Effect of Using Different Vehicles on the Physicochemical, Antimicrobial, and Biological Properties of White Mineral Trioxide Aggregate

Bruno Cavalini Cavenago, PhD,[*†] *Aldo Enrique del Carpio-Perochena, PhD,*[*] *Ronald Ordinola-Zapata, PhD,*[*] *Carlos Estrela, PhD,*[‡] *Gustavo Pompermaier Garlet, PhD,*[§] *Mario Tanomaru-Filho, PhD,*[‖] *Paulo Henrique Weckwerth, PhD,*[¶] *Flaviana Bombarda de Andrade,*[*] *and Marco Antonio Hungaro Duarte, PhD*[*]

Abstract

Introduction: This study evaluated the physicochemical, antimicrobial, and biological properties of white mineral trioxide aggregate (MTA) mixed with aqueous and propylene glycol extracts of *Arctium lappa* L. and *Casearia sylvestris* Sw. **Methods:** The setting-time test was performed according to American Society for Testing and Materials 266/2008 and flowability by American National Standards Institute/American Dental Association 57/2012 standard specifications. The following parameters were assessed: volumetric change by micro–computed tomography; pH level and calcium ion release measured after 3 hours and 1, 3, 7, and 15 days; antimicrobial effect by the dentin decontamination method with intratubular *Enterococcus faecalis* viability, verified by using confocal laser scanning microscopy; and biocompatibility by histologic-morphometric analyses of inflammatory infiltrate at subcutaneous implant sites after 15, 30, and 60 days. In addition, rat alveolar tissues with implants were processed to measure tumor necrosis factor-alpha and interleukin-10 cytokines by enzyme-linked immunosorbent assay. **Results:** The plant extracts associated with MTA significantly increased the final setting time; however, they did not influence volumetric change ($P > .05$) and maintained medium alkalinity and calcium ion release. Propylene glycol extracts showed higher flowability. *Casearia sylvestris* increased the cementing effect against *E. faecalis* after 24-hour and 168-hour periods. Histologic evaluation of inflammatory infiltrate showed no significant differences between plant extracts groups and the distilled water group for all periods. Tumor necrosis factor-alpha and interleukin-10 expression was similar among groups ($P > .05$). **Conclusions:** *Casearia sylvestris* extracts increased the antimicrobial effect of MTA and did not influence biocompatibility but changed some physicochemical properties. *(J Endod 2017;■:1–8)*

Key Words

Biocompatibility, *Enterococcus faecalis*, material testing, phytotherapy

Significance

The effects of phytotherapy products (aqueous and propylene glycol extracts of *Arctium lappa* L./*Casearia sylvestris* Sw.) on white MTA (physicochemical, antimicrobial, and biological) properties require further study.

Mineral trioxide aggregate (MTA), which was developed in the 1990s as a root-end filling material (1), was selected for this clinical application because of its satisfactory sealing ability (2, 3), biocompatibility (4–6), and capacity for stimulating mineralization and repair processes (6, 7). However, this cement has shown handling difficulties, extended setting time, possibility of washout (8, 9), and limited antimicrobial activity (10, 11).

Periapical tissue contamination by bacterial species frequently isolated from secondary endodontic infections (12) must be considered when performing endodontic surgery. Dentin, a mineralized but permeable tissue consisting of dentinal tubules, is colonized and infected by viable bacteria (13) at the time of root-end preparation. The antibacterial properties of root-end filling cements could add benefit by eliminating persistent bacteria from the dentinal tubules. Despite the low antibacterial action reported, there have been no reports in the literature on the potential disinfection of the dentinal tubules provided by MTA.

Some studies (10, 14, 15) have proposed improved handling of MTA by replacing the distilled water or associating it with antiseptic solutions to add to or enhance the antimicrobial effect of MTA. The addition of 2% chlorhexidine provided significant increase in activity against *Enterococcus faecalis* (10), but this mixture increased the cytotoxicity (14) and interfered with the physical properties of MTA (15). In

From the *Department of Operative Dentistry, Endodontics and Dental Materials, School of Dentistry of Bauru, University of São Paulo, Bauru, São Paulo, Brazil; †Department of Restorative Dentistry, Federal University of Paraná, Curitiba, Paraná, Brazil; ‡Department of Stomatologic Sciences, Federal University of Goiás, Goiânia, Goiás, Brazil; §Department of Biological Sciences, School of Dentistry of Bauru, University of São Paulo, Bauru, São Paulo, Brazil; ‖Department of Restorative Dentistry, Araraquara Dental School, São Paulo State University, Araraquara, São Paulo, Brazil; and ¶Center of Health Sciences, Sagrado Coração University, Bauru, São Paulo, Brazil.

Address requests for reprints to Dr Bruno Cavalini Cavenago, Av. Prefeito Lothário Meissner no. 632, 80210-170 Curitiba, Paraná, Brazil. E-mail address: brunocavenago@hotmail.com

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general, subcutaneous implantation in rats has shown that white MTA induced severe inflammatory reactions within short periods, which were reduced to chronic infiltrate of moderate to mild intensity over time (4, 5).

Plant extracts with antimicrobial properties associated with MTA could act as alternatives. The herbaceous plant *Arctium lappa* L., originating from Eurasia and found in various regions throughout the world, is commonly used in folk medicine for treating infectious diseases. Another plant, *Casearia sylvestris* SW., is a common species in Latin America. Both herbal medicines have shown antimicrobial action (16, 17) and anti-inflammatory properties (18–20).

However, no studies have reported the effects of phytotherapy products mixed with MTA on its properties. This study proposed to evaluate the physicochemical, antimicrobial, and biological properties of white MTA mixed with aqueous and propylene glycol extracts of *Arctium lappa* L. and *Casearia sylvestris* Sw.

Materials and Methods

Preparation of Plant Extracts

The *Arctium lappa* L. (AL) and *Casearia sylvestris* Sw. (CS) leaves were harvested in Bauru and Botucatu, respectively, and identified in the Sagrado Coração University herbarium in Bauru, Brazil. The leaves were desiccated in an air-circulating oven at a controlled temperature until constant weight was achieved. A laboratory knife-mill was used to grind the prepared leaves.

Aqueous extracts (AqE) were obtained by diluting 25 g powdered leaves with 180 mL distilled water (DW) at 50°C for 24 hours. After this time, the solutions were filtered and stored in amber-colored flasks.

Propylene glycol extracts (PglyE) were obtained by macerating 25 g powdered leaves in 200 mL extracting solution. This association was maintained at 25°C for 8 days, with sporadic agitation in an amber-colored container.

Sample Preparation and Study Design

White MTA (Angelus, Londrina, Brazil) was mixed with AL-AqE and AL-PglyE and with CS-AqE and CS-PglyE and DW. Cement was manipulated in the ratio of 3 parts powder to 1 part liquid. For AL-PglyE and CS-PglyE, the proportions of 20% PglyE and 80% DW, respectively, were used.

Physicochemical Tests

Setting Time

Setting-time tests were carried out according to American Society for Testing and Materials C266-08 specifications, but samples were made according to the International Organization for Standardization (ISO) 6876:2012 standard. Twenty-five metal rings measuring 10 mm in diameter and 2 mm thick were immediately filled with cements (n = 5) and maintained in an incubator at 37°C ±1°C and 95% ± 5% humidity.

The initial setting time was determined with a 113.4 g Gilmore needle at 60-second intervals until the time when no indentations could be verified on the specimen surface. A 453.6 g Gilmore needle was used in the same manner to determine the final setting time. The times were registered in minutes with a digital chronometer.

Flowability

Flowability, which was based on the American National Standards Institute/American Dental Association standard no. 57 (2012), was measured by placing 0.5 mL freshly prepared cement (n = 3) on a glass slab. After 3 minutes, the cement was covered with another glass slab weighing 20 ± 2 g, and a 100 g weight was placed on this assembly. The weight and top glass slab were removed after 10 minutes. The samples were photographed with a ruler, which was used as scale for the ImageJ software (National Institutes of Health, Bethesda, MD) to measure the perimeter of digitalized samples. The results are expressed in millimeters.

Volumetric Change

Fifty acrylic teeth (n = 10) with standardized root-end cavities were filled with the experimental cements by using an MTA carrier device. The specimens were scanned with a desktop x-ray microfocus computed tomography scanner (SkyScan 1174v2; Bruker, Kontich, Belgium). The scanning procedure parameters were as follows: 50 kV x-ray tube voltages, 800 μA anode current, voxel size of 9.47 μm, and 1.0° rotation step in 360° rotation. Digital data were elaborated by reconstruction software (NRec-onv1.6.4.8, SkyScan), and CTan software (CTanv1.11.10.0, SkyScan) was used to measure the sample volume (mm^3).

After the scanning procedures and final setting time, the samples were individually placed in glass flasks containing 15 mL ultrapure water and stored at 37°C for 168 hours. After this time the samples were scanned again by using the same parameters as those adopted for the first scan, sample volumes were measured, and the reduction in volume was calculated and converted into percentage values.

pH Level and Calcium Release

Fifty acrylic teeth (n = 10), prepared in the same manner as those for the volumetric change test, were individually placed in glass flasks containing 10 mL deionized DW and stored at 37°C for 3 hours and 1, 3, 7, and 15 days. After each experimental time, the teeth were transferred to a new flask with 10 mL ultrapure water. The water in which the specimen was immersed was measured with a pH meter (model 371; Micronal, São Paulo, SP, Brazil) calibrated with known pH values (4, 7, 14).

The calcium ion release was measured with an atomic absorption spectrophotometer (AA6800; Shimadzu, Tokyo, Japan) equipped with a calcium ion–specific hollow cathode lamp, according to the protocol cited in a previous study (8). Calcium ion release readouts were compared with a standard curve obtained from readouts of the standard solutions, and data obtained were statistically analyzed.

Antimicrobial Evaluation

Sixty maxillary bovine incisors were used to obtain 3-mm-long cylindrical dentin samples from the apical section. For standardization, the root canals were prepared up to size 120 K-file (Dentsply Maillefer, Ballaigues, Switzerland). Samples were immersed in 2.5% NaOCl and then in 17% EDTA solution for 5 minutes and finally rinsed with DW. Subsequently, 2 coats of nail varnish were applied on the external root surface. The samples were individually placed in 1.5 mL microtubes and sterilized at 121°C.

Dentin Infection and Sample Preparation

The *Enterococcus faecalis* (ATCC 29212) culture was obtained by incubation in sterile brain-heart infusion broth (BHI) (Difco, Kansas City, MO) at 37°C for 24 hours. The following 5-day contamination protocol (21) was used.

On day1, the bacterial culture concentration of 3×10^8 colony-forming units/mL was standardized by using a spectrophotometer and incubated at 37°C for 7 hours to achieve exponential growth. Each microtube containing a dentin cylinder received 800 μL sterilized BHI and was shaken in an ultrasonic bath for 15 minutes. The BHI was