JB, Sem DS (2011). An *in vitro* spectroscopic analysis to determine the chemical composition of the precipitate formed by mixing sodium hypochlorite and chlorhexidine. J. Endod. 7:983-988.
- Saunders WP, Saunders EM (1994). Cronal leakage as a cause of failure in root-canal therapy: A review. Endod. Dent. Traumatol. 10:105-8.
- Siren EK, Haapasalo MP, Ranta K, Salmi P, Kerosuo EN (1997). Microbiological finding and clinical treatment procedures in endodontic cases selected for microbiological investigation. Int. Endod. J. 30:91-95.
- Stowe TJ, Sedgley CM, Stowe B, Fenno JC (2004). The effects of ChlorhexidineGluconate (0.12%) on the antimicrobial properties of tooth colored ProRoot mineral trioxide aggregate. J. Endod. 26:429-431
- Sundqvist G (1992). Ecology of the root canal flora. J. Endod. 18:427-430.
- Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD (1995). Antibacterial effects of some root end filling materials. J.Endod. 21:403-406.
- Torabinejad M, Walton RE (2009). Endodontics principle and practice 4<sup>th</sup> ed, st. Louis: Elsevier Inc. P:49-53.
- Zarrabi MH, Javidi M, Naderinasab M, Gharechahi M (2009). Comparative evaluation of antimicrobial activity of three cements: new endodontic cement(NEC), mineral trioxide aggregate(MTA) and portland. J. Endod. 51:337-442.