Dental Research Journal

Original Article

Evaluation of the film thickness and antibacterial property of mineral trioxide aggregate mixed with propylene glycol as a root canal sealer

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ABSTRACT

Background: The aim of this *in vitro* study was to compare the film thickness and antibacterial properties of mineral trioxide aggregate-propylene glycol (MTA-PG) as a sealer in comparison with MTA Fillapex and AH26 sealers.

Materials and Methods: In these *in vitro* study the antibacterial property of the sealers was evaluated using direct contact test in fresh and set states. *Enterococcus faecalis* was incubated in direct contact with fresh and set materials. The growth of exposed bacteria was evaluated by counting colony-forming units (CFUs) after 10 min and 1 h in the culture medium. The film thickness of sealers was measured according to the International Standard Organization 6876/2012. The data were statistically analyzed using an independent *t*-test and repeated measures of ANOVA. The level of significance was set at 0.05.

Results: CFU means in AH26 was significantly more than other groups ($P < 0.0001$), but there was no difference between MTA-PG and MTA Fillapex. The mean of CFUs in set AH26 after 1 h exposure was significantly >10-min exposure ($P = 0.006$). The mean film thickness values of MTA Fillapex, MTA-PG, and AH26 were 57.3, 50.9, and 78.3 μm, respectively.

Conclusion: MTA-Fillapex and MTA-PG showed distinct antibacterial effect. AH26 showed more antibacterial effect in fresh state in comparison with set state. The film thickness of MTA-PG and MTA-Fillapex was significantly less than AH26.

Key Words: Antibacterial agents, canals sealer, mineral trioxide aggregate, propylene glycol

Received: October 2018
Accepted: August 2019

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INTRODUCTION

The quality of root canal therapy has been improved by providing sealers and obturation materials with high sealing ability and biocompatibility. Mineral trioxide aggregate (MTA) as a biomaterial has several applications in endodontics because of various desirable characteristics including high biocompatibility and low cytotoxicity,[1] release of calcium hydroxide ($Ca(OH)_2$),[2] sealing ability against the bacteria and saliva,[3] antibacterial features,[4] ability of setting in the presence of bleeding or in wet condition,[5] adequate compressive strength, and acceptable hardness.[4] However, inappropriate consistency and difficult handling are the two main drawbacks of MTA.[5] The efforts have been made to improve these properties, including adding propylene

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How to cite this article: Milani AS, Moeinian A, Barhaghi MH, Abdollahi AA. Evaluation of the film thickness and antibacterial property of mineral trioxide aggregate mixed with propylene glycol as a root canal sealer. Dent Res J 2020;17:142-6.

glycol (PG) to MTA to enhance its manipulation.[6-8] PG (1,2-propanediol) is a dihydric alcohol without toxicity, carcinogenicity, or genotoxicity and has been used as a solvent in several drug mixtures and food products.[9] Studies have been shown that mixing MTA with PG could improve its flow and pushout bond strength in addition to increasing the pH of dentin and cementum due to enhanced release of $Ca(OH)_2$,[9,10] without affecting its desirable biocompatibility.[7] Since adding PG to MTA results in better consistency and manipulation, this mixture may also be used as a sealer. Recently, a new generation of sealers with calcium silicate base has been introduced.[11,12] Due to the similarity in composition to MTA, these sealers are sometimes called MTA-based sealers. Despite favorable properties of MTA-based sealers such as MTA-Fillapex, they have shown some drawbacks such as higher cytotoxicity and lower sealing ability in comparison to resin-based sealers.[13,14] Furthermore, some studies reported lower biocompatibility of MTA-Fillapex than MTA.[15] Moreover, although MTA-Fillapex has shown antibacterial and antibiofilm properties in short terms,[16,17] its effect after setting was insignificant.[17] These drawbacks have been attributed to the low percentage of MTA in the sealer composition and presence of resin and other additives.[13,14] Remained microorganisms in the root canal system have the potential to survive despite chemomechanical preparations. Therefore, root canal filling materials such as sealers should have immediate and ideally long-term antibacterial property to remove these microorganisms.[17] The antibacterial characteristic of MTA-PG has not been studied, and therefore, the main goal of this study was to evaluate the antibacterial property of MTA-PG as a sealer in comparison with two commonly used resin-(AH26) and MTA-based (MTA Fillapex) sealers. Meanwhile, another property of ideal sealer is its penetration ability between the gutta-percha cones and into the root canal irregularities. This ability depends on the flow and viscosity of sealer.[18] The American Dental Association has not introduced any criterion for direct measurement of viscosity; however, film thickness has been proposed as an indirect criterion for assessing viscosity. Since the film thickness of MTA-PG sealer is unknown, the second goal of the present study was to compare the film thickness of MTA-PG, AH26, and MTA Fillapex sealers.

MATERIALS AND METHODS

This *in vitro* study was approved by the Research and Ethics Committee of Tabriz University of Medical Sciences (Tbzmed.REC.1394.604). In this *in vitro* study, standard suspension of *Enterococcus faecalis* (ATCC 29212, Reference Laboratories of Iran Research Center, Tehran, Iran) was used, and antibacterial assessment was carried out by direct contact test (DCT). In this method, the bacteria were kept in direct contact with testing materials, and then, growth of the bacteria was evaluated.[19,20]

Antibacterial properties of materials in the fresh condition

In this study, eight microtubes with the volume of 1.5 ml were used for each of the following testing materials prepared under aseptic conditions: AH26 sealer and MTA Fillapex (Angelus, Londrina, Brazil) were prepared according to the manufacturer's orders. In addition, MTA-PG sealer was prepared by mixing MTA (Angelus, Londrina, Brazil), with 50% PG (Merck, Germany) and 50% distilled water (DW). The DW/PG ratio was determined by volume, and the powder/liquid ratio was the same for MTA-PG groups (1 g powder to 0.33 mL liquid). The floor and one-fourth of the inner walls of each microtube were covered by the prepared sealers, and after 20 min, 10 μL of 0.5 McFarland standard suspension of *E. faecalis* prepared at the sterile Brain–Heart Infusion Broth (BHI-Oxid LTD, Hanks, USA) was added to microtubes. Then, the microtubes were incubated at 37°C for 1 h. In this way, the evaporation of suspension liquid could guarantee the direct contact. After that, the sealers in the microtubes were immersed in 500 μL of BHI broth, and after stirring for 2 min, they were incubated at 37°C. Finally, after incubation for 10 min and 1 h, 10 μL of solutions existing in each microtube was separately transferred to the BHI agar culture mediums, and after incubation for 24 h, bacterial colony count was done for each plate. Some of the samples were diluted up to 100 times to enable counting. In the negative control group (n = 3), no material was placed in microtubes and bacterial suspension was not added. Although, in the positive control group (n = 3), no material was placed in the microtubes, bacterial suspension was added to microtubes.

Antibacterial properties of materials in the set condition

The preparation procedures were exactly the same as the fresh condition. However, in this condition, bacterial suspension was placed on the testing materials after 72 h.[20]

Film thickness assessment

The film thickness assessment was carried out according to the International Standard Organization (ISO) 6876/2012 instruction. Two flat glass plates with 5-mm thickness and 200 ± 10-mm surface area were placed on each other, and the total thickness was measured by a digital micrometer to the nearest 10 μm. AH26 sealer and MTA Fillapex (Angelus, Londrina, Brazil) were prepared according to the manufacturers' orders, and MTA-PG sealer was prepared by mixing MTA (Angelus, Londrina, Brazil), with 50% PG (Merck, Germany) and 50% DW. The DW/PG ratio was determined by volume, and the powder/liquid ratio was the same for MTA-PG groups (1 g powder to 0.33 mL liquid). Immediately after mixing, 0.5 ml of each sealer was transferred onto the first plate and the second plate was placed over it. After 180 ± 5 s, the force of 150 N was applied vertically on the upper plate. The total thickness of the plates and the sealer between them was measured using micrometer after 10 min from the mixing time (7 min from the time of applying the force). The total thickness of the plates was deducted from this amount and the film thickness of the sealer was obtained. The test was repeated for three times for each sealer, and the mean value was recorded as film thickness of that sealer.

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS version 20.0, SPSS, Chicago, IL, USA). The data were statistically analyzed using repeated measures of ANOVA. Two independent sample *t*-tests were used to compare the colony-forming unit (CFU) amounts in each group at two different times (10 min and 1 h). The level of significance (P) was set at 0.05.

RESULTS

The means and standard deviations of CFUs of *E. faecalis* related to each endodontic sealer in fresh and set conditions are presented in Table 1. The controls confirmed the validity of experimental procedures, as no bacterial growth was seen in negative control and bacterial growth occurred in the positive control. There was a significant difference of mean CFU among the groups ($P < 0.001$). The results of Tukey's *post hoc* test showed that CFU means in AH26 was significantly more than other groups ($P < 0.0001$), but there was no difference between the CFU means of MTA-PG and MTA-Fillapex. There was a significant difference between the two-time evaluations in AH26 group. The mean of CFUs in set AH26 after 1 h exposure was significantly >10-min exposure ($P = 0.006$). The mean film thickness values of MTA-Fillapex, MTA-PG, and AH26 were 57.3, 50.9, and 78.3 μm, respectively. The film thickness of MTA-PG and MTA-Fillapex was significantly less than AH26.

DISCUSSION

In this study, the antibacterial characteristic of MTA-PG as a sealer was compared with two common resin-based (AH26) and MTA-based (MTA-Fillapex) sealers in fresh and set conditions. Furthermore, the film thickness of MTA-PG as a sealer was compared with MTA-Fillapex and AH26 sealers. In the present study, DCT was used to assess the antibacterial properties of the sealers. Other methods such as agar-diffusion test (ADT) have also been used with some major drawbacks.[21] There may be chemical interactions between media and testing materials, and there is no study definitely correlating the inhibition zone diameters in ADT with clinical performance of disinfectants. The antibacterial effect of some sealers is related to their pH. Therefore, the buffering effect of agar has a vital role in determination of diameter of inhibition zone.[21] However, DCT is a repeatable and quantitative method which is widely used for the evaluation of antibacterial effect of sealers and root-end filling materials since direct contact of bacteria with the sealers is obtained in this method.[20,22] The data from this method are related to bactericidal and not

Table 1: Mean and standard deviation of *Enterococcus faecalis* colony-forming units in evaluated groups

Groups	Evaluation time	CFU in fresh condition	Set in set condition
MTA-PG	10 min following contact	0	0
	1 h following contact	0	0
MTAFillapex	10 min following contact	0	0
	1 h following contact	0	0
AH26	10 min following contact	0	0
	1 h following contact	292.6±64.3×100	393.9±171.7×100

CFU: Colony-forming units; MTA: Mineral trioxide aggregate; PG: Propylene glycol

bacteriostatic effect.[22] This is important because the bacteria could begin to grow after reduction of the bacteriostatic effect of sealers. Furthermore, DCT method is an appropriate method for the evaluation of antibacterial effect of solid surfaces since it is almost independent of sealer solubility or penetration. We used *E. faecalis* as a test bacterium because it has been shown to be associated with resistant endodontic infections.[23,24] This bacterium has been routinely used in *in vitro* studies.[25,26] In this study, all the groups in the fresh conditions showed no bacterial growth in both 10 min and 1 h time periods. Furthermore, both MTA-Fillapex and MTA-PG groups in the set conditions showed no bacterial growth in the tested time periods. However, set AH26 sealer group showed positive results in both the time periods and significant increase of bacterial amount was seen in 1 h. Antibacterial property of sealers differs against various bacteria. Mohammadi *et al*.[27] and Shantiaee *et al*.[28] concluded that the antibacterial effect of AH26 on *Staphylococcus aureus* and *Streptococcus mutans* was significantly more than Apexit sealer. Morgental *et al*.[17] reported that fresh MTA-Fillapex sealer showed significant antibacterial effect, but in a 7-day set condition, this property was insignificant. However, in the present study, MTA-Fillapex showed significant antibacterial effect in the fresh and set conditions. The set samples in the present study were 3-day set, which can explain the difference in results. The antibacterial effect of MTA-Fillapex decreased over time.[29] Furthermore, studies have also evaluated the antibacterial characteristic of MTA-Fillapex and AH26 on *E. faecalis* biofilms. Faria-Júnior *et al*.[16] reported effective antibacterial property of set samples of MTA-Fillapex sealer against *E. faecalis* biofilms which was in coordination with this study. According to the results of this study, the film thickness of all sealers was >50 μm (the maximum amount recommended by the ISO).[18] The film thickness of AH26 was significantly more than other sealers. However, MTA-PG showed the lowest film thickness (51 μm). Studies have evaluated the film thickness of MTA-based sealers and reported the range of 24 μm.[18] Furthermore, Razmi *et al*. reported the film thickness of AH26 sealer in the acceptable ISO range (24 μm).[30] In this study, the film thickness measurements were performed according to the ISO 6876/2001 recommendations. The higher amounts of film thickness in this study in comparison with the similar studies could be attributed to the different accuracies of digital caliper used in the studies.

The present study showed promising results about the antibacterial property of MTA-PG mixture as a root canal sealer. However, further studies including animal studies and also with other bacterial species of oral flora are recommended.

CONCLUSION

MTA-Fillapex and MTA-PG showed distinct antibacterial effects. AH26 sealer demonstrated more antibacterial effect in the fresh state in comparison with the set state. The film thickness of MTA-PG and MTA-Fillapex was significantly less than AH26.

Acknowledgment

The authors would like to thank the Research Vice Chancellor and Dental and Periodontal Research Center of Tabriz University of Medical Sciences.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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