Archives of Oral Biology 60 (2015) 1497–1502

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/aob

An *in vitro* evaluation of the antibacterial properties of three mineral trioxide aggregate (MTA) against five oral bacteria

Ryan Jin-Young Kim[a,1], Myung-Ook Kim[b,1], Ki-Sun Lee[c], Dong-Yul Lee[d], Joo-Hee Shin[e,*]

[a] Department of Conservative Dentistry, Seoul National University Gwanak Dental Hospital, Seoul, South Korea
[b] Postgraduate student, Korea University University Graduate School of Medicine, Seoul, South Korea
[c] Institute for Clinical Dental Research, Korea University Guro Hospital, Seoul, South Korea
[d] Department of Orthodontics, Korea University Guro Hospital, Seoul, South Korea
[e] Department of Conservative Dentistry, Korea University Guro Hospital, 97 Gurodong-Gil, Guro-Gu, Seoul, South Korea

ARTICLE INFO

Article history:
Received 27 March 2015
Received in revised form 13 July 2015
Accepted 16 July 2015

Keywords:
Base
Disc diffusion test
Endodontics
MTA
Pulp capping
Zone of inhibition

ABSTRACT

Objective: The purpose of this study was to evaluate the antibacterial ability of three MTA (MTA-Angelus, Endocem MTA, and ProRoot MTA) against five typical oral bacteria (*Streptococcus mutans*, *Enterococcus faecalis*, *Lactobacillus rhamnosus*, *Lactobacillus paracasei*, and *Porphyromonas gingivalis*).
Design: For disc diffusion test, each test material was placed into agar plates after inoculation of each bacterial strain. The zones of inhibition of bacterial growth were then measured. Antibacterial broth test was performed by adding the test material into the media. Colony-forming units were counted after incubation with bacteria. The data were analyzed using ANOVA and the Tukey's test.
Results: Disc diffusion test showed that the antibacterial activity against *S. mutans*, *L. rhamnosus*, *L. paracasei*, and *P. gingivalis* ranked in decreasing order of MTA-Angelus > ProRoot MTA > Endocem MTA ($p < 0.05$). An inhibitory effect against *E. faecalis* was only observed in Endocem MTA. Antibacterial broth test showed that the antibacterial activity against all bacteria was Endocem MTA > MTA-Angelus > ProRoot MTA ($p < 0.05$).
Conclusion: Discrepant results were obtained from the disc diffusion and antibacterial broth test, with MTA-Angelus and Endocem MTA being most effective, respectively. Both tests revealed that the most resistant bacteria was *E. faecalis*, which was not susceptible at all, except to Endocem MTA in disc diffusion test.

1. Introduction

Mineral trioxide aggregate (MTA) has become the material of choice in various endodontic procedures such as pulp capping, pulpotomy, apexogenesis/apexification, repair of root resorption and lateral or furcal perforations, and retrograde filling, because of its superior properties including sealability (Torabinejad, Rastegar, Kettering, & Pitt Ford, 1995), biocompatibility (Torabinejad, Hong, Pitt Ford, & Kettering, 1995a), and bioactivity (Enkel et al., 2008). However, the main drawbacks of MTA are difficulty in handling, long setting time, and discoloration potential (Parirokh & Torabinejad, 2010). The main ingredients are tricalcium silicate, dicalcium silicate, and bismuth oxide, with small quantities of iron and aluminum (Ferris & Baumgartner, 2004). Since the introduction of ProRoot MTA (Dentsply, Tulsa, OK, USA) in 1998, novel commercially available MTA-based products including MTA-Angelus (Angelus, Londrina, PR, Brazil) and Endocem MTA (Maruchi, Wonju, Korea) have been developed in an attempt to improve these shortcomings by modifying the composition and/or concentration of each ingredient. Endocem is an MTA-derived pozzolan cement with a lesser setting time (4 min ± 30 s) and similar biocompatibility and osteogenicity compared to conventional MTA (Choi et al., 2013). As the characteristics of a material may change along with composition modification, numerous studies have evaluated the biological and physical properties of these MTA products.

Considering the indispensable role of microorganisms in the development and progress of pulpal and periapical disease as well as the failure of endodontic treatment, the eradication of microorganisms from the root canal system in endodontic treatment and prevention of bacterial ingress to the root canal system during restorative treatment are the key factors in successful clinical outcome (Baumgartner & Falkler, 1991; Fabricius, Dahlen, Ohman,

* Corresponding author. Fax: +82 2 837 6245.
E-mail address: endoshin11@gmail.com (J.-H. Shin).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.archoralbio.2015.07.014
0003-9969/

& Moller, 1982; Fouad, Zerella, Barry, & Spangberg, 2005; Kakehashi, Stanley, & Fitzgerald, 1965; Moller, Fabricius, Dahlen, Ohman, & Heyden, 1981; Siqueira, Rocas, Souto, de Uzeda, & Colombo, 2000; Sundqvist, 1992). Therefore, an ideal dental material should also possess antibacterial property, but not at the expense of its other biological and physical properties.

There is to date limited information, however, on the comparative antibacterial activity of MTA-based products against some of the predominant clinically relevant bacteria including *Enterococcus faecalis* in endodontic disease, *Porphyromonas gingivalis* in periodontal disease, *Streptococcus mutans* in the initiation of dental caries, *Lactobacillus rhamnosus*, and *Lactobacillus paracasei* in the progression of dental caries.

This study was conducted to compare the antibacterial effects of three MTA products (MTA-Angelus, Endocem MTA, and ProRoot MTA) against these five typical oral bacteria, and thereby to develop a clinical recommendation for their specific use based on their antibacterial activity.

2. Materials and methods

2.1. Compositions of tested cements

The chemical composition of the tested materials (MTA-Angelus, Endocem MTA, and ProRoot MTA) were analyzed by X-ray fluorescence spectrometer (ZSX100e, Rigaku, Akishima, Japan). The powders of each material were pressed into rigid pellets, which were then evaluated twice qualitatively and quantitatively using the fundamental parameter method.

2.2. Disc diffusion test

The antibacterial activity was evaluated using five standard bacterial strains: *S. mutans* (ATCC 25175), *E. faecalis* (ATCC 4082), and *P. gingivalis* (ATCC 33277) obtained from American Type Culture Collection (ATCC, Manassas, VA, USA), and *L. rhamnosus* (KCTC 3237) and *L. paracasei* (KCTC 3165) obtained from Korean Collection for Type Cultures (KCTC, Daejeon, Korea).

S. mutans and *E. faecalis* were cultivated in brain-heart infusion (BHI) (Difco, Detroit, MI, USA) broth, and *L. rhamnosus and L. paracasei* were inoculated in de Man, Rogosa and Sharpe (MRS) (Becton, Dickinson and Co., Flanklin Lakes, NJ, USA) broth aerobically at 37 °C for 1 day. *P. gingivalis* was grown in BHI broth supplemented with Hemin and Vitamin K at 37 °C and incubated anaerobically for 3 days to a concentration of 0.5 McFarland turbidity standard, which corresponds to approximately 3×10^8 colony-forming units (CFU)/mL. A sterile cotton-tipped swab was used to inoculate the bacterial suspension onto the agar plate to achieve a lawn of growth. MRS (Becton) agar was used for the cultivation of *L. rhamnosus and L. paracasei*. For *P. gingivalis*, Brucella Blood Agar plate (Hanil KOMED, Sungnam, Korea) was used. As for *E. faecalis* and *S. mutans*, Muller-Hinton Agar (Hanil KOMED) and Tryptic Soy Agar (Becton) were used, respectively, for cultivation. Thereafter, 4 equidistant wells with a diameter of 3.5 mm and a depth of 4 mm were made in each plate (total 32 wells in 8 plates for each bacterial strain) by removing the agar with a sterile hand tissue punch (Osung, Kimpo, Korea).

The tested MTA were mixed with a sterile spatula on a sterile glass slab according to the manufacturer's instruction. The mixed MTA were placed into the wells on the agar plate using a sterile MTA carrier (Dentsply-Tulsa Dental, Johnson City, TN, USA) and gently pressed into place. All plates were maintained at room temperature for 2 h to allow prediffusion of the test materials and then incubated at 37 °C for 3 days, except the *P. gingivalis* plates, which were incubated for 7 days anaerobically. After incubation, the diameter of the zone of inhibition was measured with a 0.5 mm precision ruler to the nearest millimeter ($n = 32$).

2.3. Antibacterial broth test

Antibacterial activity of the tested MTAs was carried out according to the methods of Clinical Laboratory Standard Institute (CLSI). Each MTA was dissolved in the selective media, as described in disc diffusion test, to a concentration of 10 mg/mL. 180 μL of the specific media for bacteria dispensed in each well of 96-well plate (SPL Life Sciences, Pocheon, Korea). 180 μL of the MTA-dissolved medium was added to the first row of 96-well plate and performed serial 2-fold dilution using a multi-channel micropipette. The bacterial suspensions (20 μL; 1.5×10^5 cells of *P. gingivalis*, 1.0×10^5 cells of the others) were inoculated in each well ($n = 6$). The plate was incubated at 37 °C in an anaerobic condition for *P. gingivalis* for 2 days and in an aerobic condition for *E. faecalis, S. mutans, L. paracasei* and *L. rhamnosus* for 1 day. Broth without MTA materials and Ampicillin (1 μg/mL) were served as controls for comparison. The cultured bacteria in the plate were suspended using up and down with multi-channel micropipette to homogenize them, and the plate was then centrifuged at 500 × g for 5 min to sink MTA particles. The bacteria level in each wells was counted by Petroff-Hausser bacteria counter (Hausser Scientific, Horsham, PA, USA).

2.4. Statistical analysis

The data were analyzed with one-way analysis of variance and the Tukey's honest significant difference post hoc test for multiple comparisons between the antibacterial effects of the three MTA materials against each bacteria tested. The level of significance was established at 5%. Statistical analysis was performed with SPSS software (IBM, Armonk, NY, USA).

3. Results

3.1. Compositions of the cements

The chemical compositions of the cements by X-ray fluorescence spectrometer are shown in Table 1. Main chemical compounds were calcium oxide (lime; CaO), silicon dioxide (silica; SiO_2), and bismuth oxide (Bi_2O_3), accounting for approximately 95% of the total mass of MTA-Angelus and ProRoot MTA, and 83% of Endocem MTA. When compared with MTA-Angelus and ProRoot MTA, the amount of CaO was relatively lower, while aluminum oxide (Al_2O_3), magnesium oxide (MgO), ferric oxide (Fe_2O_3), and

Table 1
Chemical compositions of each MTA material (mass%).

	MTA-Angelus	Endocem MTA	ProRoot MTA
CaO	68.85	54.47	63.06
Bi_2O_3	13.13	14.24	14.46
SiO_2	14.28	14.25	17.06
Al_2O_3	3.1	5.83	1.74
SO_3	0.02	3.32	1.96
MgO	0.34	3.21	0.75
Fe_2O_3	0.03	2.53	0.28
K_2O	–	1.34	0.05
P_2O_5	0.07	0.06	0.28
TiO_2	–	0.28	0.07
F	–	0.21	–
SrO	0.17	0.08	0.09
Na_2O	0.01	0.11	0.12
MnO	–	0.05	0.03
V_2O_5	–	–	0.03
NiO	–	0.01	0.01
Cl	–	0.01	0.01