Effect of 3 Bioceramic Materials on Stem Cells of the Apical Papilla Proliferation and Differentiation Using a Dentin Disk Model

Amber A. Miller, DDS,[*†] *Koyo Takimoto, DDS,*[*] *James Wealleans, DMD,*[*†] *and Anibal Diogenes, DDS, PhD*[*]

Abstract

Introduction: There is a complex interaction between biomaterials placed as a coronal barrier with stem cells and dentin in regenerative procedures. In this study, the effect of Biodentine (BD; Septodont, Saint-Maurdes-Fossés, France), Endosequence BC Root Repair Material-Putty (ES; Brasseler, Savannah, GA), Endosequence BC Root Repair Material-Putty Fast set (ES-fast, Brasseler), and ProRoot (Dentsply Tulsa Dental Specialties, Johnson City, TN) mineral trioxide aggregate (MTA) on the viability and differentiation of stem cells of the apical papilla (SCAP) was evaluated using an *ex vivo* dentin disk model. **Methods:** Standardized human dentin disks were treated using an established protocol. Disk lumens were filled with BD, ES, ES-fast, or MTA, and SCAP were cultured directly onto the samples. Cell viability was measured at 7 days, whereas differentiation into a mineralizing phenotype was evaluated by real-time reverse-transcription polymerase chain reaction and alizarin red staining at 21 days in culture. Results were analyzed using 1-way analysis of variance with the Bonferroni post hoc test or the Mann-Whitney *U* test ($P \leq .05$). **Results:** All materials promoted SCAP viability and proliferation with a greater response in the BD and ES groups. Also, a greater expression of alkaline phosphatase messenger RNA and dentin sialophosphoprotein was noted in the BD and ES groups, whereas MTA promoted a greater expression of the osteoblastic marker IBSP. Interestingly, no difference in alizarin red staining was observed with MTA, BD, or ES, which were significantly greater than ES-fast. **Conclusions:** These data suggest that BD and ES promoted greater survival and differentiation of SCAP and the increase of the odontoblastic marker DSPP, whereas MTA appeared to promote greater osteoblastic differentiation. Thus, BD and ES can be considered for regenerative and vital pulp therapies. *(J Endod 2018;■:1–5)*

From the *Department of Endodontics, University of Texas Health Science Center at San Antonio; and †Air Force Post Graduate Dental School, JBSA-Lackland, Texas.

Address requests for reprints to Dr Anibal Diogenes, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229-3900. E-mail address: Diogenes@uthscsa.edu

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Significance

The placement of a bioactive coronal barrier is an important step in REPs. Alternative bioceramic materials with less staining potential than MTA are available. In this study, Endosequence Root Repair Material-Putty and Biodentine promoted greater viability and differentiation of stem cells of the apical papilla into a mineralizing phenotype with greater odontoblastic marker expression than MTA. These materials should be considered as a coronal barrier in REPs and vital pulp therapy.

The management of immature teeth with both vital and necrotic pulps is a clinical challenge. These teeth may become diseased by various etiologic factors such as trauma, dental developmental anomalies, and caries (1). Maintaining pulp vitality or reattaining vitality through regenerative endodontic procedures (REPs) should be considered the treatment of choice, particularly in immature permanent teeth with pulp necrosis and open apices because it promotes the completion of root development and normal physiologic responses (2). It has been shown that a substantial number of mesenchymal stem cells (MSCs) are transferred from the apical tissues during REPs in immature (3) and mature teeth (4). These cells are hypothesized to be derived from the apical papilla (5) but could also be sourced in various apical tissues including bone, the periodontal ligament, and granuloma. However, the apical papilla appears to be the primary source of these cells because it has a favorable anatomic location and a relatively greater number of stem cells. Furthermore, the apical papilla has been shown to survive dental infections in both an animal model (6) and a clinical study (5). Therefore, during REPs, a dental restorative material is applied directly to the blood clot containing stem cells, including stem cells of the apical papilla (SCAP). Bioceramic materials, in particular calcium silicate–based materials, have emerged as the dental materials of choice for these procedures because of their biocompatibility, sealing, and antimicrobial potential (7–10). However, the effect of different bioceramic materials in contact with dentin on SCAP has never been previously evaluated.

It is important to note that there is a complex interaction of stem cells, including SCAP, with bioactive materials and conditioned dentin (11). The role of dentin on stem cell survival and differentiation has been evaluated by many elegant studies (12–15), showing that the treatment of dentin with irrigants and medicaments has a dramatic effect on stem cell fate (12). Also, there is strong evidence that bioactive materials can potentially change the bioavailability of the growth factors embedded in the dentin matrix and therefore modulate stem cell fate (16). Thus, there is a need to investigate the complex interaction of calcium silicate–based materials with conditioned dentin and stem cells.

Since the introduction of mineral trioxide aggregate (MTA; Dentsply Tulsa Dental Specialties, Johnson City, TN), there have been several other calcium silicate–based materials developed, including Biodentine (BD; Septodont, Saint-Maurdes-Fossés, France), Endosequence BC Root Repair Material-Putty (ES; Brasseler, Savannah, GA), and Endosequence BC Root Repair Material-Putty Fast Set (ES-fast, Brasseler), among others. MTA has been widely used for REPs (17), pulp capping (18),

perforation repair (19), and root-end filling because of its well-studied biocompatibility and robust osteo- and cementoconductive properties (7). However, its use in REPs is associated with significant coronal staining (20). BD, a tricalcium silicate, has excellent bioactivity, favoring proliferation and differentiation of stem cells while having excellent handling properties and a low risk of discoloration (21–23). ES is a newer calcium silicate–based nanoparticle material that has been shown to be biocompatible with both dimensional and chemical stability (24–26). In addition, it has acceptable handling and radiopacity and a low risk for discoloration (27–29). Far less is known about the Endosequence Root Repair Material fast set paste formulation. To date, there have been no published studies on the effects of ES and ES-fast on SCAP.

Several studies have revealed that MTA and BD can affect the proliferation and differentiation of stem cells from dental pulp tissues, tooth germs, and periodontal ligament tissues as well as SCAP (21, 24, 30). However, these studies did not include dentin as a substrate and source of growth factors and morphogens for stem cell growth and differentiation. Because the goal of these procedures is to regenerate a pulp-dentin complex, this study aimed to include all the components, namely stem cells, biomaterials, and dentin. Thus, using a dentin disk model, we sought to determine the effect of 4 different calcium silicate–based materials on the viability and differentiation of SCAP into a mineralizing phenotype. For the first time, this study directly compared 2 different formulations of ES with MTA and BD, providing further evidence for the selection of bioceramic materials in both regenerative and pulp capping procedures.

Materials and Methods

Teeth Collection and Dentin Disk Preparation

This study was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, San Antonio, TX, and Joint Base San Antonio-Lackland. Third molar teeth diagnosed with vital pulp but with an indication for extraction were collected.

Dentin disks were prepared by removal of gingival and periodontal tissues with a sterile surgical blade followed by a wash in cold Hank's Balanced Salt Solution (HBSS; Sigma-Aldrich, St Louis, MO) for 5 minutes. Next, an IsoMet 1000 precision saw (Buehler, Lake Bluff, IL) was used to obtain dentin disks by axially sectioning the pulp chamber of the extracted molars. Dentin disks were standardized to transverse sections of 2-mm thickness from the cementoenamel junction (CEJ) to the crown to generate 1 to 2 slices per molar. The dentin disk lumens were prepared to a standardized inner diameter of 2.5 mm with the use of a high-speed dental handpiece and carbide or a diamond dental bur and constant irrigation with cold HBSS. The outer diameter of the dentin disk was standardized to 5.75 mm using a metal circular template. The dentin disks were stored in sterile HBSS at 4°C until used.

Restorative Material Preparation

The materials in this study included BD, gray ProRoot MTA, ES, and ES-fast. All materials were prepared according to the manufacturers' instructions. MTA powder was mixed for 30 seconds with sterile water. BD was prepared by squeezing out the liquid of a single-dose container into the powder-containing capsule. The capsule was placed in a triturator (Dentsply Tulsa Dental Specialties) and mixed for 30 seconds at 4000 rpm. ES and ES- fast came as ready-to-use putty/paste.

Irrigation and Medicament Treatment Protocol

To simulate a disinfection protocol similar to that used in REPs, dentin disks were rinsed with sodium hypochlorite (1.5%) for 5 minutes, EDTA (17%) for 5 minutes, and saline for 5 minutes. Next, calcium hydroxide paste (Ultracal; Ultradent, South Jordan, UT) was applied to the dentin disk lumen and stored in a 37°C 5% CO_2 humidity incubator for 2 weeks. Next, dentin disks were thoroughly washed with the same initial protocol that consisted of sodium hypochlorite (1.5%), EDTA (17%), and sterile saline. After the washes, excess moisture was removed by blotting the samples in sterile paper towels. The disk lumen was occluded with BD, ES, ES-fast, or MTA according to the manufacturer's instructions. The dentin disks with the materials were placed in a 96-well plate and transferred to a 37°C 5% CO_2 humidity incubator for 12 hours to allow for the initial set of the materials. Dentin disks in a 96-well plate were rehydrated for 30 minutes in sterile HBBS before seeding of SCAP.

SCAP Cell Culture

Next, a previously characterized SCAP cell line was used (31). Cells were cultured in a basal cell culture medium comprised of alpha-minimum essential medium (Sigma-Aldrich) containing 10% fetal bovine serum (Gibco, Life Technologies, Grand Island, NY) and 1% L-glutamine:penicillin:streptomycin solution (Gemini Bio-Products, West Sacramento, CA) at 37°C in 5% CO_2. All experiments were performed with cells from the third to sixth passages.

For each experiment, the same passage of cells was used. A total of 100,000 SCAP in 0.5 mL media were seeded in 96-well culture plates containing the dentin disks with the biomaterials. In addition, SCAP were cultured directly in the 96-well culture plates in the absence of treated dentin disks to serve as a control. The media was changed every 3 days. The survival/proliferation experiments were assessed after 7 days in culture using OZBlue (OZBlue Biosciences, San Diego, CA) according to the manufacturer's recommendations. The differentiation was assessed after 21 days in culture with quantitative reverse-transcription polymerase chain reaction (qRT-PCR) (n = 6/group) or quantitative alizarin red staining (Millipore, Darmstadt, Germany) (n = 6/group) as previously shown (22).

Cell Counting for Viability

After a 7-day culture period, OZBlue was added to each 96-well plate media for all groups and incubated for 2 hours at 37°C 5% CO_2 humidity. The absorbance was measured at 490 nm using a FlexStation 3 Benchtop Multimode Microplate Reader (Molecular Devices, Sunnyvale, CA). After viability determination, SCAP were further cultured for a total of 21 days, and differentiation was evaluated with qRT-PCR and alizarin red staining.

Evaluation of Gene Expression by qRT-PCR

A total of 5 groups were tested by qRT-PCR after being maintained in culture for 21 days: group 1, SCAP+ dentin disks without biomaterials; group 2: SCAP+ dentin disks with MTA; group 3, SCAP+ dentin disks with BD; group 4, SCAP+ dentin disks with ES; and group 5, SCAP+ dentin disks with ES-fast. The total RNA was isolated using the RNeasy Mini Kit (Qiagen, Valencia, CA), and any potential genomic DNA contamination was removed by DNAse treatment (DNA-free kit; Ambion, Austin, TX) under a standardized protocol. The total RNA samples were used as a template in real-time polymerase chain reactions using the TaqMan Assays on Demand (Life Technologies, Foster City, CA). TaqMan Assays on Demand consisted of previously validated primers and probes specific for the following genes: dentin sialophosphoprotein (DSPP, assay # Hs00171962_m1), alkaline phosphatase