Effects of an Experimental Calcium Aluminosilicate Cement on the Viability of Murine Odontoblast-like Cells

Wei Wei, DMD, MS,[*] Yi-pin Qi, DDS,[†] Sergey Y. Nikonov, DDS,[‡] Li-na Niu, DDS, MS,[§] Regina L.W. Messer, PhD,[‖] Jing Mao, DMD, PhD,[*] Carolyn M. Primus, PhD,[¶] David H. Pashley, DMD, PhD,[‖] and Franklin R. Tay, BDSc (Hons), PhD[‖#]

Abstract

Introduction: Quick-setting calcium aluminosilicate cement with improved washout resistance is a potential substitute for calcium silicate cements in endodontics. This study examined the effect of an experimental calcium aluminosilicate cement (Quick-Set; Primus Consulting, Bradenton, FL) on the viability of odontoblast-like cells. **Methods:** The biocompatibility of Quick-Set and white ProRoot MTA (WMTA; Dentsply Tulsa Dental Specialties, Tulsa, OK) cements and their eluents was evaluated using a murine dental papilla–derived odontoblast-like cell line (MDPC-23); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was used to examine the effects of the 2 hydraulic cements on mitochondrial metabolic activity. Flow cytometry and confocal laser scanning microscopy were used to identify the effects of the 2 cements on cell death–induced plasma membrane permeability to fluorescent dyes and DNA stains. **Results:** After the first week of immersion in culture medium, Quick-Set and WMTA were more cytotoxic than the Teflon-negative control ($P < .05$), and the cells exhibited more apoptosis/necrosis than Teflon ($P < .05$). After the second week of immersion, the 2 cements were as biocompatible as Teflon ($P > .05$), with cells exhibiting minimal apoptosis/necrosis. Eluents from the set cements at 1:1 dilution were significantly more cytotoxic that eluents at 1:10 or 1:100 dilution ($P < .05$). **Conclusions:** Quick-Set and WMTA exhibited similar cytotoxicity profiles. They possess negligible *in vitro* toxicologic risks after time-dependent elution of toxic components. *(J Endod 2012;38:936–942)*

Key Words

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, biocompatibility, calcium aluminate, hydraulic cement, flow cytometry, MTT assay, vital cell staining

Over 1.8 million pulp cappings, 3.2 million pulpotomies, 23,000 apexification procedures, and 150,000 root-end filling procedures are performed annually in the United States alone (1). For the past decade, endodontists have embraced the use of calcium silicate hydraulic cements (ie, cements that set in the presence of water), such as mineral trioxide aggregate (MTA) for these procedures and for root and furcal perforations. MTA possesses excellent biocompatibility, bioactivity, and sealing properties and the ability to induce reparative hard-tissue formation. Despite these outstanding properties, there are potential disadvantages associated with the use of MTA including a long setting time, difficulty of manipulation, low resistance to washout, and potential staining of coronal dentin (2–5). Although other calcium silicate–based materials with improved handling properties have been introduced recently (eg, Biodentine; Septodont, Saint-Maur-des-Fossés, France) (6), the shortcomings of MTA and MTA-like materials necessitate the development of more ideal cements for endodontic applications.

Hydraulic aluminate cements for biomedical uses derived from industrial compositions are modifications of high-alumina cements used for refractory linings in furnaces and some civil engineering purposes. The aluminate cements were developed in the early 20th century to be more chemically durable than Portland cement (7). The hydraulic calcium phases typically contain 30% to 50% alumina, whereas Portland cements contain less than 5%. Calcium aluminate cements have been developed for use as bone cements (8, 9); they have strengths higher than 100 MPa, pull-out strengths equal to polymethyl methacrylate bone cement, and formed calcium alumina chlorohydrate phases. In dentistry, this type of cement has been formulated into dentin replacement restorative materials (DoxaDent; Doxa AB, Uppsala, Sweden) (10–13) and more recently as a bioactive, hybrid-glass-ionomer dental luting cement (Ceramir C&B, Doxa AB) (14–17). Calcium aluminate powder has been blended with radiopacifier powders and mixed with water or salt solution for comparisons with MTA products for the same endodontic indications (EndoBinder; Binderware, São Carlos, SP, Brazil) (18–22).

Another related calcium aluminosilicate cement (Capasio; Primus Consulting, Bradenton, FL) has been previously evaluated for its physical properties (23) and *in vitro* bone nodule formation by rat calvarial osteoblasts (24). Capasio is composed primarily of bismuth oxide, dental glass, and calcium aluminosilicate with a water-based gel. The material is slightly less basic (pH = 10.9) than white MTA (pH = 11.6) upon the final setting, has a setting time of 9 minutes, penetrates dentinal tubules,

From the *Department of Stomatology, Tongji Hospital, Huazhong University of Science and Technology, Wuhan, China; [†]Department of Operative Dentistry and Endodontics, Guanghua School of Stomatology, Sun Yat-sen University, Guangzhou, China; [‡]Private Practice, Krasnoyarsk, Russia; [§]Department of Prosthodontics, School of Stomatology, Fourth Military Medical University, Xi'an, China; Departments of [‖]Oral Biology and [#]Endodontics, Georgia Health Sciences University, Augusta, Georgia; and [¶]Primus Consulting, Bradenton, Florida.

Dr Primus is the inventor of Quick-Set. Dr Nikonov's visit to the Georgia Health Sciences University was supported by a Fulbright scholarship.

Address requests for reprints to Dr Franklin R. Tay, Department of Endodontics, College of Dental Medicine, Georgia Health Sciences University, Augusta, GA 30912-1129. E-mail address: ftay@georgiahealth.edu

0099-2399/$ - see front matter

doi:10.1016/j.joen.2012.03.020

and shows improved acid resistance and washout resistance versus MTA. Recently, Capasio powder has been refined and renamed as Quick-Set (Primus Consulting), and the cationic surfactant was removed from the liquid gel component, which was thought to interfere with cell cytocompatibility (24). The quick setting, fine particles, high radiopacity, and slightly elevated pH properties of Capasio were preserved in Quick-Set.

Because endodontic cements are applied in intimate contact with periradicular tissues, their biocompatibility is a prerequisite in expediting the repair of bone loss caused by host immunologic responses to microbial infections. Thus, the objective of the present study was to examine the effect of Quick-Set on the early proliferative events of murine odontoblast-like cells before their differentiation. The null hypothesis tested was that there is no difference between experimental calcium aluminosilicate cement and commercially available calcium silicate endodontic cement in affecting the viability of odontoblast-like cells.

Materials and Methods

Quick-Set and white ProRoot MTA (WMTA; Dentsply Tulsa Dental Specialties, Tulsa, OK) were mixed with the proprietary gel or deionized water, respectively, in a powder/water ratio of 3:1. The mixed materials were placed in presterilized Teflon molds (5-mm diameter and 3-mm thick) (DuPont, Wilmington, DE), covered with presterilized Mylar sheets (DuPont), and allowed to set completely in a 100% humidity chamber for 24 hours. The positive control consisted of disks prepared from Intermediate Restorative Material (IRM; Dentsply Caulk, Milford, DE), which is a zinc oxide–eugenol cement. Teflon disks of the same dimensions were used as the negative control. All set materials were sterilized with ultraviolet light for 4 hours before testing.

Cell Culture

The biocompatibility of the set cements was evaluated with an *in vitro* cell culture cytotoxicity model using a murine dental papilla–derived odontoblast-like cell line (MDPC-23) (25, 26). The cells were plated in a 24-well format at a density of 5,000 cells/cm^2 in 0.5 mL growth medium and incubated at 37°C in a humidified 5% CO_2 atmosphere for 24 hours until fully established. The growth medium consisted of Dulbecco modified Eagle medium (Lonza, Wakersville, MD) and 10% fetal bovine serum (Invitrogen Corp, Carlsbad, CA) supplemented with 2 mmol/L L-glutamine and 100 U/mL penicillin streptomycin.

Because cytotoxic components can diffuse out of set hydraulic cements into periradicular tissues, both cement disks and their eluents were evaluated using a cycling regimen developed in our laboratory (27–30). A weekly cycle consisted of direct evaluation of the toxicity of the cement disks over the plated cells for 3 days and indirect evaluation of the effect of eluents derived from the set cements on the plated cells. The latter was achieved by immersion of the disks in growth medium for 4 days to collect eluents. Accordingly, during the first part of each weekly cycle, cement and control disks (n = 12) were placed individually in transwell inserts with a 3-μm pore size (BD Falcon, Franklin Lakes, NJ) to prevent direct contact of the cells by the specimen. After the inserts were placed over the plated cells, an additional 2 mL of growth medium was added to each well to ensure that the level of the culture medium was above the sides of the transwell insert. The disks were exposed to the plated cells for 3 days, without further change in culture medium, before testing for succinic dehydrogenase activity. During the second part of each weekly cycle, the disks were retrieved and incubated at 37°C with growth medium (1 disk/2 mL) for 4 days to collect the eluents (n = 12) from the set cement before using the same disks for the next cycle. For each disk, the same growth medium was used for eluent collection throughout the entire testing period. This cycling regimen was repeated weekly for 2 weeks (ie, 2 cycles) until the material disks were rendered noncytotoxic (ie, >90% of the mean succinic dehydrogenase activity exhibited by the Teflon-negative control) (27). Each eluent concentrate collected after the 2-week aging period was diluted with fresh growth medium to 1:1, 1:10, and 1:10^2 of its original concentration to achieve a final volume of 2 mL (27). Each diluted, eluent-containing growth medium was then used as the respective culture medium for freshly plated murine dental papilla–derived odontoblast-like cell line (MPDC-23) cells for testing cell viability.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Assay

Cell viability was evaluated by incubating 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) with MDPC-23 cells that had been exposed to cement disks for 3 days in a normal growth medium and an eluent-containing growth medium in various dilutions for 3 days. The cells were incubated in MTT-succinate solution for 60 minutes and fixed with Tris-formalin. The purple MTT formazan produced in the cells, as a result of mitochrondrial succinic dehydrogenase activity, was dissolved *in situ* using dimethyl sulfoxide-NaOH, and the optical density was measured using a microplate reader at 562 nm. The optical density of blank dimethyl sulfoxide-NaOH was subtracted from all wells. The formazan content of each well was computed as a percentage of the mean of the Teflon controls, which was taken to represent 100% biocompatibility.

Because the normality and equal variance assumptions of the raw data were violated, they were logarithmically transformed before analysis with 2-factor repeated-measures analysis of variance (ANOVA). Data from the IRM-positive control were excluded from the analyses. For the evaluation of the set cements, statistical analysis was performed to examine the effect of the "material," the "immersion period," and the interaction of those 2 factors on cell viability. For the evaluation of the eluents derived from the set cements, statistical analysis was performed to examine the effect of the "material," the "dilution factor," and the interaction of those 2 factors on cell viability. For each analysis, post hoc multiple comparisons were performed using the Holm-Sidak test. Statistical significances for all analyses were set at $\alpha = 0.05$.

Flow Cytometry

The viability of MDPC-23 cells exposed to the set materials after different cycles of culture medium immersion was evaluated. Cells were plated at the same density as previously described. For each week, the established cells were first exposed to the respective materials for 3 days as previously described. The cells were then washed twice with phosphate-buffered saline and detached from the culture wells with 0.25% trypsin. The harvested cells were centrifuged to discard the supernatant and resuspended at 1×10^4 cells/mL in 1× binding buffer included in the Apoptosis and Necrosis Quantification Kit (Biotium, Inc, Hayward, CA). The cells were stained with fluorescein isothiocyanate (FITC)–annexin V (AnV) ($\lambda_{abs}/\lambda_{em}$= 492/514 nm, green fluorescence) and ethidium homodimer-III (Etd, $\lambda_{abs}/\lambda_{em}$ = 528/617 nm, red fluorescence) and incubated for 15 minutes in the dark. The stained MDPC-23 cells were subjected to fluorescence-activated cell sorting (FACS) using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) to determine the percentage distribution of viable (AnV/Etd negative), early apoptotic (AnV positive, Etd negative), late apoptotic (secondary necrosis; AnV/Etd positive), and