Comparison of the Biological Properties of ProRoot MTA, OrthoMTA, and Endocem MTA Cements

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Abstract

Introduction: OrthoMTA (BioMTA, Seoul, Korea) and Endocem MTA (Maruchi, Wonju-si, Korea) were recently developed to overcome the disadvantages of ProRoot MTA (Dentsply, Tulsa, OK). This study aimed to compare the biological properties of OrthoMTA and Endocem MTA with those of ProRoot MTA using the preosteoblastlike cell line MC3T3-E1. **Methods:** The setting times of calcium silicate–based cements (CSCs) and their effects on the pH of distilled water during storage were determined according to ISO standards. MC3T3-E1 cells were cultured with ProRoot MTA, OrthoMTA, and Endocem MTA. The viability of the cells was assessed using the Cell Counting Kit-8 assay (Dojindo Laboratory, Kumamoto, Japan) on the supernatants of CSCs, and the cells' osteopontin production was determined by an enzyme-linked immunosorbent assay on a culture with the materials on days 3 and 7 of incubation. **Results:** Endocem MTA exhibited a significantly shorter setting time (15.3 $\pm$ 0.5 minutes) than did ProRoot MTA and OrthoMTA (318.0 $\pm$ 56.0 and 324.3 $\pm$ 2.1 minutes, $P < .05$). Additionally, all CSCs caused their storage water to become highly alkaline after 7 days. OrthoMTA was significantly more cytotoxic than ProRoot and Endocem MTA ($P < .05$). ProRoot MTA induced significantly more OPN production than OrthoMTA and Endocem MTA on both days 3 and 7 ($P < .05$). **Conclusions:** ProRoot MTA appeared to be superior to OrthoMTA and Endocem MTA in terms of biological properties although Endocem MTA exhibited the shortest setting time and presented lower cytotoxicity with osteoblastlike cells. *(J Endod 2014;■:1–5)*

Key Words

Calcium silicate–based cements, cytotoxicity, Endocem MTA, MC3T3-E1 cell, OrthoMTA, osteopontin, ProRoot MTA

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ProRoot mineral trioxide aggregate (MTA) (Dentsply, Tulsa, OK), a type of calcium silicate–based cement (CSCs), is used worldwide in endodontic treatment, not only as a root-end filling material (1) but also for direct pulp capping and apexification and in regenerative endodontic procedures (2, 3). ProRoot MTA, which has supplanted other historic endodontic cements because of its superior physical and biological properties, is mainly composed of fine hydrophilic powders of tricalcium silicate, tricalcium aluminate, tricalcium oxide, and other oxides that can set in the presence of water. An *in vitro* study showed that the MG-63 human osteoblastlike cell line attaches to MTA, and it was concluded that MTA is a biocompatible material (4). In addition, an *in vivo* study in monkeys suggested that MTA has many of the required properties of root-end and perforation filling materials such as biocompatibility, sealing ability, and capacity of inducing regeneration of the dentoalveolar structure (5).

However, ProRoot MTA sets relatively slowly. Although this characteristic may allow less shrinkage, this material may wash out from the preparation under hemorrhagic condition when it is used as a root-end filling material, which may cause treatment failure (6). Moreover, ProRoot MTA has a discoloration potential that can result in the discoloration of teeth and contains several toxic elements (7, 8). It is necessary to overcome these drawbacks of ProRoot MTA that are related to the health problems of patients. In addition, because of its difficulty in manipulation and its high cost, many endodontists may hesitate to use it (7).

Recently, 2 new types of CSCs were developed and marketed as novel endodontic materials, namely, OrthoMTA (BioMTA, Seoul, Korea) and Endocem MTA (Maruchi, Wonju-si, Korea), to overcome these drawbacks. According to the manufacturers, both of them are relatively cheap and easy to manipulate. OrthoMTA, developed mainly for orthograde root canal obturations as well as retrograde fillings and perforation repairs, consists of tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, gypsum, free calcium oxide, and bismuth oxide. It also has a bioactive characteristic; it causes the apical foramen to release calcium ions, which leads to the formation of an interfacial hydroxyapatite layer (9). In addition, the manufacturer claims that it has less heavy metal contents than ProRoot MTA. Endocem MTA consists of calcium oxide, aluminum oxide, silicate oxide, magnesium oxide, and bismuth trioxide (10), and its developers assert that it has a clinically faster working time than other CSC materials and shows good clinical results in vital pulp therapies. Recently, 1 study showed Endocem MTA represented less discoloration potential than ProRoot MTA (11). However, there are relatively few studies on these novel CSC materials.

The murine osteoblast precursor cell line MC3T3-E1 is often used for *in vitro* studies on bone cells because when it is subjected to strain, it expresses specific hard tissue–related genes, such as bone morphogenic protein 2, runt-related transcription factor 2, and mothers against decapentaplegic homolog 5 (12). It also expresses osteopontin (OPN), which is 1 of many bone mineralization markers and 1 of the main noncollagenous proteins in mineralized tissues (13). OPN is also an adhesion molecule in the extracellular matrix of calcified tissues that participates in wound healing, inflammatory reaction, and immunologic responses (14). A study investigating human periodontal regeneration indicated that OPN is expressed at the border between newly formed cementum and bone (15).

To the best of our knowledge, there are no studies comparing the biological properties of OrthoMTA and Endocem MTA with those of ProRoot MTA on MC3T3-E1 cells. The present study was performed to compare the properties of OrthoMTA and Endocem MTA

with those of ProRoot MTA, namely, their setting times, induced changes in pH, and their effects on MC3T3-E1 cell viability and OPN expression.

Materials and Methods

Sample Preparation

The setting times of the CSCs and their effect on the pH of distilled water during storage were determined according to International Organization for Standardization criterion 6876 (16).

Measurement of Setting Time

To prepare the 6 specimens of each CSC, metal molds (inner diameter = 10 mm, depth = 3 mm) were used to generate standard volumes and shapes. The molds were placed on a microscopic glass slide and transferred to a cell culture incubator at 37°C and 95% relative humidity. An indenter needle (400-g load) was used to assess the setting time, which was recorded when the needle failed to mark 3 separate areas of a specimen within the ring mold. Samples were measured every minute, and this test was repeated 3 times.

pH Changes

The 6 specimens of each CSC were prepared by filling plastic tubes (diameter = 1 mm, length = 3 mm). Each filled tube was then sealed into a flask containing 10 mL deionized water and stored at 37°C at a relative humidity of 95%. After 24 hours of immersion, the tubes were carefully removed and placed into another flask containing an equal amount of fresh deionized water. On day 7 (4 days after the last change of water), the pH of each aqueous specimen was measured by using a pH meter (Model 340; Corning Inc, Corning, NY) that was calibrated with standard 4.0 and 7.0 pH solutions.

Cell Cultures

The MC3T3-E1 cell line was donated by the Department of Endocrinology, Division of Internal Medicine, Ulsan University, Seoul, Korea. The cells were thawed and suspended in alpha-minimum essential medium (Gibco, Grand Island, NY) supplemented with 10% (v/v) fetal bovine serum (Gibco), 1% (w/v) antibiotics/antimycotics (the stock solution contained 100 U penicillin G sodium/mL, 100 μg/mL streptomycin sulfate, and 0.25 μg/mL amphotericin B in saline), 1.0 mmol/L sodium pyruvate, 0.1 mmol/L nonessential amino acids, and 1.5 g/L sodium bicarbonate (Gibco). The suspension was then placed in a 100-mm culture dish and cultured in an atmosphere of 5% (v/v) CO_2 at 37°C.

Cell Viability Test

CSCs were mixed with a sterile metal spatula according to the manufacturers' instructions into sterilized molding rings (inner diameter = 5 mm, thickness = 2 mm). The set discs were then removed from the rings, and 10 discs of each material were placed into 1 mL Dulbecco modified Eagle medium (Gibco-Invitrogen Corporation, Paisley, UK) in an autoclaved glass vial and incubated at 37°C. After 24 hours, the discs were removed by using sterile forceps, placed into fresh Dulbecco modified Eagle medium, and incubated for another 24 hours. The CSC-conditioned supernatant was then harvested. The unconditioned supernatant was used as a negative control.

Thereafter, 4.5×10^5/mL MC3T3-E1 cells were cultured with 1 mL each supernatant for 24 hours in an atmosphere of 5% (v/v) CO_2 at 37°C. Cell proliferation was then determined by using a Cell Counting Kit-8 (Dojindo Laboratory, Kumamoto, Japan) according to the manufacturer's instructions. The absorbance of the colored formazan was measured by using a microplate reader (Spectra Max 340; Molecular Devices, Sunnyvale, CA) at 450 nm.

To evaluate the direct effect of the CSCs, 1×10^5/mL MC3T3-E1 cells were cultured with CSC discs (inner diameter = 5 mm, thickness = 2 mm) in α-MEM for 24 hours in an atmosphere of 5% (v/v) CO_2 at 37°C. The 24-well plates were photographed with a microscope (Carl Zeiss, Gottingen, Germany) to assess the affinity of the cells for the CSC discs.

Effect of CSCs on OPN Production

After allowing the MC3T3-E1 cells to attach to the wells using an insert in the supplemented α-MEM described previously for 24 hours, the medium was switched to mineralizing medium, which was α-MEM containing 2% fetal bovine serum supplemented with ascorbic acid (50 mg/mL) and beta-glycerol phosphate (10 mmol/L). The mineralizing medium was changed on day 3, and the supernatants of the culture medium of each group were collected on days 3 and 7. OPN levels in the supernatants were determined by a Quantikine Human OPN Immunoassay Kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. A microplate reader (Spectra Max 340) was used to measure the absorbance at 450 nm.

Statistical Analysis

All experiments were performed in triplicate. These data are expressed as the mean $\pm$ standard deviation. The significance ($P < .05$) of differences between the groups was determined by using 1-way analysis of variance followed by Tukey tests.

Results

Setting Times and Effects of CSCs on Distilled Water pH

Endocem MTA had a significantly shorter setting time (15.3 $\pm$ 0.5 minutes) than ProRoot MTA (318.0 $\pm$ 56.0 minutes) and OrthoMTA (324.3 $\pm$ 2.1 minutes, $P < .05$). For ProRoot MTA, OrthoMTA, and Endocem MTA, the pH values of the storage water on day 7 were 11.90, 11.42, and 11.33, respectively, which did not differ significantly (Table 1).

Effects of CSCs on MC3T3-E1 Cell Viability

Cell Counting Kit-8 analysis of the viability of the cells after incubation with the CSC-conditioned media revealed that OrthoMTA was associated with significantly lower cell proliferation than the negative control, ProRoot MTA, and Endocem MTA (Fig. 1*A*, $P < .05$). When the MC3T3-E1 cells were incubated directly with the 3 CSCs, OrthoMTA again was associated with lower cell proliferation than the other 2 CSCs. The cells also showed greater affinity for the ProRoot MTA and Endocem MTA discs than for the Ortho MTA discs (Fig. 2*A–D*).

Effects of CSCs on OPN Production

The OPN levels of the ProRoot MTA group were higher than those of the other groups on days 3 and 7 ($P < .05$). The concentration of OPN in the ProRoot MTA group was significantly higher ($P < .05$) than that in the other CSC groups at each time point throughout the entire test periods (Fig. 1*B*).

Discussion

Ideal endodontic filling materials should show good biocompatibility, excellent apical sealing, easy handling, low cost, and long-term

TABLE 1. Means ($\pm$ standard deviation) of the Setting Time and pH of ProRoot MTA, OrthoMTA, and Endocem MTA given by ISO Standardization

Material	Setting time (min)	pH (day 7)
ProRoot MTA (*n* = 6)	318.0 ($\pm$56.0)	11.90
OrthoMTA (*n* = 6)	324.3 ($\pm$2.1)	11.42
Endocem MTA (*n* = 6)	15.3 ($\pm$0.5)	11.33

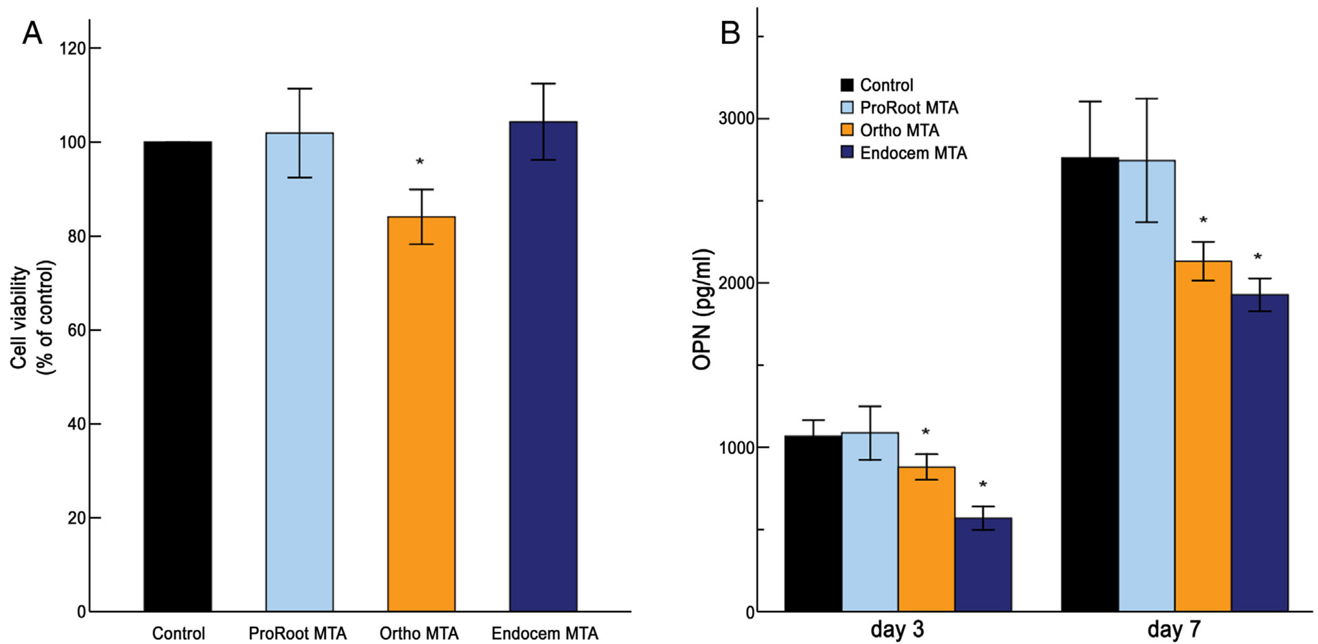

Figure 1. (*A*) MC3T3-E1 cells were incubated with MTA-conditioned media for 48 hours; after which, the viability of the cells was assessed by using the Cell Counting Kit-8 assay. The bar represents the mean ± standard deviation. ($P < .05$ compared with the negative control [unconditioned medium]). OrthoMTA was significantly more cytotoxic than ProRoot MTA or Endocem MTA. (*B*) MC3T3-E1 cells were incubated with ProRoot MTA, OrthoMTA, or Endocem MTA discs, and OPN levels in the medium on days 3 and 7 were measured by using an immunoassay. The bar represents the mean ± standard deviation. ($P < .05$ compared with the control). The Endocem MTA and OrthoMTA groups had significantly lower OPN levels than the control and ProRoot MTA groups at both time points.

clinical success (17). Unfortunately, there is currently no available material that exhibits all of these properties. The present study showed that ProRoot MTA and OrthoMTA exhibited setting times longer than 5 hours. This setting time of ProRoot MTA is not consistent with the observations of Torabinejad et al (2 hours 45 minutes) (18) and is not a clinically acceptable property of filling materials.

Recently, as mentioned earlier, Endocem MTA was developed to overcome this shortcoming. Endocem MTA is the rapidly setting

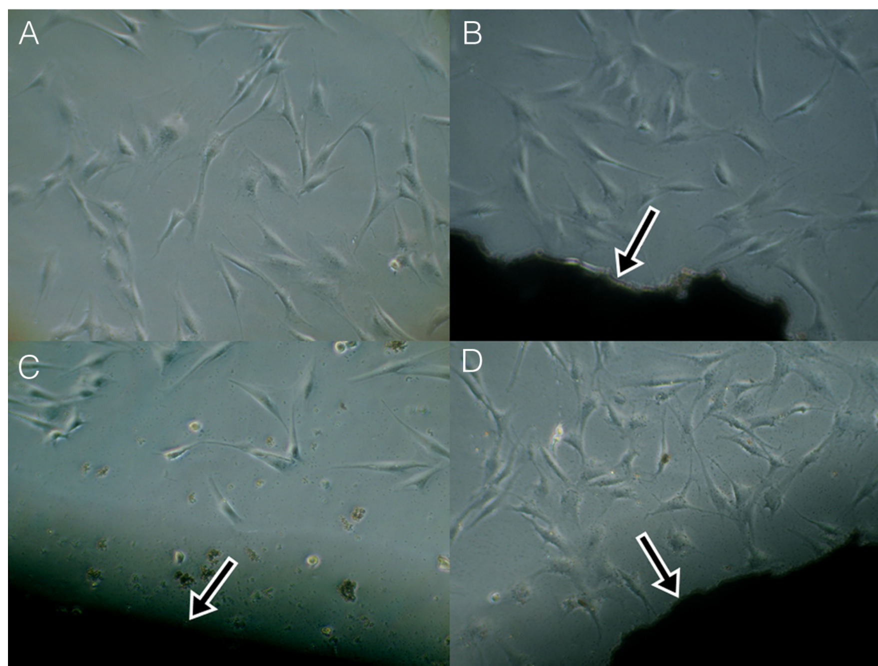

Figure 2. MC3T3-E1 cells were incubated with ProRoot MTA, OrthoMTA, or Endocem MTA discs for 24 hours. Photographs show that the Ortho MTA group had lower cell proliferation than the negative control, ProRoot MTA, and Endocem MTA groups (*A*): negative control, (*B*): ProRoot MTA, (*C*): OrthoMTA, (*D*): Endocem MTA). *Arrows* indicate the test materials (×100 magnification; microscope [Carl Zeiss]).

MTA-like cement that consists of small particles of pozzolan cement (10). In the present study, Endocem MTA showed a much shorter setting time than ProRoot MTA or OrthoMTA (ie, 15 minutes). This setting time provides a sufficient working time when performing retrograde filling or perforation repairs. Our observation is consistent with that of Choi et al (10) who used a similar testing method although the setting time of Endocem MTA in our study was longer than that reported by Choi et al (4 minutes $\pm$ 30 seconds). This study also showed that all 3 CSCs caused the pH of distilled water to markedly increase to alkaline levels upon storage. Because of the highly alkaline pH values of these materials, CSCs can be bactericidal, similar to calcium hydroxide, and may also induce limited necrosis of resorptive cells on the root surface (19).

The cytotoxicity of endodontic cements is of great concern because irritation of the surrounding tissue can delay periapical healing (20). Cell culture studies have been used for more than 40 years to investigate the cytotoxicity of endodontic materials (21). Three studies have shown ProRoot MTA cement to exhibit minimal cytotoxicity (20, 22, 23). The present study also exhibited that OrthoMTA was significantly more cytotoxic than the other 2 CSCs. The cytotoxic effect of OrthoMTA may be caused by the toxicity of the raw material itself, which may denature the adjacent cells and the proteins in the medium. This result is in agreement with that of a previous MTT cell viability assay study (9) and indicates that both ProRoot MTA and Endocem MTA are not toxic to preosteoblastlike MC3T3-E1 cells. Although OrthoMTA was found to be a safe biomaterial when the purity of heavy metals was considered (24, 25), the fact that OrthoMTA was significantly more cytotoxic than the other CSCs in this study suggests that the 3 CSCs may differ in terms of the effect of ions released from the materials.

In addition to the biological properties described previously, another ideal characteristic of an endodontic filling material is that it can induce osteogenesis. The OPN glycophosphoprotein (60 kDa) is secreted by osteoblastic cells and is found in both mineralized and nonmineralized tissues. Given that it strongly inhibits hydroxyapatite formation *in vitro*, OPN is believed to play an important role in modulating apatite crystal growth in bone (26). Moreover, OPN is required for stress-induced bone remodeling (27). In our previous study, ProRoot MTA was found to increase the OPN expression of human dental pulp cells in a time-dependent manner (28).

In the present study, enzyme-linked immunosorbent assay analysis of MC3T3-E1 cells incubated with CSC discs revealed that OPN production was high in both the negative control and ProRoot MTA groups on both days 3 and 7. This finding suggests that the mineralization induced by MC3T3-E1 cells with or without ProRoot MTA may promote the expression of OPN at an early stage (28). Moreover, compared with the negative control, the Endocem MTA and OrthoMTA groups induced significantly lower OPN production. This result is not in accordance with the results of a previous report (9), which showed that OPN production is upregulated by Endocem MTA. Although the duration of this experiment was not long enough to confirm the observations reported in other investigations (29, 30), we did find that the OPN levels on day 7 were higher than those on day 3 in all groups.

In conclusion, the present study confirmed that ProRoot MTA exhibits an excellent property, namely, it can induce OPN production by osteoblastic cells, which would not inhibit osteogenesis. In addition, although Endocem MTA exhibited a clinically acceptable short setting time and lower cytotoxicity for preosteoblastic cells, the biological properties of ProRoot MTA were superior to those of OrthoMTA and Endocem MTA. Further studies that examine the cytotoxicity and hard tissue regenerative potential of CSCs over longer durations are warranted.

Acknowledgments

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The authors deny any conflicts of interest related to this study.

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