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BASIC RESEARCH-BIOLOGY

Drug-Silica Coassembled Particles Improve Antimicrobial Properties of Endodontic Sealers

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ABSTRACT

Introduction: The purpose of this study was to assess the antimicrobial activity and flow of root canal sealers after incorporating novel highly loaded antimicrobial drug-silica coassembled particles (DSPs). **Methods:** DSPs were synthesized through coassembly of silica and octenidine dihydrochloride (OCT) antimicrobial surfactant. DSPs were loaded (1% and 2% wt) into epoxy resin sealer (AH Plus [AH]; Dentsply DeTrey GmbH, Konstanz, Germany) or calcium silicate–based sealer (EndoSequence Bioceramic Sealer (BC); Brasseler, Savannah, GA). OCT release from DSP-modified sealers was determined using liquid chromatography. Antimicrobial activity of sealers against planktonic or biofilm form *Enterococcus faecalis* was assessed using direct contact and membrane restricted tests. Sealer flow was tested according to ISO6876:2012. **Results:** OCT release from BC + 1% or 2% DSPs was above the minimum inhibitory concentration following 2 days throughout the 30-day experiment, whereas OCT release from AH + 1% or 2% DSP was significantly below the minimum inhibitory concentration against *E. faecalis* (4 μg/mL) over the whole 30-day experimental period. All materials (with or without DSPs) killed planktonic bacteria initially. AH ± 1% or 2% DSPs had no antimicrobial activity after 7 days. BC + 1% or 2% DSPs maintained antibacterial activity over the 30-day period. Both modified and unmodified sealers completely inhibited the growth of *E. faecalis* biofilms after 24 hours of contact. DSPs decreased the flow of AH and BC sealers; for AH, the reduction was proportional to the amount of DSPs added. All modified and unmodified sealers, except for AH + 2% DSPs, were within the acceptable limits of ISO 6876 flow tests. **Conclusions:** DSPs enhanced the antimicrobial performance of BC but not AH, whereas the material's flow remained compliant with ISO 6876 standards. Depending on the sealer, DSPs may enhance antimicrobial efficacy in root canal treatment and potentially improve treatment outcome. *(J Endod 2021;■:1–7.)*

KEY WORDS

Antimicrobial; controlled release; *Enterococcus faecalis*; epoxy resin sealer; silicate-based sealer

SIGNIFICANCE

This study reports that the addition of drug-silica coassembled particles could enhance the antimicrobial efficacy of root canal sealers while keeping their flow within the acceptable limits of the ISO6876 flow test, potentially improving treatment outcome.

Root canal sealers are intended to entomb remaining microbes and prevent further microbial contamination. Most root canal sealers have short-term antimicrobial activity, mainly because of the release of their unreacted and unbound components[1]. Continued antimicrobial activity from sealers is beneficial for reducing and preventing the growth of residual microbes[2]. Sustained release of antimicrobial agents from sealer by the direct addition of antimicrobial agents has been suggested[3]. However, there have been concerns of increased bacterial resistance and negative effects on the physical properties of root canal sealers[2]. Additionally, the release of antimicrobial components of sealers could result in their disintegration and could compromise dentin-sealer interfacial stability[4].

Epoxy-based resin sealer is frequently used as a benchmark when testing sealer performance because of its favorable physical and initial antibacterial properties[5]; however, its antimicrobial activity drops with time[6]. Calcium silicate–based cements have been introduced recently as root canal sealers with claims of enhanced bioactivity[7], low solubility, and maintenance of an alkaline pH[8].

Antimicrobial nanoparticle-based treatments of endodontic infections are gaining popularity because of potential enhanced antimicrobial efficacy[2]. The addition of nanoparticles to root canal sealers could improve their direct and diffusible antimicrobial properties[9], increase sealers' substantivity, and increase antimicrobial activity within dentinal tubules[2]. However, the addition of particles into the sealer's

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matrix may alter its flow and affect its sealing ability[10]. A novel synthesis of highly loaded (34% wt, ~50% vol) antimicrobial drug-silica coassembled particles (DSPs) was recently reported[11]. DSPs were synthesized through a coassembly of silica and an antimicrobial surfactant template consisting of octenidine dihydrochloride (OCT)[11]. Drug release from DSPs significantly outlasted conventional OCT-loaded mesoporous silica, and when included in a resin dental adhesive, the release was limited to the restoration-tooth interface, potentially lasting for the life of the restoration while minimizing systemic exposure, suggesting potential for use in root canal sealers[11,12]. OCT is highly biocompatible with no known antibacterial resistance and is used as a mouth rinse, wound cleansing agent, and topical antiseptic, among other applications[13]. OCT does not react with sodium hypochlorite[14]; has antimicrobial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, and *Candida albicans*; and could be used as an endodontic irrigant[15].

Therefore, it can be hypothesized that the incorporation of DSPs within endodontic sealers will allow for sustained and controlled interfacial release of OCT from the material. This may improve the long-term antimicrobial properties of the sealers while maintaining the sealer's flow in compliance with the relevant ISO standard. The aim of this study was to synthesize and investigate the antimicrobial properties and flow characteristics of root canal sealers after incorporating novel highly loaded antimicrobial DSPs.

MATERIALS AND METHODS

Bacterial Strain and Culture Conditions

Overnight cultures of *E. faecalis* ATCC 47077 (American Type Culture Collection, Manassas, VA) were prepared by collecting bacterial colonies from a blood agar plate and inoculating brain-heart infusion (BHI) broth. BHI suspensions were incubated at 37°C. Bacterial cells were collected and resuspended in fresh BHI broth to adjust the cell density according to the concentration required for the following experiments.

Measurement of Minimum Inhibitory Concentration of OCT against *E. faecalis*

The minimum inhibitory concentration (MIC) of OCT against *E. faecalis* ATCC 47077 was determined using a similar previously described methodology[16]. Stock solutions were prepared by dissolving powdered drug in phosphate-buffered saline (PBS) via sonication followed by sterilization through a 0.2-μm syringe filter. *E. faecalis* ATCC 47077 was grown to the mid-log phase at 37°C in BHI. *E. faecalis* culture was diluted 1:20 in 5 mL BHI and incubated for approximately 3 hours. Dilutions of OCT solutions were made in BHI and added in 100-μL aliquots to a 96-well plate. When optical density (OD_{600}, measured at a wavelength of 600 nm) of *E. faecalis* in BHI reached 0.1, 100 μL bacterial growth media was added to the corresponding 100-μL OCT wells within the plate, with uninoculated BHI as a control. Plates were incubated for 24 hours at 37°C. Growth and no growth were determined by visual inspection with OD confirmation via a microplate reader (Cytation 3; Bio-Tek, Winooski, VT).

Synthesis of and Characterization of DSP-loaded Sealers

OCT DSPs were prepared as previously described.[11] Briefly, OCT (TCI America, Philadelphia, PA) was dissolved in deionized water with sodium hydroxide; during mixing, tetraethyl orthosilicate (Sigma-Aldrich, St Louis, MO) was added. The particles were collected from the synthesis solution via centrifugation after 24 hours, washed 3 times in deionized water, centrifuged, and dried at 60°C for 24 hours[11]. DSP size and structure were verified by transmission electron microscopy, scanning electron microscopy, and X-ray diffraction[12]. Particles of 424 ± 75 nm in diameter with OCT content of 34% wt (~50% vol) were used in the current study[12]. DSPs were added gradually at either 1% or 2% wt to epoxy resin sealer (AH Plus Jet [AH], Dentsply DeTrey GmbH, Konstanz, Germany) or calcium silicate–based sealer (EndoSequence Bioceramic Sealer (BC); Brasseler, Savannah, GA). These concentrations of DSPs were determined after a pilot study assessing the flow of materials with descending concentrations from 10% to 1% wt; the 1%–2% range of added DSPs did not affect the setting of the materials.

OCT Release from the Modified Sealers

A 96-well microtiter plate was used to assess OCT release from the modified sealers. The bottoms of the wells were coated with either unmodified AH or BC or modified sealers with 1% or 2% wt DSPs. Two hundred microliters PBS was added to each well. Media were replaced every 24 hours for the first 5 days and then every 5 days until the end of the incubation period (30 days). Incubation media were analyzed using ultra-performance liquid chromatography in combination with mass spectrometry to quantify the mass of OCT release using known standards and calibration curves, as described previously[11]. The ultra-performance liquid chromatography–mass spectrometry system consisted of the Acquity H-Class LC system connected to the Xevo G2-XS QTof operating in the positive ion mode with the Acquity BEH C18 column and MassLynx with Quanlynx software version 4.1 (all components from Waters, Mississauga, ON). Incubation media were checked for contamination at each time point.

Antimicrobial Activity of DSP-modified Sealers against Planktonic *E. faecalis*

The direct contact test (DCT) was used as described previously[17]. An area of 3 mm on the side of each well of a 96-well microtiter plate was coated with either unmodified or modified AH or BC (1% or 2% wt DSPs). Uncoated wells served as the control. The materials used were freshly mixed (0 days) or stored for 7 or 30 days in 100% humidity at 37°C. Ten microliters of *E. faecalis* bacterial suspension in BHI (OD_{600} = 0.5) was placed on the surface of the mixed material to allow direct contact between the bacteria and the tested materials. Plates were incubated at 37°C for 1 hour followed by the addition of 200 μL PBS to each well. Subsequently, media were serial diluted and plated, and colonies of surviving bacteria were calculated for each material and transferred to $\log_{10}$ (colony-forming units [CFUs]/mL).

Antimicrobial Assay of DSP-modified Sealers against *E. faecalis* Biofilm

The DCT and the membrane restricted test (MRT) were used to investigate the antimicrobial activity of sealers according to Kapralos et al[18]. Briefly, 10 μL *E. faecalis* culture in BHI (OD_{600} = 0.1) was applied on the outer surface of cell culture inserts. The inserts were then placed with the bottom up on BHI agar plates. Plates were incubated at 37°C for 24 hours. The inserts were then removed from the agar and washed gently with PBS to remove unattached bacteria. For DCT, experimental and stock sealers were directly applied to the biofilm formed on the surface of the inserts. For MRT, the sealers were applied on the inner surface of the inserts. Biofilms with no exposure to sealer were used as a positive control, and inserts with sealers and no bacterial growth were used as a negative control. After 24 hours (37°C), sealers were separated from inserts. Sealer samples and inserts were put in separate vials containing 10 mL PBS and vigorously vortexed. Samples were serial diluted and plated, and colonies of

surviving bacteria were calculated for each material as before.

Flow Test

The flow of stock and modified sealers was tested according to ISO 6876:2012 standardized protocols[19]. Briefly, 0.05 ± 0.005 mL sealer was placed on the center of a glass plate using a graduated syringe. At 180 ± 5 seconds after the commencement of mixing, a second glass plate was centrally placed on top of the sealer with an additional mass on this plate for a total of 120 ± 2 g. Ten minutes after the commencement of mixing, the weight was removed, and the maximum and minimum diameters of the compressed discs of sealer were measured.

Statistical Analysis

All experiments were conducted in triplicate. The 1-way analysis of variance test and the Tukey post hoc test were used for comparisons between groups. The level of significance was set at $P < .05$. Statistical analyses were performed using SPSS 20.0 software (IBM Corp, Armonk, NY).

RESULTS

OCT Release and Antimicrobial Activity of Modified Sealers against *E. faecalis*

The MIC of OCT against *E. faecalis* was found to be 4 μg/mL. Cumulative OCT release over 30 days from AH + 1% or 2% wt DSPs was below the MIC at <0.4 μg/cm^2 (1 μg/mL) (Fig. 1). BC + 1% and 2% wt DSPs sealers showed a higher and continuous release of OCT over 30 days (47 and 52 μg/mL for BC + 1% or 2%, respectively, or ~30 μg/cm^2). The amount of released OCT from BC over 30 days was <3.3% and 1.9% of the total loaded amount of OCT for BC + 1% and 2% wt DSPs, respectively, whereas the released amount from AH was negligible (less than 1/1000 of the total OCT load). BC + 1% wt DSPs had significantly less OCT release during the first 20 days compared with BC + 2% wt DSPs ($P < .05$); however, there was no significant difference in the release between these groups on days 25 and 30 ($P > .05$).

Antimicrobial Activity of DSP-modified Sealers against Planktonic *E. faecalis*

All freshly mixed sealers showed no bacterial growth compared with 6.63 ± 0.93 log CFU/mL for the control (no sealer) group (Fig. 2). AH with or without DSPs lost its antimicrobial activity after 7 days with no significant difference between the modified and unmodified sealers ($P > .05$). Unmodified BC showed reduced antimicrobial activity over time but was still able to reduce the number of bacteria by log 2 after 7 days (4.14 ± 0.09 log CFU/mL) and log 1 after 30 days (5.12 ± 0.1 log CFU/mL; $P < .001$). BC with 1% and 2% wt DSPs maintained antibacterial activity (no bacterial growth) over the 30-day experimental period.

Antimicrobial Assay on *E. faecalis* Biofilm

The control group showed bacterial growth of 8.1 ± 0.96 log CFU/mL. All freshly mixed materials showed complete killing of the bacterial biofilm after 24 hours in both DCT and MRT tests (Fig. 3).

Flow Test

Both AH and BC unmodified sealers were within the ISO 6876:2012 flow parameters with flow diameters >17 mm (Fig. 4). DSPs significantly decreased the flow of all modified sealers ($P < .05$). The reduction in flow was related to the DSPs' mass within the materials for the AH but not BC groups ($P < .05$). Except for AH + 2% wt DSPs (15.7 ± 0.35 mm), all modified sealers were within the acceptable ISO 6876 flow parameters.

DISCUSSION

In the present study, DSPs were synthesized, characterized, and incorporated into the root canal sealers AH and BC at 1% or 2% wt to enhance their long-term antimicrobial effects. The results of the present study showed that DSPs improved the antimicrobial activity of BC against *E. faecalis* throughout the 30-day experimental period while maintaining the flow of the sealer within ISO parameters. DSPs did not improve the antimicrobial properties of AH, and the addition of 2% wt DSPs reduced its flow to become incompliant with the ISO standards.

The current study investigated the OCT release from the set sealer for up to 30 days. Most studies to date have shown short-term (up to a week) release and/or release of antimicrobial only before setting.[10] The antimicrobial activity of the sealers against planktonic cells was tested using DCT for 30 days. It is important to assess the longer-term antimicrobial activity of sealers as they might be exposed to bacterial biofilms over time because the coronal seal may delay but not completely prevent microbial leakage over time[20]. DCT is a reliable test that measures quantitatively the contact antimicrobial effect of sealers (insoluble materials). It is reproducible and can be used in standardized settings simulating the contact of microorganisms with sealers within the root canal system[17,18].

The antimicrobial activity of the freshly mixed sealers against biofilms was tested using the DCT and MRT because there may not be direct contact between sealers and endodontic pathogens. The filter membrane protocol used in this study was adopted from a previous study[18]. It was not possible to test set sealers in the current model because preset sealer could not bond to the membrane and could not be removed as 1 part from the molds and attached to the membrane. Assessing only the impact of the material/OCT and not its interaction with the tooth was another limitation of the current investigation; in future investigations, the antimicrobial activity of the sealer should also be assessed using an *in vitro* tooth model[12].

Endodontic infections are mainly caused by complex multispecies biofilms[21]. However, the use of multispecies biofilm to test the antimicrobial properties of newly developed endodontic materials is limited by proper species selection and specificity of culture and transport media methods[22]. *E. faecalis* is occasionally detected as the only microorganism in the root canals of infected teeth, making this species appropriate for the initial assessment of endodontic sealers[23]. The strain of *E. faecalis* (ATCC 47077) used in the current study is a good representation of endodontic pathogens[24]. The current study showed that *E. faecalis* ATCC 47077 has intermediate susceptibility to OCT (4 μg/mL) compared with an MIC of 2 μg/mL for *E. faecalis* ATCC 29212 (American Type Culture Collection)[25], highlighting the importance of strain selection for endodontic assessment[26].

The amount of OCT released from BC sealers was continuous and above the MIC over the 30 days and significantly higher than the amount released from AH sealers. The OCT contained within the silica DSPs is weakly retained through intermolecular forces and is released upon DSP exposure to a suitable solvent such as aqueous media[11]. BC's hydrophilicity and higher susceptibility to dissolution likely explains the higher release of OCT observed compared with AH[27]. The results show that BC + 1% and 2% wt DSPs released only 1/30 and 1/52 of the total loaded OCT over 1 month, respectively. Although this may indicate a potential length of activity of 30–52 months under the "open sink" conditions used in the current study, it is expected that OCT release will last significantly longer in a clinical scenario, calculated to be in the order of decades[12]. DSP-containing sealer will be encapsulated within the canal systems and not exposed to frequent media replacement and associated dilution as in the current experimental conditions. Drug release outside the root canal system should remain

FIGURE 1 – Cumulative OCT release (μg/mL left y-axis, μg/cm^2 right y-axis) over 30 days from DSP incorporated sealers. The mean ± standard deviation, $N = 3$. Different letters represent significant difference between different groups at the same time point ($P < .05$). AH, AH Plus; AH1%, AH Plus with 1% wt DSPs; AH2%, AH Plus with 2% wt DSPs; BC, Bioceramic sealer; BC1%, Bioceramic sealer with 1% wt DSPs; BC2%, Bioceramic sealer with 2% wt DSPs.

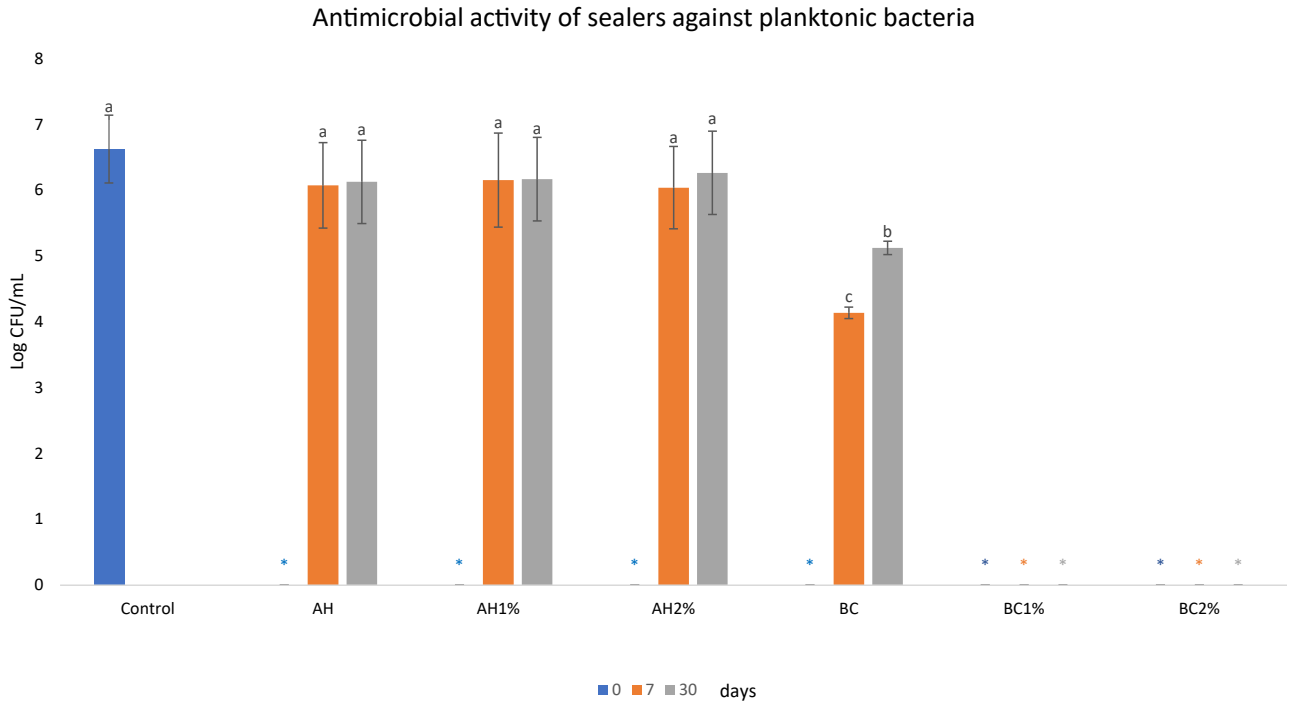

FIGURE 2 – The antimicrobial activity of sealers against planktonic bacteria. Mean log10 of CFU/mL after direct contact of DSP sealers with planktonic *E. faecalis* 47077 cells and control (no sealer) group. The mean ± standard deviation, $N = 3$. Different letters represent a significant difference between groups ($P < .05$). *Zero value (no bacterial growth). AH1%, AH Plus with 1% wt DSPs; AH2%, AH Plus with 2% wt DSPs; BC, Bioceramic sealer; BC1%, Bioceramic sealer with 1% wt DSPs; BC2%, Bioceramic sealer with 2% wt DSPs; Control, no exposure to sealer.

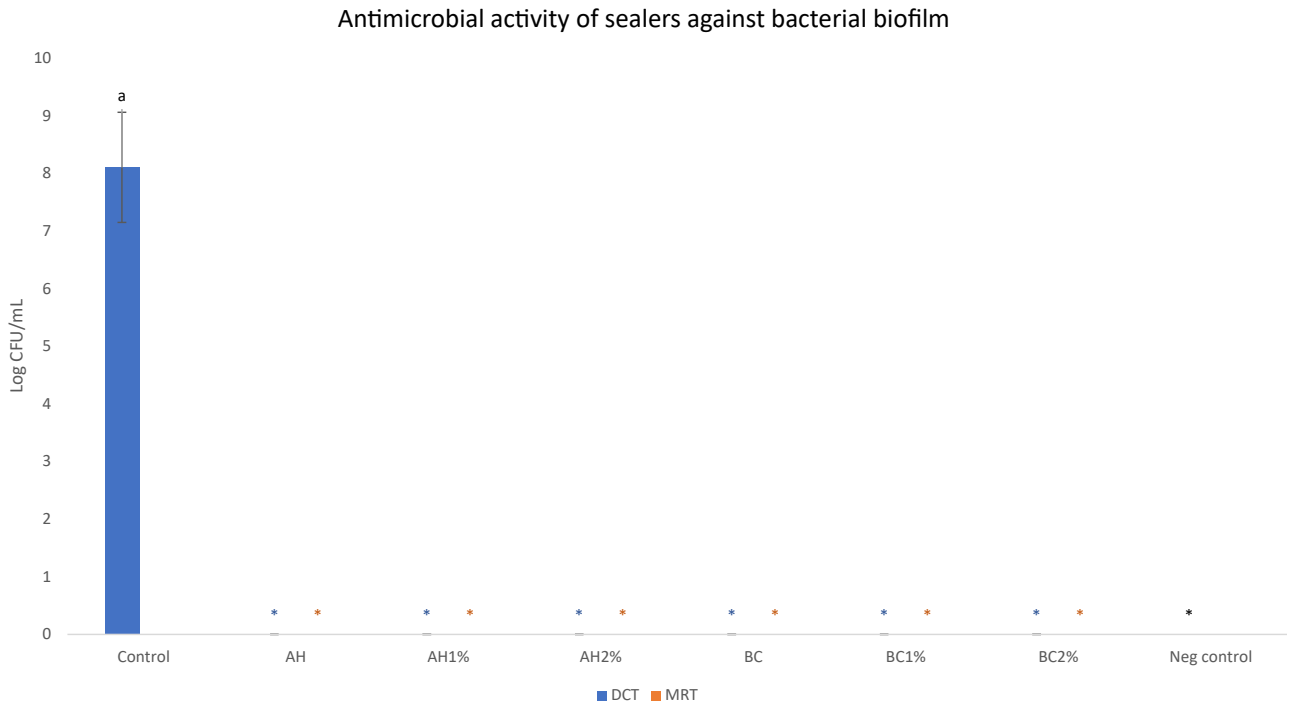

FIGURE 3 – The antimicrobial activity of sealers against bacterial biofilms. The mean log10 of CFU/mL after DCT and MRT of DSP sealers against biofilms of *E. faecalis* cells and the control group are displayed. The mean ± standard deviation, $N = 3$. Different letters represent a significant difference between groups ($P < .05$). *Zero value (no bacterial growth). AH1%, AH Plus with 1% wt DSPs; AH2%, AH Plus with 2% wt DSPs; BC, Bioceramic Sealer; BC1%, Bioceramic Sealer with 1% wt DSPs; BC2%, Bioceramic Sealer with 2% wt DSPs, Control, positive control (no exposure to sealer); Neg control, negative control (no bacteria).

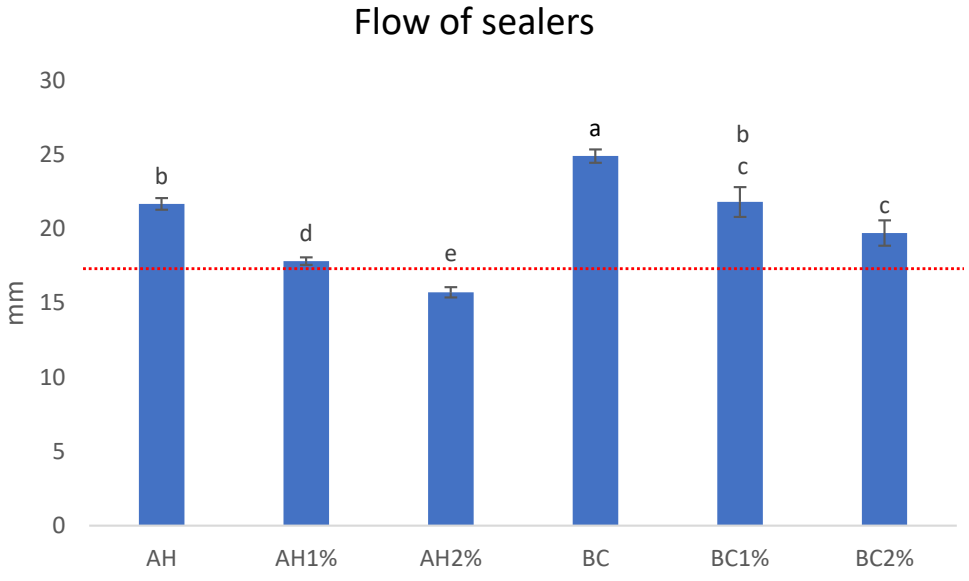

FIGURE 4 – The flow (mm) of unmodified and DSP-incorporated sealers. The mean ± standard deviation, $N = 3$. Different letters represent a significant difference between groups ($P < .05$). The horizontal line represents the ISO 6876:2012 minimum acceptable flow = 17 mm. AH1%, AH Plus with 1% wt DSPs; AH2%, AH Plus with 2% wt DSPs; BC, Bioceramic sealer; BC1%, Bioceramic sealer with 1% wt DSPs; BC2%, Bioceramic sealer with 2% wt DSPs.

negligible and ineffective for the same reasons, thus minimizing systemic exposure and the effect on the broader oral microbiota.

The inherent initial antimicrobial activity of unmodified AH is attributed to the release of low amounts of formaldehyde, unpolymerized bisphenol A diglycidyl ether, and amines during setting[28]. The current study showed that freshly mixed AH killed all planktonic bacterial cells, whereas after setting, it completely lost its antimicrobial activity. These results were consistent with other studies that showed loss of AH antimicrobial activity after setting[1,17,18]. DSPs had no effect on the antimicrobial properties of AH, possibly because of the

immobilization of the particles within the cured sealer and the inability of water to penetrate the set hydrophobic sealer and solubilize and release OCT from DSPs[29]. The biofilm experiments confirmed the ability of freshly mixed AH to completely kill all bacteria of *E. faecalis* in both the DCT and MRT because of the release of antimicrobial substances through the membrane during setting[18,28].

The antimicrobial activity of BC is attributed to the high pH it generates[8] and bioactive glass dissolution that interferes with bacterial cellular integrity via calcium phosphate precipitates[30]. The results of the current study showed that stock BC improved antimicrobial activity compared with AH, corroborating a previous study[17]. Incorporating DSPs within BC improved the antimicrobial activity of the sealer significantly throughout the experimental period, which could be attributed to OCT release due to the hydrophilicity of the sealer compared with AH.

The flow of endodontic sealers affects their ability to fill irregularities, accessory canals, and voids between core material and root canal dentin and penetrate dentinal tubules[31]. The flow of both sealers decreased with the addition of DSPs, corroborating previous studies that found that the addition of filler increased the viscosity of the resin[32]. The effect of filler on sealer viscosity is dependent on the particle's shape, concentration, and dimensions, impacting particle-particle and particle-resin interactions[33]. For AH, the reduction was proportional to the amount of DSPs added with no noticeable effect on the setting time of both sealers. All modified and unmodified sealers were within the acceptable parameters of ISO 6876 flow, except for AH + 2% wt DSPs.

CONCLUSIONS

Within the limitations of this *in vitro* study, it can be concluded that the incorporation of DSPs within bioceramic sealer, but not epoxy sealer, improved the material's long-term antimicrobial properties, whereas its flow remained compliant with the relevant ISO standard. DSPs could potentially reduce interfacial biofilm formation and proliferation and the incidence of secondary root canal infections when added to BC.

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The authors deny any conflicts of interest related to this study.

This is an independent study, although authors C.S. and Y.F. have filed a technology disclosure to the Research and Innovation Office, and a PCT application has been submitted based on the technology and material presented in the manuscript.

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