Experimental Sealers Containing Metal Methacrylates: Physical and Biological Properties

Tharsis Christini de Almeida Rossato, DDS, [*] *Julia Adornes Gallas, DDS,* [†] *Wellington Luiz Oliveira da Rosa, DDS, MD,* [*] *Adriana Fernandes da Silva, DDS, MD, PhD,* [‡] *Evandro Piva, DDS, MD, PhD,* [‡] *Sonia Luque Peralta, DDS, MD, PhD,* [§] *and Rafael Guerra Lund, DDS, MD, PhD* [‡]

Abstract

Introduction: The aim of this study was to evaluate the physical properties, the antimicrobial effect, and the biocompatibility of dual polymerization experimental sealers after the incorporation of dibutyltin (ET) or calcium (EC) methacrylate at concentrations of 0.5%, 1%, 2%, and 5%. **Methods:** RealSeal (RS; SybronEndo, Glendora, CA) was used as a commercial control. Materials were evaluated regarding film thickness, degree of conversion, radiopacity, antimicrobial effect against Enterococcus faecalis using the modified direct contact test, and cell viability. Data were analyzed using analysis of variance followed by the Tukey test or the Student-Newman-Keuls test in SigmaPlot 12.0 (Systat Software, Inc, Point Richmond, CA) ($P = .05$). **Results:** The film thickness of the dibutyltin and calcium were greater than experimental sealers following the standards given by ISO 6876:2012. For degree of conversion, dual polymerization was not influenced by the addition of metal methacrylate. Regarding the modified direct contact test, calcium and dibutyltin at all concentrations showed antimicrobial activity when compared with the positive control after 48 hours of contact ($P < .05$). In cell viability, ET at all concentrations showed high cytotoxicity similar to RS, and EC at concentrations of 0.5%, 1%, and 2% showed moderate cytotoxicity that was less than 5% of calcium and RS. **Conclusions:** It was concluded that calcium and dibutyltin methacrylate incorporation in experimental sealers promoted the antimicrobial effect. The incorporation of calcium methacrylate at 0.5%, 1%, and 2% seemed to be a good treatment option to provide antimicrobial activity associated with moderate cytotoxicity and adequate physical properties. *(J Endod 2017;■:1–5)*

Key Words

Antimicrobial activity, calcium, endodontics, methacrylate-based resin sealer, physical properties, tin

Significance

Metal methacrylates incorporated in endodontic sealers showed moderate cytotoxicity, antimicrobial potential, and adequate physicochemical properties.

Root canal filling and subsequent restoration are the main stages of endodontic treatment. The proliferation of microorganisms during root canal treatment can occur as a result of failures in the filling or the intracanal medication or from an ineffective biomechanical preparation (1). Thus, the prognosis of endodontic treatment is greatly improved with the biological sealing of the periapical region and the elimination or reduction of microorganisms in the root canal system, such as *Enterococcus faecalis* (2, 3). The *Enterococcus* species constitute a small portion of the initial flora in untreated root canals and are commonly found in canals of teeth with failed endodontic treatment (4). As a result, the endodontic sealer should provide hermetic sealing in dentinal tubules and in the apical foramen to avoid bacterial invasion; additionally, the sealer should be biocompatible and have adequate fluidity and radiopacity (5).

Studies have reported that endodontic sealers with calcium hydroxide (ECs) have acceptable biocompatibility (6, 7). Calcium methacrylates may release calcium ions that play an important role in the repair process (8). Additionally, dibutyltin methacrylate presents tin, which has been used for decades as a prophylactic agent in preventive dentistry; previous findings revealed the independent action on inhibiting bacterial biofilm formation, reducing the acid portion of this microbial polysaccharide matrix (9, 10). In addition, endodontic sealants have undergone a major breakthrough in endodontics in recent years. After photoactivation, the sealer may present good adhesion and adaptation to canal walls, greatly improving the marginal leakage (11). Some methacrylate resin–based sealers have already been introduced in the dental market, such as RealSeal (RS; SybronEndo, Glendora, CA) (12). RS was introduced in 2006, combining the priming and bonding steps in a single container in an effort to save time and reduce technique sensitivity; components of RS are listed in Table 1(11). These materials may create monoblocks within the root canal space,

From the *Postgraduate Program in Dentistry and [‡]Department of Restorative Dentistry, School of Dentistry, Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil; [†]Postgraduate Program in Dentistry, School of Dentistry, University of São Paulo, Ribeirão Preto, São Paulo, Brazil; and [§]School of Dentistry, Metropolitan College of Fortaleza, FAMETRO, Fortaleza, Ceará, Brazil.

Address requests for reprints to Dr Rafael Guerra Lund, School of Dentistry, Federal University of Pelotas, Gonçalves Chaves St, 457/502, 96015560 Pelotas, Rio Grande do Sul, Brazil. E-mail address: rafael.lund@gmail.com

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TABLE 1. Composition of Endodontic Sealers Evaluated

Endodontic sealer	Composition
RealSeal*	Urethane dimethacrylate, polyethylene glycol dimethacrylate, ethoxylated bisphenol-A dimethacrylate, bisphenol glycidyl dimethacrylate, barium borosilicate glass, barium sulfate, silica, calcium hydroxide, bismuth oxychloride with amines
Experimental endodontic sealer	Exothane 8,† ethoxylated bisphenol A dimethacrylate (ethoxylation = 30),† PEG 400 dimethacrylate,† triethylene glycol dimethacrylate,† camphorquinone,† dihydroxy ethyl-p-toluidine,† hydroxybutyl toluene,‡ silica,§ 28% ytterbium trifluoride,‡ sulfinic,‡ and benzoyl peroxide‡
E + calcium methacrylate	E composition + calcium methacrylate at 0.5%, 1%, 2%, and 5%
E + dibutyltin methacrylate	E composition + dibutyltin methacrylate at 0.5%, 1%, 2%, and 5%

*SybronEndo, Sybron Dental Specialties, Glendora, CA; batch number: 14G3.

†Esstech Inc, Essington, PA.

‡Sigma-Aldrich, St Louis, MO.

§Aerosil 380; Degussa, Essen, Germany.

with the advantages of simultaneously improving the seal and providing fracture resistance of the filled canals (13).

To the best of our knowledge, no studies evaluating experimental ECs or endodontic sealers with dibutyltin methacrylates (ETs) have been published to date. Therefore, the purpose of this study was to evaluate the physical, biological, and microbiological properties of experimental ECs and ETs. The hypothesis evaluated was that the incorporation of calcium or dibutyltin methacrylates would improve antimicrobial properties without compromising the physical and biological properties of the experimental endodontic sealers.

Materials and Methods

The composition of ECs and ETs is shown in Table 1 as well as in the commercial reference RS. Two endodontic sealer pastes were mixed at the same proportion and photoactivated with a light-emitting diode for 20 seconds (Radii Curing Light; SDI, Bayswater, Victoria, Australia). RS has a self-mixing system, and its samples were photoactivated for 40 seconds.

Antimicrobial Assay

An antimicrobial assay was performed in triplicate using the modified contact direct test against *Enterococcus faecalis* ATCC 4083 (American Type Culture Collection, Manassas, VA) isolated from a periapical abscess (14). It was cultured overnight at 37°C in tryptic soy agar plates in an aerobic atmosphere. The strain was inoculated in tryptic soy broth, and the bacterial turbidity was adjusted to an optical density of 0.5 at 600 nm. Five-millimeter diameter cylinders with 1-mm thicknesses from all sealers were photoactivated for 20 seconds on each side. Subsequently, 10 μL bacterial suspension was placed above the surface of the materials that were tested. Strain suspensions (10 μL) placed in uncoated wells served as nonexposed controls (C+). Materials incubated without bacteria served as the negative controls. All samples were incubated aerobically for 1, 24, and 48 hours at 37°C in >95% humidity; then, 240 mL tryptic soy broth was added and gently mixed with a pipette for 1 minute. Serial dilutions were prepared in saline, plated onto tryptic soy agar, and incubated in an aerobic environment for 24 hours at 37°C. Colony-forming units were counted, and the colony-forming units/mL was calculated (14).

Cell Viability Assay

Cell viability was performed using mouse fibroblasts (L929, 20×10^3/well) according to ISO 10993:2009 (15). Discs of each material ($n = 6$) were photoactivated for 20 seconds and stored in 1 mL Dulbecco modified Eagle medium for 24 hours to obtain the eluate. Cytotoxicity was assessed after 24 hours using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolidium bromide (Sigma-Aldrich, St Louis, MO). The formazan content of each well was computed as a percentage of the control group (untreated cells). The cytotoxic response was rated as severe (30%), moderate (30%–60%), slight (60%–90%), or noncytotoxic (>90%) (16).

Physical Properties

Degree of Conversion. The carbon double bond length, such as m-dash carbon conversion of the TFMs ($n = 3$), was determined using Fourier transform infrared spectroscopy (Prestige 21 spectrometer; Shimadzu Corporation, Kyoto, Japan) equipped with an attenuated total reflectance attachment incorporating a horizontal diamond crystal with a 45° mirror angle (PIKE Technologies, Madison, WI). The light-emitting diode curing unit was rigidly held in position, enabling standardization of the distance between the fiber tip and the top of the sample at 5 mm. Infrared analysis was performed at a controlled room temperature of 23°C (±2°C) and 60% (±5%) relative humidity. Approximately 5 μL of each sample was dispensed directly onto the diamond crystal to evaluate the degree of conversion (DC). The spectra of uncured and cured material were obtained after 20 seconds of photoactivation. TFMs were acquired between 1.690 and 1.575 cm^{-1}, averaging 12 scans at the 4 cm^{-1} resolution transmission mode to provide a single spectrum. The spectra of each unpolymerized TFMs were also captured. Descriptive analysis was performed to evaluate the sealer polymerization.

Film Thickness. This assay was performed with 2 square glass plates with a thickness of 5 mm and a contact area of approximately 200 mm^2 (ISO 6876:2012 [17]) using a loading device (150 N ± 3) ($n = 3$). Aiming to standardize the amount of sealer for all groups, an amount of 0.05 mL sealer for each specimen and a standardized syringe were used. The sealer film thickness (FT) was measured as the difference between plates with and without sealer ($M_2 - M_1$) (18).

Radiopacity. Five samples were obtained for each material (5-mm diameter and 1-mm thickness). The rings were placed on occlusal radiographic film (Insight; Kodak Company, Rochester, NY) and radiographed with an X-ray apparatus (Kodak 2200 intraoral X-ray system) operating at 70 kV and 10 mA with an exposure time of 0.36 seconds and a focus film distance of 30 cm according to the specifications no. 57 American National Standards Institute/American Dental Association (*ADA Professional Product Review*, 2008) and ISO 6876 (*Dentistry—Root Canal Sealing Materials*, 2012, British Standards Institution, London, UK). After processing, the optical density or gray tones of images were measured and performed by ImageJ 1.4 software (National Institute of Mental Health, Bethesda, MD). The "histogram" was used to measure gray shades, ranging from 0 to 255 pixels. Five different points of each specimen were randomly selected to obtain the mean radiopacity value in pixels; then, this value was further