Effects of 3 Endodontic Bioactive Cements on Osteogenic Differentiation in Mesenchymal Stem Cells

Bin-Na Lee, DDS, MSD, PhD,[*†] *Kkot-Nim Lee, DDS, MSD, PhD,*[‡] *Jeong-Tae Koh, DDS, MSD, PhD,*[†‡] *Kyung-San Min, DDS, MSD, PhD,*[§] *Hoon-Sang Chang, DDS, MSD, PhD,*[*] *In-Nam Hwang, DDS, MSD, PhD,*[*] *Yun-Chan Hwang, DDS, MSD, PhD,*[*†] *and Won-Mann Oh, DDS, MSD, PhD*[*]

Abstract

Introduction: Because a root-end filling material comes into contact with the surrounding cells or tissues, understanding the cell-material interfacial activity is important. Thus, the purpose of this study was to assess the biocompatibility of 3 endodontic bioactive cements (MTA [Dentsply, Tulsa, OK], Bioaggregate [BA; Innovative Bioceramix, Vancouver, BC, Canada], and Biodentine [BD; Septodont, St Maur des Fosses, France]) and to investigate the effect of cements on the differentiation of mesenchymal stem cells. **Methods:** Cell viability, mineralization, and differentiation were evaluated using an 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) assay and alkaline phosphatase (ALP) staining. The expressions of ALP, osteocalcin, and bone sialoprotein at the gene level were detected by reverse-transcription polymerase chain reaction and real-time polymerase chain reaction. **Results:** Cell viability of BD in concentrations of 1, 1/2, and 1/4 was significantly lower than MTA and BA ($P < .05$). There was no statistically significant difference in cell viability between materials in concentrations of 1/10 and 1/50 ($P < .05$). The messenger RNA level of osteogenic genes increased significantly in the MTA and BA groups compared with controls ($P < .05$). However, although the messenger RNA level of osteogenic genes increased in the BD group, there was no statistically significant difference compared with controls. MTA, BA, and BD led to an increase in ALP staining compared with controls. **Conclusions:** In conclusion, MTA, BA, and BD have effects on osteoblast differentiation in mesenchymal stem cells, suggesting that these cements may be useful for root-end filling material. *(J Endod 2014;40:1217–1222)*

Key Words

Bioaggregate, Biodentine, mineral trioxide aggregate

Periradicular surgery is usually performed in the presence of persistent periradicular pathosis or when orthograde endodontic treatment is considered unfeasible or contraindicated. Because most endodontic failures are attributable to inadequate cleaning and egress of antigens into periradicular tissues, a number of investigators have recommended the placement of root-end filling in the roots of almost all teeth that require root-end resection (1). To achieve satisfactory periradicular surgery, an ideal root-end filling material should satisfy the following requirements: biocompatibility with normal tissues, high sealing ability, desirable ability of periapical tissue regeneration, effective inhibition of pathogenic microorganisms, sufficient radiopacity to distinguish the material from surrounding tissue, and excellent workability and handling properties (2–5). Materials that have been advocated for use as root-end filling materials include amalgam, composite resin, reinforced zinc oxide–eugenol cements (IRM; Dentsply, Tulsa, OK), super ethoxybenzoic acid (Super-EBA; Bosworth Co, Skokie, IL) cements, and glass ionomer cements (6).

Mineral trioxide aggregate (MTA) (Dentsply) is a powder consisting of fine hydrophilic particles of tricalcium silicate, tricalcium aluminate, tricalcium oxide, silicate oxide, and other mineral oxides, which set in the presence of moisture. The hydration of powder results in a colloidal gel that solidifies to a hard structure within approximately 4 hours. MTA has a pH of 12.5 after setting, which is similar to calcium hydroxide (7, 8). MTA is a material that has been used worldwide, with several clinical applications such as apical barriers in teeth with immature apices, repair of root perforations, root-end filling, direct pulp capping, and pulpotomy (9). Compared with other root-end filling materials, MTA has significant advantages in terms of its biocompatibility and osteoconduction ability (2, 10, 11).

Bioaggregate (BA; Innovative Bioceramix, Vancouver, BC, Canada), a white nanoparticle ceramic cement, is a novel root-end filling material composed primarily of calcium silicate, calcium hydroxide, and hydroxyapatite (12). BA has biocompatibility and antibacterial effects similar to those of MTA and is a possible alternative to MTA (13–15).

Based on the outstanding biological properties of MTA, a new calcium silicate–based cement called Biodentine (BD; Septodont, St Maur des Fosses, France) has been developed. The powder is mainly composed of tricalcium silicate, calcium carbonate, and zirconium oxide. The liquid contains water, calcium chloride (used as a setting accelerator), and a modified polycarboxylate (a super-plasticizing agent). A single dose of liquid is dropped in a disposable cap containing powder and then mixed with an amalgamator for 30 seconds. The cement can be applied directly in the restorative cavity

From the Departments of *Conservative Dentistry and ‡Pharmacology and Dental Therapeutics, School of Dentistry, Dental Science Research Institute, Chonnam National University, Gwangju, Korea; †Research Center for Biomineralization Disorders, Chonnam National University, Gwangu, Korea; and §Department of Conservative Dentistry, School of Dentistry, Chonbuk National University, Jeonju, Korea.

Address requests for reprints to Dr Yun-Chan Hwang or Dr Won-Mann Oh, Department of Conservative Dentistry, School of Dentistry, Chonnam National University, Young-bong ro 77, Bukgu, Gwangju, Korea. E-mail addresses: ychwang@chonnam.ac.kr or wmoh@chonnam.ac.kr

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2014.01.036

with a spatula and a plugger as a bulk dentin substrate without any conditioning pretreatment (16). As part of the chemical setting reaction of BD, calcium hydroxide is formed. Investigating its interactions with the pulp cells showed its biocompatibility and its ability to induce odontoblast differentiation and mineralization in cultured pulp cells (17, 18).

Because a root-end filling material comes into contact with the surrounding cells or tissues, understanding the cell-material interfacial activity is important. For this reason, many studies have focused on the biocompatibility of root-end filling materials with cells, the biological behavior of cells in the presence of root-end filling materials, and the influence of these materials in terms of related cellular signal pathways. Interactions between root-end filling materials and injured periradicular tissue in the initiation and the development of wound healing and regenerative processes remain unclear. Thus, the purpose of this study was to assess the biocompatibility of 3 endodontic bioactive cements and to investigate the effect of cements on the differentiation of mesenchymal stem cells.

Materials and Methods

Cell Culture

The C3H10T1/2 cells were cultured in Dulbecco modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (FBS, Invitrogen) and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin, Invitrogen). The cells were cultured until subconfluence at 37°C in a humidified atmosphere containing 5% CO_2.

To induce osteogenic differentiation, the cells were incubated in a medium containing DMEM supplemented with 2% FBS, antibiotics, 50 μg/mL ascorbic acid, and 5 mmol/L β-glycerophosphate, and the medium was termed "differentiation medium."

Preparation of Material Extracts

MTA, BA, and BD were mixed following the manufacturers' instructions under aseptic conditions, and discs were prepared by using a sterile cylindrical polyethylene tube (10-mm diameter and 3-mm height). To obtain the complete setting, discs were kept for 6 hours at 37°C and 95% relative humidity. After setting, discs were demolded and exposed to ultraviolet light for 1 hour on each surface to ensure sterility and transferred into 24-well culture plates. Discs were incubated in 1.5 mL DMEM containing 2% or 10% FBS and antibiotics at 37°C in a humidified atmosphere containing 5% CO_2 for 24 hours. The supernatant collected was referred to as "material extract." All material extracts were sterile filtered using 0.20-μm filters (Minisart; Sartorius Stedim Biotech, Goettingen, Germany).

Cell Viability Assay

The C3H10T1/2 cells were seeded in 96-well culture plates at a density of 1×10^4 cells per well and preincubated in a culture medium of DMEM containing 10% FBS and antibiotics for 24 hours to achieve attachment of the cells before adding the material extracts. The medium was changed to the material extracts of the experimental groups and incubated for 5 days. To observe a dose-response relationship, the material extracts were serially diluted with culture medium to achieve a total of 5 concentrations (1, 1/2, 1/4, 1/10, and 1/50).

Cell viability was examined using an XTT assay (Ez-Cytox Enhanced Cell Viability Assay Kit; Daeil Lab Service Co, Seoul, Korea) according to the manufacturer's recommendations. Briefly, 10 μL Ez-Cytox (tetrazolium salts) was added to the medium, and the cells were incubated at 37°C for 3 hours. The absorbance was measured at 420 nm with a background subtraction of 650 nm using a spectrophotometer (VERSAmax Multiplate Reader; Molecular Devices, Sunnyvale, CA).

Reverse-transcription Polymerase Chain Reaction and Quantitative Real-time Polymerase Chain Reaction

The C3H10T1/2 cells were seeded in 6-well culture plates at a density of 2×10^5 cells per well and preincubated in a culture medium for 24 hours. The culture medium was changed to a differentiation medium containing 1/10 concentration of material extracts for 24, 48, and 72 hours. The untreated C3H10T1/2 cells were used as the control. The total RNA was isolated from the cultures using a TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Complementary DNA was synthesized from the Maxime RT PreMix Kit (iNtRON Biotechnology, Seongnam, Korea). Each reaction consisted of an initial denaturation at 95°C for 1 minute followed by 3-step cycling: denaturation at 95°C for 30 seconds, annealing at a temperature optimized for each primer pair for 30 seconds, and extension at 72°C for 30 seconds. After the required number of cycles (25–30 cycles), the reactions underwent a final extension at 72°C for 5 minutes. The primer sequences were as follows: alkaline phosphatase (ALP), forward 5′-TACATTCCCCATGTGATGGC-3′ and reverse 5′-ACCTCTCCCTTGAGTGTGGG-3′; osteocalcin (OC), forward 5′-CTCCTGAGTCTGACAAAGCC-3′ and reverse 5′-GCTGTGACATCCATTACTTG-3′; bone sialoprotein (BSP), forward 5′-ACACTTACCGAGCT TATGAG-3′ and reverse 5′-TTGCGCAGTTAGCAATAGCA-3′; and β-actin, forward 5′-TGGATG GCTACGTACATGGCTGGG-3′ and reverse 5′-TTCTTTGCAGCTCCTTCGTTGCCG-3′. All the primers were synthesized by Bioneer Co (Daejeon, Korea). Each polymerase chain reaction (PCR) product was loaded in 1.5% agarose gels by electrophoresis and visualized by ethidium bromide staining. Reverse-

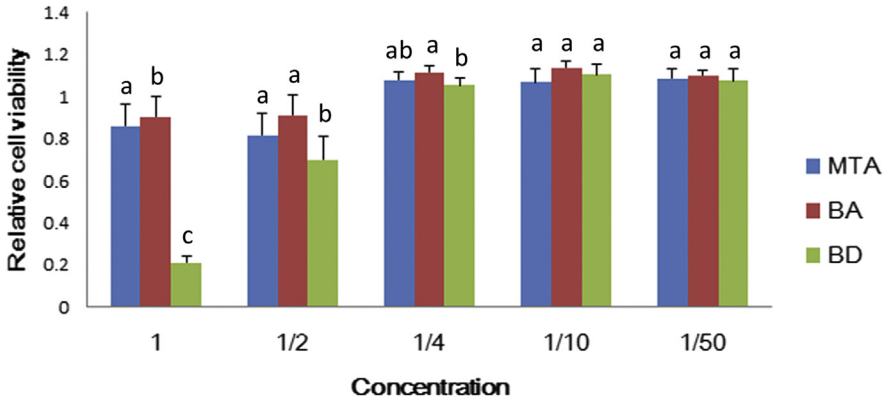

Figure 1. The effect of MTA, BA, and BD on cell viability assayed by XTT. Results are expressed as the relative cell viability (percentage of control). Different letters represent statistically significant differences between the test materials ($P < .05$).