Odontogenic Effect of a Fast-setting Pozzolan-based Pulp Capping Material

Su-Jung Park, DDS, PhD, Seok-Mo Heo, DDS, PhD,† Sung-Ok Hong, DDS, MSD,* Yun-Chan Hwang, DDS, PhD,‖ Kwang-Won Lee, DDS, PhD,‡ and Kyung-San Min, DDS, PhD‡§*

Abstract

Introduction: Mineral trioxide aggregate (MTA) is widely used as a pulp capping material. Recently, a MTA-derived fast-setting pozzolan cement (Endocem; Maruchi, Wonju, Korea) was introduced in the endodontic field. Our aim in this study was to investigate the odontogenic effects of this cement *in vitro* and *in vivo*. **Methods:** Human dental pulp cells (hDPCs) were cultured, and the effects of Endocem and a previously marketed MTA (ProRoot; Dentsply, Tulsa, OK) on biocompatibility were evaluated by assessing cell morphology and performing a cell viability test. Chemical composition of each material was analyzed by energy-dispersive X-ray spectroscopic analysis. Odontoblastic differentiation was analyzed by alkaline phosphatase activity and alizarin red S staining. The expression of odontogenic-related markers, namely dentin sialophosphoprotein, dentin matrix protein 1, and osteonectin, was evaluated by real-time polymerase chain reaction, Western blotting, and immunofluorescence analysis. Pinpoint pulp exposures were made on rat teeth and then capped with ProRoot or Endocem. After 4 weeks, reparative tertiary dentin formation and inflammatory responses were investigated histologically. **Results:** The biocompatibility of Endocem was similar to that of ProRoot. Energy-dispersive X-ray spectroscopic analysis showed that ProRoot and Endocem contained similar elemental constituents such as calcium, oxygen, and silicon. Alkaline phosphatase activity and mineralized nodule formation increased in ProRoot- and Endocem-treated cells compared with medium only–treated cells in the control group ($P < .05$). The expression of odontogenic-related markers was significantly higher in the ProRoot- and Endocem-treated groups than the control group ($P < .05$), but there was no significant difference in the expression of these markers between the 2 experimental groups ($P > .05$). Four weeks after the pulp capping procedure, continuous tertiary dentin had formed directly underneath the capping materials and the pulp exposure area in all samples in the 2 treated groups. Furthermore, most specimens either had no inflammation or minor pulpal inflammation. **Conclusions:** Our results indicate that ProRoot and Endocem have similar biocompatibility and odontogenic effects. Therefore, Endocem is as effective a pulp capping material as ProRoot. *(J Endod 2014;40:1124–1131)*

Key Words

Dental pulp, fast setting, mineral trioxide aggregate, odontogenic, pozzolan, tertiary dentin

Pulp capping, which involves sealing the pulp to stimulate the formation of tertiary dentin, is indicated to treat reversible pulpal injuries caused by physical or mechanical trauma. Mineral trioxide aggregate (MTA) has been successfully used for pulp capping in both experimental and clinical settings (1–5). However, it requires a long setting time; therefore, clinicians usually apply wet cotton to MTA and then make another appointment for further treatment to allow the MTA sufficient time to set. Numerous attempts have been made to shorten the setting time of MTA by adding chemical accelerators to MTA (6–9). However, there are 2 related problems with this approach. First, although these approaches did decrease the setting time, it was still too long to offer a significant clinical advantage. Second, additives might have an adverse effect on the physical and biological properties of MTA (9–11).

An MTA-derived pozzolan cement (Endocem; Maruchi, Wonju, Korea) that sets quickly has recently been marketed to endodontists. Choi et al (12) reported that Endocem has a much shorter setting time (around 4 minutes) and higher washout resistance than the previously marketed MTA brand (ProRoot; Dentsply, Tulsa, OK). They also showed that it had similar biocompatibility and mineralization potential as ProRoot in MG63 cells. However, they conducted their studies using a root-end surgery model, not a pulp capping model. No previous study has examined the odontogenic effects of Endocem versus ProRoot. Therefore, we investigated the odontogenic effects of these 2 cement types both *in vitro* and *in vivo* by examining their effects on odontoblastic differentiation of human dental pulp cells (hDPCs) and assessing tertiary dentin formation on capped rat teeth, respectively. Our 2 null hypotheses were as follows: (1) ProRoot and Endocem induce odontoblastic differentiation of hDPCs to a similar extent and (2) there is no difference between these 2 materials with respect to tertiary dentin formation or pulpal inflammation in experimentally exposed rat teeth.

From the *Department of Conservative Dentistry, School of Dentistry, Wonkwang University, Iksan, Korea; Departments of †Periodontology and ‡Conservative Dentistry, School of Dentistry, Chonbuk National University and Institute of Oral Bioscience, Jeonju, Korea; §Research Institute of Clinical Medicine of Chonbuk National University-Biomedical Research Institute of Chonbuk National University Hospital, Jeonju, Korea; and ‖Department of Conservative Dentistry, School of Dentistry, Chonnam National University, Gwangju, Korea.

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

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2014.01.004

Materials and Methods

Primary Culture of hDPCs

Human dental pulp tissue was obtained from a freshly extracted human third molar. Pulp tissue was cut into fragments and cultured in minimal essential medium alpha (MEM-α; Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (FBS, Invitrogen) along with 100 U/mL penicillin and 100 U/mL streptomycin (Invitrogen). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2. Cell cultures between the fourth and sixth passages were used. All experimental procedures were approved by the Institutional Review Board (IRB#: 2013-02-010-003) of Chonbuk National University Hospital, Jeonju, Korea.

Preparation of Material Extracts

ProRoot and Endocem were mixed according to the manufacturers' instructions. Mixed cement was placed into a paraffin wax mold (1-mm thickness and 5-mm diameter). Then, the cement was stored in an incubator at 95% ± 5% relative humidity at 37°C for 1 day. One tablet of each cement was stored in 10 mL MEM-α containing 10% FBS for 3 days to produce the extracts used for the treatment of hDPCs.

Cell Viability Test

Cells were seeded in 24-well culture plates at a density of 2×10^4 cells per well and preincubated in growth medium for 24 hours. Then, cells were treated with the prepared extracts (experimental groups) or medium only (control group). After exposure to the material extracts for 1, 2, 3, and 7 days, cell viability was examined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, 200 μL MTT solution (0.5 mg/mL in phosphate-buffered saline [PBS], Invitrogen) was added to each well followed by a 2-hour incubation. Then, 200 μL 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. Spectrophotometric absorbance was measured at 540 nm using an enzyme-linked immunosorbent assay plate reader (Synergy 2; Bio-Tek, Winooski, VT). Statistical analysis was performed by 1-way analysis of variance (ANOVA) followed by the multiple-comparison Tukey test ($P = .05$).

Measurement of pH

Specimens (1-mm thickness and 5-mm diameter) of ProRoot or Endocem were prepared and allowed to set for 1 day. After setting, 1 tablet was placed in 10 mL deionized water. The pH value of the water was then measured using a pH meter (Orion 3 Star; Thermo Scientific, Singapore). Before measurement, the pH apparatus was calibrated with solutions with pH levels of 7.0 and 4.0. Between each measurement, the electrode was washed with ultrapure water and blot dried. Data were then analyzed by an independent samples *t* test to compare the 2 materials ($P = .05$).

Scanning Electron Microscopic Analysis

Materials were condensed into 1 × 5-mm round wax molds under aseptic conditions. The materials were allowed to set for 24 hours in a humidified incubator at 37°C. Next, the disks were placed at the bottom of 24-well tissue culture plates. Cells were seeded at 1×10^5 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. Samples were then dehydrated in increasing concentrations of ethanol (70%, 80%, 90%, 95%, and 100%) for 20 minutes at each concentration and immersed in n-butyl alcohol (Junsei Chemical Co, Tokyo, Japan) for 20 minutes. Scanning electron microscopy was performed using the SN-3000 system (Hitachi, Tokyo, Japan) operated at 10 kV.

Energy-dispersive X-ray Spectroscopic Analysis

Energy-dispersive X-ray spectroscopic (EDS) analysis was executed using the Apollo-X detector (EDAX, Mahwah, NJ), which was attached to a scanning electron microscope, for chemical element analysis of the surface of ProRoot and Endocem. The high magnification of ×10,000 was selected to discern the chemical compositions of specific crystal types within a sample. Via this process, a spectrum was obtained, and elements could be identified. Semiquantitative, standardless analyses of these spectra were performed to derive the atomic percent concentrations of constituent elements.

Alkaline Phosphatase Activity

Cells (5×10^4) were inoculated in 6-well culture plates and preincubated in medium for 24 hours. After hDPCs were incubated for 1, 2, and 3 days in the presence of material extract, cells were scraped into cold PBS and then sonicated with a cell disruptor (Heat System Ultrasonics, Plainview, NJ) in an ice-cold bath. Alkaline phosphatase (ALP) activity in the supernatant was determined using the method reported by Lowry et al (13) using p-nitrophenyl phosphate as a substrate. Absorbance was measured at 410 nm using an enzyme-linked immunosorbent assay reader (Beckman DU-650; Beckman Coulter, Fullerton, CA). One-way ANOVA and Tukey tests were used for statistical analyses ($P = .05$).

Alizarin Red S Staining to Detect Mineralized Nodule Formation

Cells were placed in a 24-well plate at a density of 2×10^4 cells per well and cultured for 24 hours for initial attachment. After exposure to the extract medium (experiment group) or medium only (control group) for 14 days, mineralization was assessed by staining with alizarin red S (Sigma-Aldrich). In brief, cells were fixed with 4% formalin for 1 hour at 4°C, washed 3 times with distilled water, and then stained with 40 mmol/L alizarin red (pH = 4.2) solution. After being washed with deionized water, the stained cell culture plate was moved to a scanner, and the stained image was acquired. For quantitative evaluation, the sample was reacted with 10% cetylpyridinium chloride (pH = 7.0) solution at room temperature for 15 minutes to dissolve the stain, and absorbance was measured at a wavelength of 540 nm with a standard solution. One-way ANOVA and Tukey tests were used for statistical analyses ($P = .05$).

Real-time Polymerase Chain Reaction Analysis

Cells (2×10^5) in MEM-α containing 10% FBS were seeded in 6-well tissue culture plates and incubated for 24 hours. The medium was then switched to the extract medium. After exposure to the extract medium for 3 days, cells were lysed, and total RNA was isolated using Trizol reagent (Invitrogen). After chloroform extraction, total RNA was recovered from the aqueous phase and precipitated using 75% isopropanol and RNase-free distilled water (USB, Cleveland, OH). Then, reverse transcription of RNA was performed using the Superscript First-Strand Synthesis Kit (Invitrogen).

SYBR green-based real-time polymerase chain reaction (PCR) was conducted and optimized using the TOPreal qPCR Premix Kit (Enzynomics, Cheongju, Korea). The final PCR mixture contained 2 μL each of forward and reverse primers (final concentration of 0.4 μmol/L for each), 2 μL SYBR green (2×), 1.6 μL $MgCl_2$ (final