# Biological Effects and Washout Resistance of a Newly Developed Fast-setting Pozzolan Cement

*Yoorina Choi, DDS,* Su-Jung Park, DDS, MSD,* Seoung-Hoon Lee, PhD,†‡ Yun-Chan Hwang, DDS, PhD,§‖ Mi-Kyung Yu, DDS, PhD,¶ and Kyung-San Min, DDS, PhD¶*

## Abstract

**Introduction:** Mineral trioxide aggregate has been widely used as a retrograde filling material. The aim of this study was to evaluate the biological effects of a newly developed fast-setting, mineral trioxide aggregate–derived pozzolan cement (Endocem). Furthermore, we explored whether this cement is resistant to washout in comparison with ProRoot. **Methods:** Biocompatibility was evaluated on the basis of cell morphology and a viability test. The expression of osteogenic genes was evaluated by performing real-time polymerase chain reaction, and calcified nodule formation was assessed by alizarin red S staining. The setting time was measured, and washout testing was performed by placing the material into fetal bovine serum. **Results:** The biocompatibility and osteogenicity of Endocem were similar to those of ProRoot. Moreover, Endocem showed a higher resistance to washout than ProRoot did. **Conclusions:** These results suggest that Endocem can be used as an available retrograde filling material because it sets faster and shows similar biological effects when compared with ProRoot. *(J Endod 2013;39:467–472)*

## Key Words

Biocompatibility, fast-setting, mineral trioxide aggregate, osteogenic, pozzolan, washout

From the *Department of Conservative Dentistry and †Department of Oral Microbiology and Immunology, School of Dentistry, and ‡Institute of Biomaterials Implant, Wonkwang University, Iksan, Korea; §Department of Conservative Dentistry, School of Dentistry, and ‖Dental Science Research Institute, Chonnam National University, Gwangju, Korea; and ¶Department of Conservative Dentistry, School of Dentistry, Chonbuk National University, Jeonju, Korea.

Yoorina Choi and Su-Jung Park contributed equally to this work.

Supported by Rising Support Project of Wonju Medical Industry Technovalley Foundation in 2012.

Address requests for reprints to Dr Kyung-San Min, Associate Professor, Department of Conservative Dentistry, Chonbuk National University School of Dentistry, 567 Baekje-daero, deokjin-gu, Jeonju-si, Jeollabuk-do, 561-756 Korea. E-mail address: endomin@gmail.com

0099-2399/$ - see front matter



http://dx.doi.org/10.1016/j.joen.2012.11.023

Endodontic surgery is the preferred approach when conventional orthograde endodontic therapy fails or cannot be performed appropriately. The key to success in endodontic surgery is hermetic sealing of the root-end filling. Therefore, root-end filling materials should have adequate sealing ability (1) and should also be biocompatible with the host tissue, insoluble in tissue fluids, and dimensionally stable (2, 3). Although various materials have been advocated as root-end filling materials such as intermediate restorative material (IRM) and SuperEBA, mineral trioxide aggregate (MTA) is preferred because of its superior sealing ability and biocompatibility (4, 5).

Several studies have shown that MTA is a biocompatible material, because it shows less cytotoxicity than any other restorative materials in various conditions and cell lines (6–9). It has been reported that MTA induces an osteogenic phenotype, as reflected by the up-regulated expression of mineralization-related genes. Therefore, MTA is regarded as a bioactive material that is osteogenic, cementogenic, and odontogenic (10–12).

Some drawbacks of MTA also have been reported, including its long setting time (3). After endodontic surgery, the retrograde-filled MTA that is not set may encounter blood or tissue fluid. Kim et al (13) reported that washout of MTA could be mediated by continuous exudates or tissue fluid. Although MTA has excellent sealing ability, its washout can prevent the complete sealing of the retrograde preparation site of the tooth and thus eventually cause failure (13, 14).

There have been efforts to overcome the long setting time of MTA by using various additives (15–17). Although the setting time of MTA with additives is shorter than that of the original form of MTA, the reported setting time is still too long to reflect clinical significance. Moreover, various studies have shown that adding additives to MTA to accelerate the setting time may have an adverse effect on its physical and biological properties (18, 19).

Recently, an MTA-derived pozzolan cement (Endocem) was introduced. Endocem sets quickly without the addition of a chemical accelerator because it contains small-particle pozzolan cement. However, the biological effects, including biocompatibility and mineralization potential, of Endocem have not been evaluated. Therefore, the purpose of this study was to evaluate the biological effects of Endocem, fast-setting MTA-derived material. Furthermore, we explored whether this cement is resistant to washout when compared with a previously marketed MTA (ProRoot).

## Materials and Methods

### Cell Morphology Analysis by Scanning Electron Microscopy

Under aseptic conditions, ProRoot (Dentsply, Tulsa, OK), Endocem (Maruchi, Wonju, Korea), and IRM (Dentsply-Caulk, Milford, DE) were mixed according to the manufacturers' instructions. Then the materials were condensed into 1-mm × 5-mm round wax molds and allowed to set for 24 hours in a humidified incubator at 37°C. The disks were all placed at the bottom of 24-well tissue culture plates. Then MG63 cells were seeded at $1 \times 10^5$ cells per well on the prepared materials. After a 72-hour incubation period, the dishes were fixed with 2.5% glutaraldehyde (Sigma-Aldrich, St Louis, MO) for 2 hours. The samples were then dehydrated in increasing concentrations of ethanol (70%, 80%, 90%, 95%, and 100%) for 20 minutes at each concentration, immersed in n-butyl alcohol (Junsei Chemical Co, Tokyo, Japan)

for 20 minutes, and dried by using a CPD 030 critical point dryer (BalTec, Balzers, Liechtenstein). Scanning electron microscopy (SEM) was performed by using a JSM-6360 (JEOL, Tokyo, Japan) system operated at 10 kV.

## Preparation of Extracts

ProRoot, Endocem, and IRM were mixed according to the manufacturers' instructions. The mixed cements were placed into a paraffin wax mold (1-mm thickness and 5-mm diameter), and the cements were stored in an incubator at 100% relative humidity and 37°C for 1 day of hydration. The cements were then sterilized in ultraviolet light for 1 hour. One tablet of each cement was stored in 10 mL of Dulbecco modified Eagle medium (DMEM) (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (FBS) (Invitrogen) along with 100 U/mL penicillin and 100 U/mL streptomycin (Invitrogen) for 3 days.

## Cell Viability Test

As determined by hemocytometry, single-cell suspensions of MG63 cells were seeded in 24-well tissue culture plates at a density of $2 \times 10^4$ cells per well in DMEM containing 10% FBS along with 100 U/mL penicillin and 100 U/mL streptomycin and incubated in a humidified atmosphere of air and 5% $CO_2$ at 37°C for 24 hours. Then cells were treated with the prepared extract (experimental groups) or medium only (control group). After exposure to the extract of the materials for 12, 24, 48, and 72 hours, viable cells were detected by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye, which forms blue formazan crystals on reduction by the mitochondrial dehydrogenase present in living cells. Briefly, 200 $\mu$L MTT solution (0.5 mg/mL in phosphate-buffered saline) was added to each well, and the wells were incubated for 2 hours. Subsequently, 200 $\mu$L dimethyl sulfoxide (Amresco, Solon, OH) was added to each well. The plates were then shaken until the crystals had dissolved, and the solution in each well was transferred to a 96-well tissue culture plate. The reduced MTT was then measured spectrophotometrically at 540 nm in a dual-beam microtiter plate reader. The statistical analysis of the data was performed by 1-way analysis of variance (ANOVA) followed by a multiple-comparison Tukey test, with the use of the SPSS program (SPSS 12.0; SPSS GmbH, Munich, Germany). Statistical significance was determined at $P < .05$.

## Real-time Polymerase Chain Reaction Analysis

Cells ($1 \times 10^5$) in DMEM containing 10% serum were seeded in 6-well tissue culture plates and incubated for 24 hours. The medium was then switched to the extract medium for the duration of the experiment. After exposure of the materials to the extract for 7 days, the cells were lysed to extract the total RNA by using Trizol reagent (Invitrogen), according to the manufacturer's instructions. In brief, the cells were lysed directly in the plates by using 1.0 mL Trizol reagent. After chloroform extraction, the total RNA was recovered from the aqueous phase and precipitated by using isopropanol and RNAse-free distilled water. Then reverse transcription of RNA was performed by using the Superscript First-Strand Synthesis kit (Invitrogen).

SYBR Green–based real-time polymerase chain reaction (PCR) was optimized and conducted by using the TOPreal qPCR premix Kit (Enzynomics, Cheongju, Korea). The final PCR mixture contained 2 $\mu$L each of the forward and reverse primers (final concentration of 0.4 $\mu$mol/L for each), 2 $\mu$L SYBR Green (2×), 1.6 $\mu$L $MgCl_2$ (final concentration, 3 mmol/L), and 5 $\mu$L of the template, and the volume was adjusted to 20 $\mu$L by using nuclease-free water. The sequences of the primers are listed in Table 1. All real-time PCR reactions (reactions, experimental samples, and controls) were performed in duplicate and conducted by using the StepOne Real-Time PCR System (Applied Biosystem, Singapore). The following protocol was used: 10 minutes at 95°C, followed by 30 cycles at 94°C for 30 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. After the PCR cycles, a DNA melting curve was generated (0 second at 95°C and 15 seconds at 65°C, with a ramping time of 20°C/s, and 0 second at 95°C, with a ramping time of 0.1°C/s) to discriminate between specific and nonspecific amplification products. One-way ANOVA and Tukey tests were used for statistical analysis ($P = .05$).

**TABLE 1.** Real-time PCR Primers

| Genes | Sequence |
|---|---|
| BSP | Forward: 5′-GCGAAGCAGAAGTGGATGAAA-3′<br>Reverse: 5′-GCTGCCGTTGCCGTTTT-3′ |
| OC | Forward: 5′-CACTCCTCGCCCTATTGGC-3′<br>Reverse: 5′-CCCTCCTGCTTGGACACAAAG-3′ |
| OP | Forward: 5′- GTGATGTCCTCGTCTGTAGCATCA-3′<br>Reverse: 5′- GTAGACACATATGATGGCCGAGG-3′ |
| GAPDH | Forward: 5′-AAGGTGAAGGTCGGAGTCAAC-3′<br>Reverse: 5′-GGGGTCATTGATGGCAACAATA-3′ |

GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

## Alizarin Red S Staining

After the MG63 cells were cultured in the presence of ProRoot, Endocem, or osteogenic medium supplemented with 50 $\mu$g/mL L-ascorbic acid (Sigma-Aldrich), 10 mmol/L $\beta$-glycerolphosphate (Sigma-Aldrich), and 100 nmol/L dexamethasone (Sigma-Aldrich) for 14 days, the cells were fixed in 70% ethanol for 30 minutes and rinsed with distilled water. The cells were stained with 40 mmol/L alizarin red S, at pH 4.2, for 10 minutes with gentle agitation. The cells were then washed with distilled water and allowed to dry. Images of the alizarin red S staining were obtained by using a scanner, and the stain intensity was analyzed by using an image analysis program (Image J; National Institutes of Health, Bethesda, MD). One-way ANOVA and Tukey tests were used for statistical analysis ($P = .05$).

## Measurement of Setting Time

The test materials were mixed according to the manufacturers' instructions. The samples (n = 10) were tested just before their anticipated setting time and at 30-second intervals until they were fully set. A Gilmore apparatus was used with a stainless steel indenter and 1/4-pound indentation force for the initial setting time measurement; a 1-pound indentation force was used for the final setting time. The apparatus was applied at a right angle to the surface of the sample for 5 seconds. The setting time was defined as the time at which the indenter failed to leave a definite mark on the surface of the sample. One-way ANOVA and Tukey tests were used for statistical analysis ($P = .05$).

## Washout Test

ProRoot, Endocem, and IRM were mixed according to the manufacturers' instructions. After mixing, each material was placed into a 5-mm × 2-mm Teflon mold (n = 10). Each mold was then placed into FBS (Invitrogen) immediately as shown in Figure 1*A*. After 24 hours, SEM was performed by using a JSM-6360 system operated at 10 kV. The washout score was evaluated by 3 dentists who had no knowledge about the source of the specimens, according to the criteria listed in Table 2. The Kruskal-Wallis test was used to evaluate the washout test score ($P = .05$).

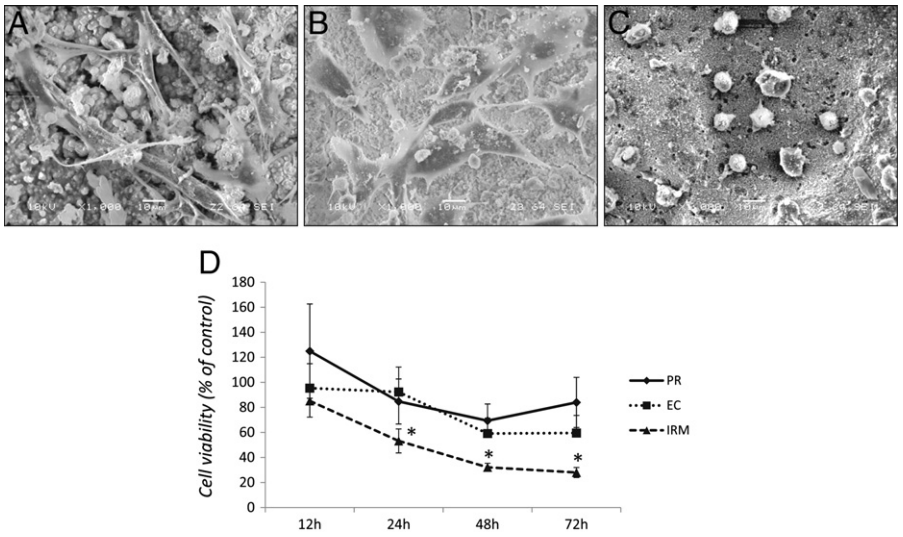


**Figure 1.** SEM observation of cells incubated for 72 hours on (*A*) ProRoot (original magnification, ×1000), (*B*) Endocem (original magnification, ×1000), and (*C*) IRM (original magnification, ×1000). (*D*) Effects of ProRoot (PR), Endocem (EC), and IRM on MG63 cells as measured by the MTT assay. Each point and bar represent the mean ± standard deviation. *Significant difference between each group; $P < .05$.

# Results

## Cell Morphologic Analysis

The cell growth and morphology of each material were evaluated by using SEM observation. As shown in Figure 2*A* and *B*, well-spread and flattened cells were observed in close contact with the surfaces of ProRoot and Endocem. In contrast, a few rounded cells were observed on the IRM surface, and no living cells were observed (Figure 2*C*). In the ProRoot and Endocem groups, many cellular extensions interacted with the surface of the cement and with the adjacent cells.

## Cell Viability Test

To evaluate cell viability in the presence of the material extracts, an MTT assay was performed. As shown in Figure 2*D*, ProRoot and Endocem showed similar cell viability throughout the experimental period ($P > .05$). However, IRM showed lower cell viability after 24 hours when compared with the other groups ($P < .05$).

## Real-time PCR Analysis

To evaluate the expression of mineralization-related markers, real-time PCR analysis was performed. Total RNA isolated from IRM-treated cells did not yield enough products for use with real-time PCR analysis. The expression of bone sialoprotein (BSP) and osteopontin (OP) mRNA increased in ProRoot-treated and Endocem-treated cells when compared with the medium only–treated cells of the control group ($P < .05$). The relative expression of BSP, osteocalcin (OC), and OP mRNA demonstrated no significant difference between the ProRoot-treated and Endocem-treated groups (Fig. 3*A–C*, $P > .05$).

**TABLE 2.** Scoring System for the Washout Test

| Score | Description |
|---|---|
| 0 | No defect |
| 1 | Defect area ranges below 25% of total area |
| 2 | Defect area ranges from 25%–50% of total area |
| 3 | Defect area ranges from 50%–75% of total area |
| 4 | Defect area ranges above 75% of total area |

Defect area is defined as hollow area on the surfaces of materials, not including marginal gaps around or porosities on the surfaces.

## Alizarin Red S Staining

To investigate the effect of ProRoot and Endocem on mineralization, MG63 cells were stained with alizarin red S. After the addition of ProRoot and Endocem, the formation of mineralized nodules in MG63 cells was significantly higher than that in the medium only–treated cells of control group at day 14 (Fig. 3*D*, $P < .05$). Cells cultured in osteogenic medium served as the positive control.

## Setting Time

The initial setting time of the ProRoot was 78 ± 5 minutes, and the final setting time was 261 ± 21 minutes. The initial setting time of the Endocem was 2 minutes ± 30 seconds, and the final setting time was 4 minutes ± 30 seconds. The initial setting time of the IRM was 6 minutes ± 30 seconds, and the final setting time was 10 minutes ± 30 seconds. The setting time of the Endocem was significantly lower than that of the ProRoot and IRM ($P < .05$) (Table 3).

## Washout Test

As shown in Figure 1*B*, the washout score was higher in the ProRoot group than in the Endocem and IRM groups after 24 hours ($P < .05$). Representative images of ProRoot, Endocem, and IRM are shown in Figure 1*C–E*, respectively. Notably, ProRoot was washed out from the Teflon mold, whereas Endocem and IRM were maintained in the mold. However, a remarkable gap was observed between IRM and the mold.

# Discussion

MTA fulfills many of the ideal properties of a root-end filling material, including biocompatibility and sealing ability (4). However, the long setting time, which is one of the major drawbacks of MTA, is still problematic for clinical applications of MTA. Notably, when the MTA is used as a retrograde filling material, MTA that was not set can interact with the tissue fluid until it sets completely. Tingey et al (20) and Nekoofar et al (21) reported that the physical properties of MTA set in tissue

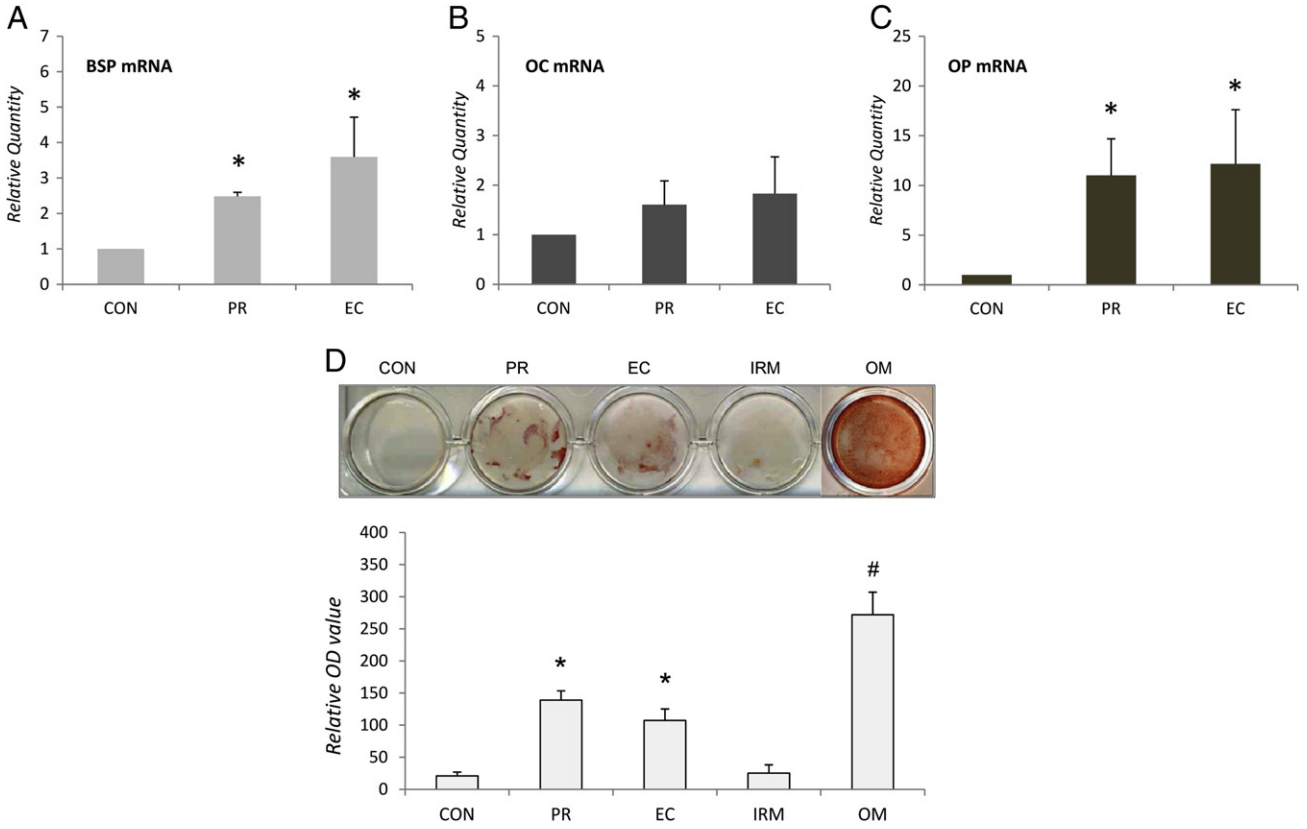


**Figure 2.** Effect of ProRoot (PR) and Endocem (EC) on expression of BSP (*A*), OC (*B*), and OP (*C*) mRNAs in MG63 cells cultured for 7 days. The relative mRNA levels were calculated by dividing the absolute levels of expression of BSP, OC, and OP mRNAs by the absolute level of expression of glyceraldehyde-3-phosphate dehydrogenase mRNA. *Significant difference among groups; $P < .05$. (*D*) Effects of PR and EC on the formation of calcified nodules in MG63 cells. Osteogenic medium (OM)-treated cells served as the positive control. The cells were cultured with MTA and OM for 14 days and stained with alizarin red S. A representative photograph of alizarin red staining is shown. Alizarin red S stain intensity was analyzed by performing densitometry. Groups identified by the same superscript symbols were not significantly different in the same gene group ($P > .05$). CON, control.

fluid, blood, or low pH conditions, which mimic clinical situations such as endodontic surgery, are different from those in moisture, which is the recommended setting condition. Furthermore, Kim et al (22) reported that MTA failed to set in the presence of FBS. There also have been reports that the washout of MTA can occur when MTA is used as a retrograde filling material (13, 14, 22). Initial looseness of MTA, ie, it is not

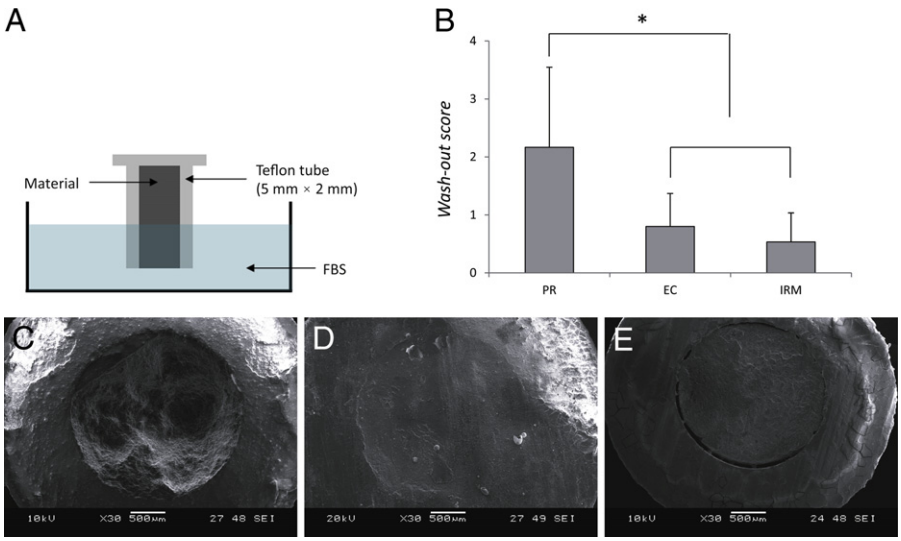


**Figure 3.** (*A*) Schematic illustration of the model used for the experiment. (*B*) Washout score of ProRoot (PR), Endocem (EC), and IRM. Each point and bar represent the mean ± standard deviation. *Significant difference among groups; $P < .05$. SEM observation indicating the washout tendency and marginal gap of PR (*C*), EC (*D*), and IRM (*E*).

**TABLE 3.** Means and Standard Deviations of Initial and Final Setting Times for Tested Materials

| | Initial setting time (min) | Final setting time (min) |
|---|---|---|
| ProRoot | 78 ± 5 | 261 ± 21 |
| Endocem | 2 ± 30 seconds* | 4 ± 30 seconds* |
| IRM | 6 ± 30 seconds# | 10 ± 30 seconds# |

Groups identified by the same superscript symbols were not significantly different in the same gene group ($P > .05$).

set or weakened physical condition, occurs in the presence of tissue fluid or blood flow and may threaten the hermetic sealing of communicating channels between the root canal system and the periradicular tissues, thereby resulting in the failure of endodontic surgery. This washout can be minimized if the root-end filling material sets quickly before it is exposed to blood or tissue fluid (17, 21, 23). In this respect, a short setting time in addition to biocompatibility and sealing ability is necessary for an ideal root-end filling material (17, 24).

The development of fast-setting MTA has been attempted by many researchers (14–17, 24). However, most of these approaches were based on the addition of chemical setting accelerators, some of which showed adverse physical and biological effects (1, 15, 16). Recently, Endocem, a fast-setting MTA-derived cement, was developed by using small particles of pozzolan cement without any chemical accelerators. A pozzolan is a siliceous or siliceous and aluminous material that possesses little or no cementitious value in itself but in finely divided form and in the presence of water will react chemically with calcium hydroxide at ordinary temperature to form compounds possessing cementitious properties (25). The chemical composition of Endocem is very similar to that of MTA, assembly of trioxide compound. Bismuth oxide ($Bi_2O_3$) is also added as a radiopacifier. The composition provided by the manufacturer, expressed as wt%, was 46.7 CaO, 5.43 $Al_2O_3$, 12.80 $SiO_2$, 3.03 MgO, 2.32 $Fe_2O_3$, 2.36 $SO_3$, 0.21 $TiO_2$, 14.5 $H_2O/CO_2$, and 11.0 $Bi_2O_3$. The pozzolanic reaction progresses like an acid-base reaction of lime and alkalies with oxides ($SiO_2$ + $A1_2O_3$ + $Fe_2O_3$) of the pozzolan (26). Two things happen. First, there is a gradual decrease in the amount of free calcium hydroxide with time. Calcium hydroxide has bad effects on mechanical and durability properties of material. Second, during this reaction there is an increase in formation of calcium silicate hydrate and calcium aluminate hydrate, stable crystals that are so effective on the strength of material (26). Furthermore, the use of small particles increases the surface contact of the particles with the mixing liquid and provides rapid setting and ease of handling (3, 27, 28). In the present study, Endocem demonstrated a much shorter setting time than ProRoot. The initial setting time of Endocem was 2 minutes ± 30 seconds, and the final setting time was 4 minutes ± 30 seconds, whereas the initial setting time of ProRoot was 78 ± 5 minutes, and the final setting time was 261 ± 21 minutes. The obtained value is similar to the value of 3 minutes and 15 seconds reported as the final setting time by the manufacturer's data sheet.

The cytotoxicity of root-end filling materials is of great concern because damage or irritation of the periapical tissue can delay wound healing (29, 30). In this study, SEM revealed that MG63 cells cultured on ProRoot and Endocem for 3 days appeared to be flat and exhibited well-defined cytoplasmic extensions that projected from the cells to the surrounding surface or adjacent cells, in contrast to cells cultured on IRM (Fig. 2*A–C*). Min et al (31) stated that cell attachment with multiple cytoplasmic extensions implies cell growth, proliferation, and differentiation. Furthermore, ProRoot and Endocem demonstrated significantly lower cytotoxicity than IRM in the MTT assay throughout the experimental period (Fig. 2*D*). These results indicate that Endocem permitted cell attachment and growth to a degree similar to that of ProRoot.

In addition to biocompatibility, osteogenic induction is regarded as an ideal characteristic for a retrograde filling material. Healing after endodontic surgery necessitates osseous repair of the medullary and cortical bone. It has been recognized that MTA stimulates the regeneration of osseous tissues surrounding the root end (4, 5, 7). In the current study, osteogenic differentiation markers including BSP, OC, and OP were examined, and alizarin red S staining was performed to evaluate the effects of the MTA-derived pozzolan cement on the formation of a mineralized matrix *in vitro*. Alizarin red S staining has been used for decades to evaluate calcium deposition by cells in culture (32). BSP is a major noncollagenous protein in mineralized connective tissue. BSP expression is highly specific for mineralizing tissues, including bone, mineralizing cartilage, dentin, and cementum (33). OC appears immediately before the start of mineralization and is a major noncollagenous protein synthesized by osteoblasts, odontoblasts, and cementoblasts. OP is believed to play a crucial role in modulating apatite crystal growth in bone (34). In this study, the relative quantity of BSP, OC, and OP mRNA was not significantly different between the ProRoot-treated and Endocem-treated groups (Fig. 3*A–C*). In alizarin red S staining, the extent of mineralization in cells treated with Endocem was similar to that in the cells treated with ProRoot and was significantly higher than that in the control and IRM groups (Fig. 3*D*). Collectively, these results indicate that the mineralization potential of Endocem is comparable to that of ProRoot.

To explore whether this fast-setting cement is resistant to washout when compared with the previously marketed MTA, a washout test was performed. FBS was used as the medium to replicate the clinical situation because of its biosafety and availability and because its biochemical profile is similar to that of human serum (20, 21). The degree of washout for ProRoot was significantly higher than that for Endocem (Fig. 1*B*). It is likely that the rapid setting of Endocem enhances its washout resistance (17). Most IRM samples showed a marginal gap between the materials and molds, whereas ProRoot and Endocem showed tight sealing between the material and the mold (Fig. 1*C–E*). This finding was in agreement with that of Torabinejad et al (34), who observed that the degree of marginal gaps was significantly higher for IRM than for MTA. On the basis of these results, Endocem can be concluded to have beneficial characteristics including "anti-washout" characteristic in tissue fluid–like medium and a lack of surrounding marginal gaps. The short setting time of Endocem may thus help to overcome the unfavorable clinical environment in endodontic surgery.

In this study, Endocem exhibited a biological effect similar to that of ProRoot. Thus, Endocem may be a substitute for ProRoot as an available retrograde filling material because of its short setting time and resistance to washout. However, long-term evaluation regarding the carcinogenicity or genotoxicity of Endocem should be performed.

## Acknowledgments

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

## References

1. Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review—part II: leakage and biocompatibility investigations. J Endod 2010;36:190–202.
2. Torabinejad M, Pitt Ford TR. Root end filling materials: a review. Endod Dent Traumatol 1996;12:161–78.
3. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review—part I: chemical, physical, and antibacterial properties. J Endod 2010;36:16–27.

4. Torabinejad M, Higa RK, McKendry DJ, Pitt Ford TR. Dye leakage of four root end filling materials: effects of blood contamination. J Endod 1994;20:159–63.
5. Economides N, Pantelidou O, Kokkas A, Tziafas D. Short-term periradicular tissue response to mineral trioxide aggregate (MTA) as root-end filling material. Int Endod J 2003;36:44–8.
6. Keiser K, Johnson CC, Tipton DA. Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts. J Endod 2000;26:288–91.
7. Osorio RM, Hefti A, Vertucci FJ, Shawley AL. Cytotoxicity of endodontic materials. J Endod 1998;24:91–6.
8. Ribeiro DA, Sugui MM, Matsumoto MA, et al. Genotoxicity and cytotoxicity of mineral trioxide aggregate and regular and white Portland cements on Chinese hamster ovary (CHO) cells in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:258–61.
9. Karimjee CK, Koka S, Rallis DM, Gound TG. Cellular toxicity of mineral trioxide aggregate mixed with an alternative delivery vehicle. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:e115–20.
10. Torabinejad M, Hong CU, Lee SJ, et al. Investigation of mineral trioxide aggregate for root-end filling in dogs. J Endod 1995;21:603–8.
11. Camilleri J, Pitt Ford TR. Mineral trioxide aggregate: a review of the constituents and biological properties of the material. Int Endod J 2006;39:747–54.
12. Thomson TS, Berry JE, Somerman MJ, Kirkwood KL. Cementoblasts maintain expression of osteocalcin in the presence of mineral trioxide aggregate. J Endod 2003;29:407–12.
13. Kim Y, Kim S, Shin YS, et al. Failure of setting of mineral trioxide aggregate in the presence of fetal bovine serum and its prevention. J Endod 2012;38:536–40.
14. Lee SJ, Cho OI, Yum J, et al. Physical properties of novel composite using Portland cement for retro-filling material. J Kor Acad Cons Dent 2010;35:445–52.
15. Kogan P, He J, Glickman GN, Watanabe I. The effects of various additives on setting properties of MTA. J Endod 2006;32:569–72.
16. Ber BS, Hatton JF, Stewart GP. Chemical modification of ProRoot MTA to improve handling characteristics and decrease setting time. J Endod 2007;33:1231–4.
17. AlAnezi AZ, Zhu Q, Wang YH, et al. Effect of selected accelerants on setting time and biocompatibility of mineral trioxide aggregate (MTA). Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:122–7.
18. Lee BN, Hwang YC, Jang JH, et al. Improvement of the properties of mineral trioxide aggregate by mixing with hydration accelerators. J Endod 2011;37:1433–6.
19. Camilleri J, Montesin FE, Di Silvio L, Pitt Ford TR. The chemical constitution and biocompatibility of accelerated Portland cement for endodontic use. Int Endod J 2005;38:834–42.
20. Tingey MC, Bush P, Levine MS. Analysis of mineral trioxide aggregate surface when set in the presence of fetal bovine serum. J Endod 2008;34:45–9.
21. Nekoofar MH, Stone DF, Dummer PM. The effect of blood contamination on the compressive strength and surface microstructure of mineral trioxide aggregate. Int Endod J 2010;43:782–91.
22. Kim Y, Lee CY, Kim E, Jung IL. Failure of orthograde MTA filling: MTA wash-out? J Kor Acad Cons Dent 2011;36:510–4.
23. Rhim EM, Huh SY, Ahn SJ, et al. Comparison of the microhardness and morphology of five different retrograde filling materials in aqueous and dry conditions. Scanning 2012;34:359–66.
24. Hsieh SC, Teng NC, Lin YC, et al. A novel accelerator for improving the handling properties of dental filling materials. J Endod 2009;35:1292–5.
25. American Concrete Institute (ACI). Cement and Concrete Terminology, ACI 116R. Farmington Hills, MI: American Concrete Institute; 2000.
26. Askarinejad A, Pourkhorshidi AR, Parhizkar T. Evaluation the pozzolanic reactivity of sonochemically fabricated nano natural pozzolan. Ultrason Sonochem 2012;19: 119–24.
27. Lin F, Meyer C. Hydration kinetics modeling of Portland cement considering the effects of curing temperature and applied pressure. Cement Concr Res 2009;39: 255–65.
28. Byun SH, Kim HC, Kim JY, et al. Effect of cement particle size on properties of ordinary Portland cement. J Kor Ceram Soc 2010;47:394–400.
29. Damas BA, Wheater MA, Bringas JS, Hoen MM. Cytotoxicity comparison of mineral trioxide aggregates and EndoSequence bioceramic root repair materials. J Endod 2011;37:372–5.
30. De Deus G, Ximenes R, Gurgel-Filho ED, et al. Cytotoxicity of MTA and Portland cement on human ECV 304 endothelial cells. Int Endod J 2005;38: 604–9.
31. Min KS, Kim HI, Park HJ, et al. Human pulp cells response to Portland cement in vitro. J Endod 2007;33:163–6.
32. Lee SY, Min KS, Choi GW, et al. Effects of simvastain and enamel matrix derivative on Portland cement with bismuth oxide-induced growth and odontoblastic differentiation in human dental pulp cells. J Endod 2012;38:405–10.
33. Min KS, Kwon YY, Lee HJ, et al. Effects of proinflammatory cytokines on the expression of mineralization markers and heme oxygenase-1 in human pulp cells. J Endod 2006;32:39–43.
34. Torabinejad M, Smith PW, Kettering JD, Pitt Ford TR. Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root-end filling materials. J Endod 1995;21:295–9.