Antibacterial activities and mineral induction abilities of proprietary MTA cements

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Mineral trioxide aggregate (MTA) cements are used in direct pulp capping and many other applications, and several types of these products have been commercialized. The aim of this study was to examine the antibacterial effects and mineral induction abilities of three conventional MTA cements and one resin-modified MTA cement. Agar diffusion tests revealed that, after setting, all four cements exhibited little antibacterial effects against Enterococcus faecalis and Streptococcus mutans, with no significant differences among the materials. After 24 h, E. faecalis and S. mutans suspensions incubated in the presence of each cement did not exhibit reduced numbers of viable bacteria, compared with those same bacterial suspensions incubated without any cement; this indicated that none of the cements inhibited bacterial growth. Furthermore, the resin-modified MTA cement exhibited lower mineral induction ability, compared with that of the three conventional MTA cements.

Keywords: Mineral trioxide aggregate, Antibacterial effect, Mineral induction

INTRODUCTION

Mineral trioxide aggregate (MTA) cements were developed by Torabinejad in the early 1990s and have been used in direct pulp capping and many other applications, such as pulpotomy, apexification, root-end filling, and perforation sealing²–⁵. ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA), the first MTA cement introduced, is mainly composed of Portland cement (i.e. a hydraulic calcium silicate cement). ProRoot MTA produces calcium hydroxide via hydration reaction during its setting process⁶. Subsequent dissolution of this calcium hydroxide promotes Ca²⁺ release and increased pH upon contact with water; this high pH reportedly exhibits antimicrobial activity against oral pathogens⁷. The antimicrobial activities of ProRoot MTA have been investigated by agar diffusion tests, in which ProRoot MTA produced inhibition zones against Streptococcus mutans, Streptococcus sanguinis, Enterococcus faecalis, Candida albicans, Fusobacterium nucleatum, and Actinomyces odontolyticus⁸–¹⁰. The calcium hydroxide formed during the ProRoot MTA setting process is also known to produce precipitates composed of hydroxyapatite in phosphate-containing fluids⁹,¹⁰; this hydroxyapatite is presumed to induce mineralization¹¹,¹², based on clinical outcomes related to its excellent biocompatibility and sealing ability when used for direct pulp capping¹³, pulpotomy¹⁴,¹⁵, or perforation sealing¹⁶. However, long setting time (180–240 min) and difficult handling characteristics have emerged as limitations of ProRoot MTA¹⁷,¹⁸.

Recently, several calcium silicate-based cements have been developed and commercialized to overcome these limitations of ProRoot MTA. NEX MTA (GC, Tokyo, Japan) is a hydraulic MTA cement that includes components similar to those within ProRoot MTA. BioMTA (BioMTA, Seoul, Korea) is a hydraulic cement mainly composed of calcium carbonate. Compared with ProRoot MTA, the curing times of NEX MTA and BioMTA are both shorter (90 and 140 min, respectively). TheraCal LC (Bisco, Schamburg, IL, USA) is a light-cured resin-modified calcium silicate-based cement that contains 45 (wt)% resin components¹⁹. TheraCal LC is a single paste in a syringe, and its flow before light-curing is similar to that of flowable resin composites. Because of the ease of handling of TheraCal LC, it is widely used for some applications within narrow regions, such as direct pulp capping. These three new products with a shorter setting time or improved handling characteristics might serve as useful alternatives to ProRoot MTA. Previously, Yamamoto et al.²⁰ compared the hydroxyapatite formation on ProRoot MTA with that on TheraCal LC and reported that the apatite-forming ability of TheraCal LC was inferior to that of ProRoot MTA. However, the antibacterial activities and mineral induction abilities of ProRoot MTA, NEX MTA, BioMTA, and TheraCal LC have not been fully compared. Therefore, the aim of this study was to evaluate the antibacterial effects and mineral induction abilities of three conventional MTA cements (i.e. ProRoot MTA, NEX MTA, and BioMTA) and one resin-modified MTA cement (i.e. TheraCal LC).
MATERIALS AND METHODS

Materials
Conventional MTA cements in this study were ProRoot MTA, NEX MTA, and BioMTA. In accordance with the manufacturers' instructions, each powder was mixed with distilled water. In addition, a light-cured resin-modified calcium silicate-based cement (TheraCal LC) was used (Table 1).

pH change
Fifty milligrams each of cement powder (ProRoot MTA, NEX MTA, or BioMTA) were placed into separate 0.4 µm-pore filter transwell inserts in a 24-well plate (Costar Transwell; Corning, NY, USA), then mixed with distilled water. The mixture was incubated in 95% humidity at 37°C for 10 min. Fifty milligrams each of TheraCal LC paste were added to separate transwell inserts and light-cured for 10 s using a light-curing unit (Pencure 2000, Morita, Kyoto, Japan). Each cement was immersed in 1.0 mL of distilled water (pH 7.0) or Brain Heart Infusion broth (BHI, pH 7.4; Becton Dickinson, Sparks, MD, USA), and incubated at 37°C. After immersion for 10 min, and for 1, 3, 6, 12, and 24 h, the pH values of all solutions were measured using a pH meter (ISFET KS723, Shindengen, Tokyo, Japan). All tests were repeated three times.

Agar diffusion tests
Agar disc diffusion tests were performed as previously reported. Briefly, bacterial strains E. faecalis SS497 and S. mutans NCTC10449 were cultivated in BHI broth at 37°C in an anaerobic atmosphere. After incubation for 24 h, bacterial suspensions were adjusted to approximately 1×10^8 colony-forming units per milliliter. Each suspension was spread onto a separate BHI agar plate (200 µL per plate).

To evaluate the antibacterial activities of freshly mixed pastes of the three conventional MTA cements, pastes of ProRoot MTA, NEX MTA, or BioMTA (i.e. cement powder mixed with distilled water) were used to fill separate wells (5-mm diameter) prepared in the agar plates inoculated with each bacterium.

To examine the effects after setting, pastes of ProRoot MTA, NEX MTA, or BioMTA were used to fill a propylene mold (5-mm inner diameter, 1-mm depth). TheraCal LC paste was used to fill the mold, then light-cured for 10 s using a light-curing unit (Pencure 2000, Morita). These specimens were incubated in 95% humidity at 37°C for 24 h to set. Each set cement was placed on an agar plate inoculated with the bacterial suspension. A sodium hypochlorite (NaOCl) solution (Neo Cleaner, Neo Dental Chemical Products, Tokyo, Japan) was diluted to 5.25% and used as a control: a 20-µL aliquot of the solution was used to impregnate a sterile paper disc (5-mm diameter, 1.5-mm thickness), which was placed on the agar plate.

All plates were incubated anaerobically for 48 h at 37°C, and the diameter of the inhibition halo was measured at three points using a caliper (Mitutoyo, Tokyo, Japan). The sizes of the inhibition zones were calculated by the following equation: Size of inhibition zone=(I−D)/2, where I=mean of three measurements of the diameter of inhibition halo (mm) and D=diameter of the well/mold or paper disc (5 mm). The tests were repeated three times.

Bacterial growth in the presence of each MTA cement
Fifty milligrams of each cement powder (ProRoot MTA, NEX MTA, or BioMTA) were mixed with distilled water, then placed into separate 0.4 µm-pore filter transwell inserts in a 24-well plate. Fifty milligrams each of TheraCal LC paste were added to separate transwell inserts and light-cured for 10 s. All cements were incubated in 95% humidity at 37°C for 10 min, then immersed in 200 µL of E. faecalis or S. mutans

Table 1 Mineral trioxide aggregate cements used in this study

| Trade name | Code | Manufacturer | Composition                                      |
|------------|------|--------------|--------------------------------------------------|
| Pro Root MTA | Pro  | Dentsply Tulsa Dental | Powder: Tricalcium silicate, dicalcium silicate, bismuth oxide, Tricalcium aluminate, calcium sulphate dihydrate (gypsum), Calcium aluminoferrite  
| NEX MTA | Nex  | GC           | Powder: Calcium oxide, Bismuth oxide, Silicon dioxide, Aluminum oxide  
| TheraCal | Thr  | Bisco        | Paste: CaO Sr glass, Fumed silica, Barium sulphate, Barium zirconate, Portland cement, Bis-GMA, PEGDMA  
| Bio MTA | Bio  | Bio MTA      | Powder: Calcium carbonate, Silicon dioxide, Aluminum oxide, Calcium zirconia complex  

Bis-GMA, bisphenol A-glycidyl methacrylate; PEGDMA, polyethylene glycol dimethacrylate
suspension at $1 \times 10^3$ colony-forming units per milliliter. After anaerobic incubation at 37°C for 24 h with gyratory shaking at 100 rpm, 100 µL of each bacterial suspension was collected and added to 9.9 mL of BHI broth. The diluted suspension was used to inoculate BHI agar (Becton Dickinson) plates, which were incubated anaerobically at 37°C for 48 h; resulting colonies were counted. The experiments were repeated three times.

Mineral induction ability
Each cement paste within the mold was immersed in phosphate-buffered saline (PBS) solution at 37°C for 14 days. After immersion in PBS, the surface precipitates that formed on the specimens before and after immersion in PBS were dehydrated through a graded series of ethanol, then gold-sputter coated. The morphologies of precipitates formed on the specimen were observed using a scanning electron microscope (SEM; JSM-6390, JEOL, Tokyo, Japan) at 5 kV under ×20 and ×100 magnifications.

To assess the elemental components of the precipitate, the surface layer of each specimen before and after immersion in PBS for 14 days was scraped using a sterilized spatula; field-emission scanning electron microscope/energy dispersive spectroscopy (FE-SEM/EDS; JSM-F100, JEOL) analysis was then performed. The energy spectra analyses were performed by EDS to determine the element composition, and the ratios of elements in each cement were calculated. The morphologies of precipitates were then observed using FE-SEM at 10 kV.

The X-ray diffraction (XRD; RINT-2000, Rigaku, Tokyo, Japan) analysis was then performed. The X-ray beam angle 2θ (degree) range was set between 20 and 40 degrees and scanned at 0.04 degrees per second. The Cu X-ray source was operated with an acceleration voltage of 40 kV and an electron beam current of 30 mA. Peak positions in the XRD patterns obtained from the specimens were compared and matched with those of the standard material in the powder diffraction file (#9-432) of the International Center for Diffraction Data 2013.

Statistical analysis
Statistical analyses were performed using SPSS Statistics 21 (IBM, Armonk, NY, USA). The homogeneity of variances was confirmed before subsequent analyses. pH change, agar diffusion test, and bacterial growth data were compared among groups by using analysis of variance (ANOVA) and Tukey’s honestly significant difference (HSD) test with a significance level of $p<0.05$.

RESULTS

pH change
The pH changes after immersion of the four cements in distilled water and BHI broth are shown in Fig. 1. The pH values in solution of ProRoot MTA, NEX MTA, and BioMTA reached approximately 12 after immersion in water for 3 h; these remained stable or slightly decreased after immersion for 24 h. The pH value in solution of TheraCal LC increased reached approximately 9–10 after immersion in water for 24 h. In contrast, the pH values obtained by immersion of each of the four cements in BHI broth reached only 7.5–8.2; these were

![Fig. 1](image)

**Fig. 1** pH changes after immersion of each of the four MTA cements in distilled water (A) or brain heart infusion broth (B) for 24 h. Bio, BioMTA; Nex, NEX MTA; Pro, ProRoot MTA; Thr, TheraCal LC

![Fig. 2](image)

**Fig. 2** Agar diffusion tests using freshly mixed conventional mineral trioxide aggregate cements. Instead of inhibition zones, white-clouded spots (arrows) were observed for Pro, Nex, and Bio where their components penetrated into brain heart infusion agars. Bio, BioMTA; Nex, NEX MTA; Pro, ProRoot MTA
significantly lower than the values observed in water ($p<0.05$, ANOVA, Tukey’s HSD test).

Agar diffusion tests
White turbid spots were observed around freshly mixed ProRoot MTA, NEX MTA, and BioMTA pastes, but there were no inhibition zones against *E. faecalis* or *S. mutans* (Fig. 2). After setting, all cements produced inhibition zones of only 0.5–1.0 mm against *E. faecalis* and *S. mutans* (Fig. 3). The inhibition zones of the four cements were significantly smaller than that of NaOCl ($p<0.05$, Tukey’s HSD test). No significant differences in the inhibition zones were observed among the four MTA cements ($p>0.05$, Tukey’s HSD test).

Bacterial growth in the presence of each MTA cement
Figure 4 demonstrates the numbers of viable bacteria after incubation of *S. mutans* or *E. faecalis* suspensions in the presence of each cement. After incubation for 24 h in the presence of each of the four cements, the numbers of surviving cells of either species were not reduced, compared with those of control bacterial suspensions incubated without any exposure to cement ($p>0.05$, ANOVA, Tukey’s HSD test).

Mineral induction ability
Figure 5 shows the SEM images before/after immersion of each cement in PBS for 14 days. Precipitates were observed on the surfaces of ProRoot MTA, NEX MTA, and BioMTA; comparatively fewer precipitates were observed on TheraCal LC. Figure 6 shows the FE-SEM images and the element compositions of precipitates formed on each specimen. The precipitates on ProRoot MTA, NEX MTA, and BioMTA displayed spherical

![Fig. 3 Inhibition zones of MTA cements against Streptococcus mutans (A) and Enterococcus faecalis (B).
Inhibition zone=(I-D)/2, where I=mean of three measurements of the diameter of inhibition halo (mm) and D=diameter of the well/mold or paper disc (5 mm). There were no significant differences among the four groups ($p<0.05$, Tukey’s HSD test). *Asterisk indicates significant differences between each cement and NaOCl ($p<0.05$, Tukey’s HSD test). Bio, BioMTA; Nex, NEX MTA; n.s., no significant differences; Pro, ProRoot MTA; Thr, TheraCal LC.

![Fig. 4 Numbers of viable bacteria after incubation of Streptococcus mutans (A) or Enterococcus faecalis (B) for 24 h in the presence of each cement. Control: Bacterial suspension without any materials. There were no significant differences among all groups ($p>0.05$, Tukey’s HSD test). Bio, BioMTA; Nex, NEX MTA; n.s., no significant differences; Pro, ProRoot MTA; Thr, TheraCal LC.](image-url)
Fig. 5 SEM images before and after immersion of each cement in PBS for 14 days. A–C: ProRoot MTA. D–F: NEX MTA. G–I: TheraCal LC. J–L: BioMTA. A, D, G, J: images before immersion of each cement in PBS. B–C, E–F, H–I, and K–L: images after immersion of each cement in PBS for 14 days.

Fig. 6 FE-SEM images and element compositions of precipitates formed on each cement after immersion in PBS for 14 days. (A) ProRoot MTA, (B) NEX MTA, (C) TheraCal LC, (D) BioMTA.

Fig. 7 X-ray diffraction patterns of precipitates on each cement before and after immersion in PBS for 14 days. (A) ProRoot MTA, (B) NEX MTA, (C) TheraCal LC, (D) BioMTA.

DISCUSSION

MTA cements that have set contain calcium hydroxide, which is produced through a hydration reaction during the setting process. The dissolution of the resulting calcium hydroxide increases pH upon contact with an aqueous environment. Several studies have reported that, in a solution containing set ProRoot MTA cement, the pH value rose to approximately 12 during 24 h of immersion in water. Our results confirmed that, when set ProRoot MTA, NEX MTA, or BioMTA cements were immersed in water, the pH values of solution reached approximately 12 at 3 h. This could explain the similarities among the results of previous reports. In addition, the pH values of solutions with set cement gradually increased during the first 3 h of immersion in water and remained stable for 24 h. The low solubility of calcium hydroxide (1.2 g/L in water at 25°C) is responsible for the slow dissolution of calcium and hydroxide ions.
In a solution containing set TheraCal LC cement, the pH value after immersion in water for 24 h was lower than that of other cements; notably, it only reached 9.0–10.0. TheraCal LC is a light-cured resin-modified calcium silicate-based cement. Because the process used for its curing/setting does not involve water, the hydration of TheraCal LC requires uptake from an aqueous environment. Yamamoto et al.\textsuperscript{30} reported that TheraCal LC exhibited lower levels of Ca\textsuperscript{2+} release in water and lower pH values than ProRoot MTA. Moreover, TheraCal LC did not form calcium hydroxide after setting, and calcium phosphate was found on its surface\textsuperscript{26}. The absence of calcium hydroxide produced during the setting of TheraCal LC resulted in the lower pH values.

When all set cements were immersed in BHI broth, the pH values in solution reached approximately 8, which was lower than the values reached in water. BHI broth contains sodium hydrogen phosphate, which presumably served as a pH buffer and prevented pH increase due to the release of hydroxide ions from the cements.

Calcium hydroxide is used for a number of endodontic treatments and is included in several materials and formulations, such as intracanal medicaments, pulp capping agents, and endodontic sealers. The pH of calcium hydroxide paste (i.e. a mixture of calcium hydroxide powder and water) is approximately 12.5–12.8, which is regarded as a strong base\textsuperscript{27}. Its high pH is achieved through the ionic dissociation of calcium and hydroxide ions, and exhibits antimicrobial activities against oral pathogens. Although the specific mechanism remains unclear, the antibacterial effects of hydroxide ions are presumably due to damage to the bacterial cytoplasmic membrane, denaturation of proteins, and damage to the DNA\textsuperscript{28}. \textit{E. faecalis} is more alkali-resistant than other species of oral bacteria (including \textit{S. mutans}), but cannot survive at pH ≥11.5\textsuperscript{28,30}. Several studies have reported that the antibacterial effects of ProRoot MTA result from the alkaline pH produced during its setting process\textsuperscript{40}. To evaluate these antibacterial effects, we performed agar diffusion tests of all four cements. Although all set cements produced inhibition zones against \textit{E. faecalis} and \textit{S. mutans}, these inhibition zones were less than those produced by paper discs impregnated with 5.25% NaOCl. In addition, no significant differences were observed among the four materials. We then performed agar diffusion tests to evaluate the antibacterial effects of freshly mixed pastes of the three conventional MTA cements. However, white-clouded spots were observed, instead of inhibition zones, for all three conventional MTA cements.

To confirm the above findings regarding the antibacterial effects of freshly mixed MTA cements, bacterial growth assays were conducted by incubating \textit{E. faecalis} or \textit{S. mutans} suspensions in the presence of each of freshly mixed cements; light-cured TheraCal LC was used also for this experiment. Unexpectedly, the results revealed that none of the cements had inhibitory effects on the growth of \textit{E. faecalis} or \textit{S. mutans} after incubation for 24 h. As described above, after immersion of all cements in BHI broth (suitable culture medium for both bacteria), the pH values reached only 8 over an incubation period of 24 h. Slow release of hydroxide ions from MTA cements was presumably neutralized by the buffering capacity of sodium hydrogen phosphate in the culture medium; therefore, both bacterial species were able to grow normally at approximately pH 8.0. Based on the results of agar disc diffusion tests and bacterial growth assays, all MTA cements exhibited little antibacterial effects against \textit{E. faecalis} and \textit{S. mutans}, because these cements could not increase pH in the culture medium used for bacterial incubation. Therefore, because of constant fluid flow and the buffering capacity of the oral cavity, all MTA cements may not exhibit antibacterial effects \textit{in vivo}.

MTA cements are known to produce hydroxyapatite in phosphate-containing fluids\textsuperscript{31-33}. In this study, to evaluate the mineral induction abilities of the four materials, the precipitates formed on ProRoot MTA, NEX MTA, BioMTA, and TheraCal LC were analyzed by using SEM/EDS after the materials had been immersed in PBS for 14 days. Notably, ProRoot MTA, NEX MTA, and BioMTA produced precipitates on their surfaces. The precipitates on these conventional MTA cements displayed spherical morphology, and contained calcium, phosphorus, and oxygen. The ability of TheraCal LC to release Ca\textsuperscript{2+} is reportedly lower than that of ProRoot MTA, due to the lower calcium content and solubility of TheraCal LC\textsuperscript{18}. Therefore, in this study, increased concentrations of Ca\textsuperscript{2+} were released from conventional MTA cements, relative to those released from TheraCal LC; these increased levels of Ca\textsuperscript{2+} reacted with PO\textsubscript{4}\textsuperscript{3-} in the surrounding solution and formed calcium phosphate on the cement surfaces. Although XRD analysis indicated that no peaks corresponding to hydroxyapatite were present in the precipitates formed on all cements, it was suggested that the resin-modified MTA cement exhibited lower mineral induction ability than that of the three conventional MTA cements. The calcium phosphate-forming abilities of the three conventional MTA cements expected to promote hard tissue formation in clinical settings. However, in a randomized clinical trial that assessed the abilities of conventional MTA and TheraCal LC to induce reparative dentin formation when used for indirect pulp capping in primary teeth, no significant differences were found between conventional MTA and TheraCal LC in terms of the thickness of reparative dentin at 6 months after capping\textsuperscript{34}. Accordingly, further studies are needed to evaluate the rate of hard tissue formation, as well as the thickness/calcification of the dentin-bridge formed, to compare clinical effectiveness among these four MTA cements.

CONCLUSION

All three conventional MTA cements (ProRoot MTA, NEX MTA, and BioMTA) and a resin-modified MTA cement (TheraCal LC) exhibited little antibacterial effects against \textit{E. faecalis} and \textit{S. mutans}, with no significant differences among the four materials. It was
suggested that the resin-modified MTA cement exhibited lower mineral induction ability than that of the three conventional MTA cements.

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