A Modified Resin Sealer: Physical and Antibacterial Properties

Juheon Seung, DDS, MS, Michael D. Weir, PhD,† Mary Anne S. Melo, DDS, MSc, PhD,‡ Elaine Romberg, PhD,* Ali Nosrat, DDS, MS, MDS,* Hockin H.K. Xu, MS, PhD,† and Patricia A. Tordik, DMD**

Abstract

Introduction: The purpose of this study was to investigate the physical and antibacterial properties of a resin sealer mixed with a quaternary ammonium compound, dimethylaminododecyl methacrylate (DMAHDM) and nanosilver (NAg). **Methods:** A pilot study was completed to determine the highest concentrations of DMAHDM and NAg that did not significantly alter the physical properties (setting time, flow, solubility, and dimensional change) of AH Plus (Dentsply Sirona, York, PA) when added to the sealer. These concentrations were selected to create a modified resin sealer (mAH Plus). A modified direct contact test evaluated antibacterial properties of AH Plus, DMAHDM + AH Plus, NAg + AH Plus, and mAH Plus at days 1, 7, and 14 against *Enterococcus faecalis.* **Results:** Concentrations of 2.5% DMAHDM and 0.15% NAg were added to AH Plus. The flow of mAH Plus was significantly decreased but still within American National Standards Institute/American Dental Association specifications. There were no significant differences in setting time, solubility, or dimensional change. On day 1, 0.15% NAg + AH Plus, 2.5% DMAHDM + AH Plus, and mAH Plus were significantly more effective against *E. faecalis* compared with AH Plus ($P < .05$). On days 7 through 14, 2.5% DMAHDM + AH Plus and mAH Plus continued to be significantly more antibacterial than AH Plus ($P < .05$). **Conclusions:** The addition of 0.15% NAg and 2.5% DMAHDM did not adversely affect the physical properties of AH Plus, and mAH Plus was significantly more antibacterial against *E. faecalis.* *(J Endod 2018;■:1–5)*

Key Words:

Dental cements, *Enterococcus faecalis*, modified direct contact test, nanoparticles, quaternary ammonium

Significance

The addition of 2.5% DMAHDM and 0.15% NAg to AH Plus did not change the physical properties of the sealer. The modified AH Plus was significantly more antibacterial against *E. faecalis* 14 days after setting when compared with AH Plus.

Despite efforts to disinfect the root canal system with chemomechanical debridement and medicaments, residual bacteria remain with the potential to result in persistent endodontic disease (1–4). Histologic evaluation of teeth with apical periodontitis shows heavy intracanal colonization of bacteria, supporting the role of bacteria as the primary cause of persistent apical pathosis (3, 5).

Often, areas of the root canal system such as isthmuses, lateral canals, and other root canal irregularities may be left untouched during chemomechanical preparation (6–9). In addition to sealing gaps between gutta-percha and the irregular walls of the root canal system, endodontic sealers may act as disinfectants to reduce the bacterial load. Epoxy resin–based sealers are widely used because of the lack of solubility and dimensional stability after setting (10–13). Studies show most sealers exhibit antibacterial properties in the short-term. The antibacterial effect is mainly before setting and only by contact (13, 14).

Quaternary ammonium (QA) compounds are antibacterial materials with 2 possible antibacterial mechanisms. First, they contain positively charged quaternary amine groups that can attract the negatively charged cell membrane of bacteria and disrupt the membrane (15). Second, QAs may exert their effect by penetrating the bacterial membrane. This is facilitated by a long hydrophobic alkyl chain, which may be added to QA. The optimal chain length is 16 alkyl groups (dimethylaminododecyl methacrylate [DMAHDM]) (15). When incorporating QA compounds into resin, the resin maintains its antibacterial property without compromising the physical properties, and exerts contact inhibition of bacteria (16–19). The QA compounds do not affect the cytotoxicity of dental materials on gingival fibroblasts (15).

Silver is a wide-spectrum antibacterial agent with low cytotoxicity (15, 20). One antimicrobial mechanism for silver is the ability to inhibit DNA replication of bacteria (21). When decreased to a nanoscale, the increased silver particle surface area exerts a potent antibacterial effect at lower concentrations (21, 22). Resin-containing nanosilver (NAg) exhibits contact inhibition like resin containing QA, and the silver ion exerts antibacterial effects far away from the resin surface (23). Leaching NAg may reduce an antibacterial effect over time. It is reasonable to hypothesize that QAs and NAg can be complementary to each other. QA exhibits

From the *Endodontics Division, Department of Advanced Oral Sciences and Therapeutics, †Biomaterials and Tissue Engineering Division, Department of Advanced Oral Sciences and Therapeutics, and ‡Operative Dentistry Division, Department of General Dentistry, School of Dentistry, University of Maryland Baltimore, Maryland.

Address requests for reprints to Dr. Patricia A. Tordik, Endodontics Division, Department of Advanced Oral Sciences and Therapeutics, School of Dentistry, University of Maryland Baltimore, 650 West Baltimore Street, Room 4217, Baltimore, MD 21201. E-mail address: ptordik@umaryland.edu

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long-term contact inhibition of bacteria, whereas NAg exerts short-term antibacterial activity by contact inhibition and silver ion leaching.

The aims of this study were to determine the effect of the addition of DMAHDM and NAg to AH Plus (Dentsply Sirona, York, PA) on selected physical properties of the sealer and to determine the antibacterial efficacy of AH Plus mixed with DMAHDM and NAg against *Enterococcus faecalis*. The hypothesis evaluated was that the incorporation of DMAHDM and NAg will improve the antibacterial properties of AH Plus without compromising the physical properties of the sealer.

Materials and Methods

DMAHDM and NAg Preparation

Synthesis of DMAHDM was completed as previously described (24). Briefly, QAs were made using the Menschutkin reaction by the reaction of tertiary amines with organohalides. To make a stock 10% solution of NAg, silver 2-ethylhexanoate powder (Strem, New Buryport, MA) was dissolved in 2-(tert-butylamino)ethyl methacrylate (Sigma-Aldrich, St Louis, MO) at 0.1 g silver salt per 0.9 g 2-(tert-butylamino)ethyl methacrylate (23). According to safety data sheets, the main composition of paste A is bisphenol A diglycidylether (25%–50% wt/wt) and formaldehyde, oligomeric reaction products with 1-chloro-2,3-epoxypropane and phenol (2.5–10% wt/wt). The main composition of paste B is N,N'-dibenzyl-5-oxanonandiamin-1,9 (10–25% wt/wt), amantadine (2.5–≤10% wt/wt), and octahydro-4,7-methano-1H-indendimethylamin 0.1–<1%. The two pastes are mixed in equal parts for use. In this study, AH Plus was mixed according to the manufacturer's instructions.

Optimal Concentrations of DMAHDM and NAg

The concentrations screened for DMAHDM were 2.5%, 5%, and 10% (wt/wt), and for NAg they were 0.05%, 0.10%, and 0.15% (wt/wt) in AH Plus. The screening was completed by a set of physical tests (setting time, flow, solubility, and dimensional change) according to American National Standards Institute (ANSI)/American Dental Association (ADA) specifications (25). For each test, 3 molds of experimental material at each concentration were prepared, and the physical properties of the mixtures were compared with AH Plus alone (total $N = 21$).

Setting Time

Teflon molds with an internal diameter of 10 mm and 2 mm of thickness were prepared. Each mold was filled with AH Plus or AH Plus + experimental materials. The samples were transferred to a chamber with 95% relative humidity and a temperature of 37°C. A Gilmore-type needle with a mass of 100 ± 0.5 g with a flat end of 2.0 ± 0.1 mm in diameter was lowered vertically onto the sample. Probing was repeated until indentations ceased to be visible. Setting times were recorded in minutes.

Flow Test

Using a graduated 1-mL syringe, 0.5 mL sealer was placed on a 60-g glass plate. After 30 seconds of mixing, another 60-g glass plate was applied. After 10 minutes, major and minor diameters of compressed circular areas of each sample were measured using a digital caliper and then averaged for comparison.

Solubility

Plastic molds with an internal diameter of 10 mm and a height of 2 mm were filled on a glass plate with freshly mixed sealer. Another glass plate was placed over the mold, and samples were allowed to set for 24 hours at 37°C and 100% humidity. The samples were weighed 3 times, and the average weight was recorded in milligrams. Individual samples were placed in a container with 5 mL distilled water and stored at 37°C for 24 hours. Samples were removed from the container and dried in a dehumidifier for 24 hours. Solubility was calculated as the percentage of weight loss.

Dimensional Change

Plastic disks with a diameter of 10 mm and a height of 6 mm were placed on a glass plate and filled with sealer. Another glass plate was placed over the mold and held firmly with a clamp. The samples were transferred to a cabinet with 100% relative humidity and kept for 30 days at 37°C. Dimensional change was measured by assessing the volume change. Measurements were taken with a digital caliper.

Physical Properties of mAH Plus

The highest concentrations of DMAHDM and NAg, or the highest concentration allowed by ANSI/ADA specifications, that made no significant changes to the physical properties of AH Plus sealer were selected to create a modified resin sealer (mAH Plus). The mAH Plus was decided to contain 2.5% DMAHDM and 0.15% NAg incorporated into AH Plus. The mechanical properties (setting time, flow, solubility, and dimensional change) of mAH Plus were then tested ($n = 5$) as described previously and compared with AH Plus.

Antibacterial Properties

E. faecalis (ATCC 29212; American Type Culture Collection, Manassas, VA) was used to evaluate the antibacterial properties of the sealers. Inoculum was prepared by resuspension of washed cells and adjusted to a density of 1×10^6 colony-forming units (CFUs)/mL using a microplate reader (SpectraMax M5; Molecular Probes, Sunnyvale, CA) at 550 nm.

Modified Direct Contact Test

A modified direct contact test (mDCT) was performed as previously described (13) at 1, 7, and 14 days after mixing on the following groups: group 1, no sealer (negative control, $n = 36$); group 2, AH Plus (control, $n = 36$); group 3, AH Plus + NAg ($n = 36$), group 4, AH Plus + DMAHDM ($n = 36$); and group 5, mAH Plus ($n = 36$).

Briefly, a 96-well microtiter plate (Sarstedt Inc, Newton, NC) was held vertically, and an area of fixed size on the side wall of the well was coated with an equal amount of each material. The coated plates were stored for 1, 7, and 14 days in 100% humidity at 37°C. A 10-μL bacterial suspension was carefully placed on the surface of each well. Specimens were incubated in 100% humidity at 37°C for up to 60 minutes of contact time with each sealer and uncoated negative control; 240 μL brain-heart infusion (BHI) broth was added to each well. After gently mixing with a pipette for 1 minute, the bacterial suspension was transferred and serially diluted in BHI. After 10-fold serial dilutions, aliquots of 10 μL were placed on BHI agar plates. The plates were incubated for 24 hours at 37°C, colonies on the plates were counted, and the CFU/mL was calculated.

Data Analysis

Statistical analysis was completed using SPSS Version 23.0 (IBM, Armonk, NY). Because all data are continuous numbers (minutes for setting time, average diameter for flow, weight loss for solubility, millimeters for dimensional change, and CFU count for antibacterial properties), 1-way analysis of variance followed by the Tukey honest significant difference test were performed to compare the differences in physical and antibacterial properties. A P value ≤.05 was set as the threshold for significance.