

ENDODONTICS

ANTIBACTERIAL ACTIVITY OF GREY MINERAL TRIOXIDE AGGREGATE (MTA) MIXED WITH DIFFERENT VEHICLES

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ABSTRACT

The aim of this study was to investigate the *in vitro* antibacterial effects of Grey MTA mixed with different vehicles on a mix of *S aureus*. MTA was mixed with water, local anesthetic and 0.2% Chlorhexidine. 24 hour set test materials were placed on the surface of the inoculated media and incubated in appropriate atmospheres for 48 hours at 37 degrees centigrade. Augmentin suspension was used as a positive control. Dry, sterile tin foil discs were used as a negative control. The antibacterial effects of each material were determined by measuring the diameter of the zones of inhibition in millimeters. Chlorhexidine group had significantly bigger zone of inhibition ($P < 0.05$). In conclusion, substituting 0.2% Chlorhexidine gluconate for water enhanced the antimicrobial activity of Grey MTA.

Key words: MTA, Grey MTA, Chlorohexidine.

INTRODUCTION

Mineral trioxide aggregate (MTA) has been developed by Dr. Torabinejad to seal communications between the tooth and the external surfaces'. This material was tested in a series of *in vivo* and *in vitro* studies, which reported good sealing ability² and tissue behavior^{3,4}. Formation of new cementum over the material was reported in experimentally perforated furcation⁵, in root end Mine and root canal filling of dog's teeth⁴. Bridge-like dentin was observed in cases of pulp capping and pulpotomy in monkey and dog teeth'. Independent studies have shown that MTA exhibits antimicrobial ability⁸. MTA has been marketed as tooth-colored ProRoot MTA (*Tulsa I Dentsply, Tulsa, OK*) since 2002. ProRoot MTA is dispensed as MTA powder that is mixed with sterile water provided by the manufacturer. Mixing MTA with Chlorhexidine gluconate has been previously reported in *Stowe et al* study⁹. He found that substituting 0.12% Chlorhexidine gluconate for water enhanced the antimicrobial activity of tooth-colored ProRoot MTA. There is no published material regarding the effect of Chlorhexidine gluconate on grey MTA. The purpose of this *in vitro*, agar-diffusion study

is to determine whether grey MTA has antimicrobial activity, and if the substitution of 0.2% Chlorhexidine gluconate in place of sterile water or local anesthetic as a mixing agent will enhance this antimicrobial activity.

MATERIALS AND METHODS

Microorganism investigated was *Staphylococcus aureus* (15 MRSA strain no 85/2082) which was obtained from microbiology department at King Khalid University Hospital in Riyadh. The product tested was: Mineral Trioxide Aggregate® (*MTA, Dentsply, Tulsa Dental, Tulsa, OK, USA*)

Antimicrobial Assays

The strains were inoculated in 5 ml of brain heart infusion (BHI) and incubated at 37°C for 48 hours. The substances were studied using the agar diffusion test. Fifteen Petri plates with 20 ml BHI agar were inoculated with 0.1 ml of the experimental suspensions, using sterile swabs that were brushed across the medium, obtaining growth in junction. Three groups were tested; ten samples per group. In group one MTA was mixed with 0.2% Chlorhexidine gluconate. In

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group two MTA was mixed with distilled water which comes with the material itself and in group three MTA was mixed with local anesthetic (2% Xylocaine with 1:100000 epinephrine, Dentsply Pharmaceutical). MTA was mixed with these vehicles according to manufacturer's instructions using metal sleeves to have consistent amount of material and were placed on the surfaces of the plates. Augmentin suspension was used as a positive control. Dry, sterile tin foil disc was used as a negative control. The plates were incubated aerobically at 37°C for 48 h. The diameters of the zones of microbial inhibition were measured by an external examiner blindly. All assays were carried out under aseptic conditions.

The data for each MTA mixture group were subjected to *t* test analysis to determine if significant differences in zones of inhibition occurred between experimental MTA/CHX, MTA / Local anesthetic and MTA/water mixtures. Then one way ANOVA was used to determine the significant difference between all groups and a post hoc tukey test was used to decide which group had significant zone of inhibition. Confidence level was set at $p > 0.05$.

RESULTS

The data for zones of inhibition are presented in Table 1. MTA was always inhibitory regardless of the mixing liquid used. The mean zones of inhibition were 11.1 mm for MTA/CHX, 9.4 mm for MTA/water and 9.3 mm

mm for MTA/local anesthesia. There were larger zones of inhibition observed around the MTA/CHX mixtures compared with the MTA/water and MTA/ local anesthesia mixtures on all plates which was significantly different from other groups ($P > 0.05$).



Fig. 1. MTA/CX group



Fig. 2. MTA/LA group



Fig. 3. MTA/water group

TABLE 1: INHIBITION ZONE (mm)

Sample	Chlor-hexidine	Water	Local anesthetic
1	12.5 mm	9mm	8.5 mm
2	11.5 mm	8.5 mm	9.5 mm
3	11 mm	10.5 mm	9mm
4	10 mm	10 mm	10.5 mm
5	11 mm	9.5 mm	10 mm
6	12 mm	9mm	9.5 mm
7	10.5 mm	10mm	10mm
8	11.5 mm	9mm	8 mm
9	11 mm	8.5 mm	9mm
10	10 mm	9mm	9mm
Mean	11.1 mm	9.4 mm	9.3 mm



Fig. 4. Positive control



Fig. 5. Negative control

DISCUSSION

The microorganism in this study was selected to represent aerobes found in infected root canals. The endodontic infection is polymicrobial in nature and all microbiota live in symbiotic relation and disruption of one member of this environment will lead to destruction of the environment. It would be better if the material was tested against *E. faecalis* because it is the most frequently isolated microorganism recovered from failed endodontically treated cases'. *C. albicans* have been demonstrated in root filled teeth with chronic apical periodontitis but *Alnazhan et al* have demonstrated that fresh and set MTA is antifungal¹². *Torabinejad et al*¹³ reported that MTA did not have an inhibitory effect against *E. faecalis*, *S. aureus*, or *F.nucleatum*. In the present study, the MTA was inhibitory to *S. aureus*; this could be explained in different ways. First, placement of the material differed. In both studies, experimental materials were placed directly on the surface of the agar before incubation but in this study, metal sleeves were used to have

exact and reproducible volumes of MTA per sample. Another explanation could be the different MTA formulation. In the earlier study¹², the Loma Linda MTA used might be different from the commercially marketed MTA available later. The MTA tested in this investigation is the third-generation of grey MTA. The antimicrobial effect of CHX on all of the microorganisms tested has been shown previously¹². In this study, MTA/ CHX mixtures produced greater zones of inhibition than the MTA/water and MTA/local anesthetic mixtures. Our results are in agreement with Stowe et al study⁹ where he placed the material into wells that prepared on the agar plates to increase the material diffusion. But in real situation the material is only in touch with tissue. He also used white MTA while Grey MTA has been used in this study. Nevertheless, it can be concluded from his study and our study that the CHX in the MTA mixtures was the active component producing the enhanced antimicrobial response.

Changing the vehicle will produce a new material so it is necessary to study the physical properties, chemical properties and elemental analysis of this new material. Further studies are needed to determine these properties and the effect of CHX on MTA.

CONCLUSION

Using 0.2% Chlorhexidine gluconate with grey MTA enhanced the antimicrobial effect of the material in vitro against *S. aureus*.

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REFERENCES

- 1 Lee SJ, Monsef M, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. *Journal of Endodontics* 1993; 19: 541-544.
- 2 Torabinejad M, Watson TF, Pitt Ford TR. (1993) Sealing ability of a mineral trioxide aggregate when used as a root end filling material. *Journal of Endodontics* 1993; 19: 591-595.
- 3 Holland R, Souza V, Nery MJ, Otoboni Filho JA, Bernabe PFE, Dezan Junior E. (1999) Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. *Journal of Endodontics* 1999; 25: 161-166.

- 4 Holland R, Souza V, Nery MJ, Otoboni Filho JA, Bernabe PFE, Dezan Junior E. Reaction of dogs' teeth to root canal filling with mineral trioxide aggregate or a glass ionomer sealer. *Journal of Endodontics* 1999; 25: 728-730.
- 5 Pitt Ford TR, Torabinejad M, McKendry DJ, Hong CU, Kariyawasan SP. Use of mineral trioxide aggregate for repair of furcal perforations. *Oral Surgery* 1995; 79: 756-762.
- 6 Torabinejad M, Hong CU, Lee SJ, Monsef M, Pitt Ford TR. Investigation of mineral trioxide aggregate for root-end filling in dogs. *Journal of Endodontics* 1995; 21: 603-608.
- 7 Pitt Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasan SP. Using mineral trioxide aggregate as a pulp-capping material. *Journal of American Dental Association* 1996; 127: 1491-1496.
- 8 Estrela C, Bammann LL, Estrela CR, Silva RS, Pecora JD. Antimicrobial and chemical study of MTA, PC, calcium hydroxide paste, Sealapex and Dycal. *Brazilian Dental Journal* 2000; 11: 3-9.
- 9 Stowe J, Sedgley CM, Stowe B, Fenno JC. The Effects of Chlorhexidine Gluconate (0.12%) on the Antimicrobial Properties of Tooth-Colored ProRoot Mineral Trioxide Aggregate. *Journal of Endodontics* 2004; 30: 429-431.
- 10 Sundqvist G, Figdor D, Persson S, Sjogren U. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* 1998; 85: 86-93.
- 11 Peciuliene V, Reynaud AH, Balciuniene I, Haapasalo M. Isolation of yeasts and enteric bacteria in root-filled teeth with chronic apical periodontitis. *International Endodontic Journal* 2001; 34: 429-34.
- 12 Al-Nazhan S, Al-Judai A. Evaluation of antifungal activity of mineral trioxide aggregate. *Journal of Endodontics* 2003; 29: 826-7.
- 13 Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root end filling materials. *Journal of Endodontics* 1995; 21: 403-6.
- 14 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. *Journal of Endodontics* 1999; 25: 351-3.