DENTAL MATERIALS 35 (2019) 1479–1489

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Novel root canal sealer with dimethylaminohexadecyl methacrylate, nano-silver and nano-calcium phosphate to kill bacteria inside root dentin and increase dentin hardness

Bashayer H. Baras[a,b,c], *Jirun Sun*[d], *Mary Anne S. Melo*[e], *Franklin R. Tay*[f], *Thomas W. Oates*[b], *Ke Zhang*[b,g,*], *Michael D. Weir*[b,*], *Hockin H.K. Xu*[b,h,i,*]

[a] *Biomedical Sciences, Biomaterials and Tissue Engineering Division, University of Maryland School of Dentistry, Baltimore, MD 21201, USA*
[b] *Department of Advanced Oral Sciences and Therapeutics, University of Maryland School of Dentistry, Baltimore, MD 21201, USA*
[c] *Department of Restorative Dental Science, College of Dentistry, King Saud University, Riyadh, Saudi Arabia*
[d] *Volpe Research Center, American Dental Association Foundation, Frederick, MD 21704, USA*
[e] *Division of Operative Dentistry, Department of General Dentistry, University of Maryland School of Dentistry, Baltimore, MD 21201, USA*
[f] *The Dental College of Georgia, Augusta University, Augusta, GA, USA*
[g] *Department of Orthodontics, School of Stomatology, Capital Medical University, Beijing, China*
[h] *Center for Stem Cell Biology & Regenerative Medicine, University of Maryland School of Medicine, Baltimore, MD 21201, USA*
[i] *Marlene and Stewart Greenebaum Cancer Center, University of Maryland School of Medicine, Baltimore, MD 21201, USA*

ARTICLE INFO

Article history:
Received 28 May 2019
Received in revised form
10 July 2019
Accepted 15 July 2019

Keywords:
Quaternary ammonium methacrylate
Silver nanoparticles
Calcium phosphate nanoparticles

ABSTRACT

Objectives. Root canal re-infection and weakening of roots are two main challenges in endodontics. The objectives of the study were: (1) to develop a novel root canal sealer containing dimethylaminohexadecyl methacrylate (DMAHDM), nanoparticles of silver (NAg), and nanoparticles of amorphous calcium phosphate (NACP), and (2) to investigate the effects on the physical, anti-biofilm, remineralizing ions, and hardness of human dentin for the first time.

Methods. Methacrylate-resin dual-cured root canal sealer contained 5% DMAHDM, 0.15% NAg, and NACP at 10%, 20% and 30% mass fractions. The flow, film thickness, and Ca and P ions release were investigated. The effects of NACP on radicular dentin hardness after treatment with sodium hypochlorite (NaOCL) and ethylenediaminetetraacetic acid (EDTA) were assessed. Antibacterial properties were measured against *Enterococcus faecalis (E. faecalis)*-impregnated dentin blocks; colony-forming units (CFU) and live/dead assays were measured.

* *Corresponding authors at*: Department of Advanced Oral Sciences and Therapeutics, University of Maryland School of Dentistry, Baltimore, MD 21201, USA.
E-mail addresses: tuzizhangke@163.com (K. Zhang), Michael.weir@umaryland.edu (M.D. Weir), hxu@umaryland.edu (H.H.K. Xu).
https://doi.org/10.1016/j.dental.2019.07.014

Enterococcus faecalis
Anti-biofilm
Remineralization

Results. Incorporating DMAHDM, NAg and NACP did not adversely influence the flow and film thickness properties. Sealer with 30% NACP neutralized the acid and increased the solution pH ($p < 0.05$). Sealer containing 30% NACP regenerated dentin minerals lost due to NaOCL and EDTA treatment, and increased the dentin hardness to match that of sound dentin ($p > 0.1$). Incorporating 5% DMAHDM and 0.15% NAg reduced biofilm CFU of *E. faecalis*-impregnated dentin blocks by nearly 3 logs when compared control group ($p < 0.05$).

Significance. The novel therapeutic root canal sealer with triple bioactive agents of DMAHDM, NAg and NACP neutralized acid, raised the pH, regenerated dentin minerals, increased root dentin hardness, and reduced dentin-block-impregnated biofilm CFU by 3 logs. This new sealer with highly desirable antibacterial and remineralization properties are promising to increase the success rate of endodontic therapy and strengthen the tooth root structures.

1. Introduction

Root canal therapy aims to remove pulpal and periapical inflammation and achieve maximal bacterial reduction [1,2]. The disinfection of the root canal space is accomplished through mechanical instrumentation, irrigants, and medicaments [1,2]. However, despite efforts to thoroughly disinfect the canal space, the main cause of root canal treatment failure remains the persistence of root canal microorganisms [1–3].

The root canal system is very complex in nature, which includes fins, isthmuses, and accessory canals that are difficult to access and disinfect [4]. In addition, the biological nature of bacteria organized in a biofilm structure poses another challenge [5]. A number of microbial species have been frequently isolated from canals with failed root canal treatment [2,5,6]. These microorganisms have demonstrated their ability to infiltrate and penetrate the dentinal tubules to a depth of 200–1500 μm, which makes them more difficult to eradicate through traditional instrumentation and irrigation techniques [7]. *Enterococcus faecalis* (*E. faecalis*), a gram-positive and facultative anaerobe, has been predominantly associated with persistent periapical infections with a detection rate of 23–77% in cases of failed endodontic treatment [8,9]. *E. faecalis* has shown to be one of the most resistant root canal microorganisms to eradicate [9]. Other bacterial species have also been frequently associated with endodontic infections. Previous studies utilized PCR detection methods and have shown a positive association between the presence of pre-operative symptoms and the presence of Streptococcus spp, Fusobacterium nucleatum and Porphyromonas gingivalis [2,8].

In addition to the application of chemical irrigants, an effective root canal filling system with potent antibacterial properties can entrap residual bacteria, insure the success of primary endodontic therapy, and prevent secondary endodontic infections. Root canal sealers come in direct contact to root dentin and can flow into small crevices in the canal wall that are otherwise difficult to access. Therefore, a highly desired property of root canal sealers is to possess antimicrobial properties [10]. Most root canal sealers show mild antimicrobial properties to some extent, mainly due to the release of some of their components that can have toxic effects on the bacterial cell, such as zinc oxide, eugenol, and paraformaldehyde. However, as the sealers set, their antimicrobial properties diminish [10,11].

One way to resolve this problem is through the incorporation of antibacterial agents into root canal sealers to substantially strengthen and prolong their antimicrobial effects. It would be a powerful approach to apply dual antibacterial agents: (1) One to target remote bacteria invading the depths of the dentinal tubules through the release of antibacterial ions; (2) the other to target bacteria that invade the sealer-dentin interface through contact-killing mechanisms. The dual antibacterial agents have the potential for complete disinfection of the root canal system and prevent recurrent infections in case of microleakage.

Silver (Ag) salts are antimicrobial and have been used in wound dressings [12]. Recently, silver salts were reduced to nanoparticles of silver (NAg) in dental resins, achieving a uniform dispersion of the NAg in the matrix [13]. Multiple mechanisms have been proposed to explain the antibacterial action of NAg that target multiple sites on the bacterial cell wall, causing disruptions in cell-wall synthesis, suppressing cell division, and preventing DNA replication [14]. When compared to traditional macro- and micro-sized silver particles, NAg with particle size of 2.7 nm can be incorporated at low filler levels while exerting strong antimicrobial properties, thus avoiding negative effects on the physical, mechanical, and color properties of the material [13,15].

Dental resins are increasingly used due to their esthetics and photo-polymerization capability [16–20]. Quaternary ammonium methacrylates (QAMs) are contact-active compounds that have been incorporated into resin composites, bonding agents, and root canal sealers [21–28]. QAMs showed strong and long-term antibacterial activities, without compromising the physical and mechanical properties [21–28]. Various chemical compositions of QAMs have been recently developed. 12-methacryloyloxydodecylpyridinium bromide (MDPB) was incorporated into a bonding agent, and a root canal filling system that demonstrated potent bactericidal effects while maintaining an effective resin-dentin bond [23,24]. In addition, quaternary ammonium polyethyleneimine (QPEI) nanoparticles were incorporated into a root canal sealer that was able to trigger bacterial

cell death within minutes of interaction, while maintaining the needed physical properties [26]. Another study incorporated ionic dimethacrylate monomers (IDMAs) having quaternary ammonium groups into methacrylate resins, which reduced bacterial colonization without affecting the viability of mammalian cells [28]. Recently, dimethylaminohexadecyl methacrylate (DMAHDM) was synthesized and immobilized in resins [29]. DMAHDM is a mono-methacrylate that has the ability to be copolymerized with the dimethacrylate dental resin matrix by forming covalent bonds through free radical polymerization and thus becomes immobilized upon polymerization. As a result, DMAHDM is not leached out or lost over time, thereby providing long-term contact-killing properties against oral biofilms [29]. DMAHDM showed strong antibacterial functions without jeopardizing the mechanical properties of the material [29]. A recent study developed an antibacterial root canal sealer through the incorporation of DMAHDM that greatly reduced bacterial viability and quantity, without compromising the physical and sealing properties of the sealer [30].

A significant challenge shown in previous studies is that the dentin structure and its organic components can inactivate and nullify the effects of antibacterial agents applied to the root canal [31]. To provide a more clinically-relevant assessment about the properties of antibacterial agents, dentin infection models have been used to account for the complex anatomy, chemical structure of the tooth, and their effects on biofilm inhibition [32,33]. To date, there has been no report on investigating the effectiveness of a root canal sealer containing DMAHDM and NAg on bacteria-impregnated root dentin.

In addition, irrigation solutions such as sodium hypochlorite (NaOCL) and ethylenediaminetetraacetic acid (EDTA) can adversely alter the structure of root dentin. Previous studies reported the effects of using these solutions on calcium (Ca) and phosphate (P) ions that comprise the major components of dentin, resulting in structural damage and reducing dentin hardness, which could lead to root fractures [34,35]. Recently, nanoparticles of amorphous calcium phosphate (NACP) were incorporated into resins and achieved high levels of Ca and P ion release [36–38]. These Ca and P ions can help regenerate the lost minerals and potentially improve the mechanical properties of root dentin. However, there has been no report on the effect of root canal sealer containing DMAHDM, NAg and NACP on the root dentin hardness.

The objectives of the study were to develop a novel root canal sealer containing triple agents DMAHDM, NAg and NACP, and investigate the effects on the physical, antibacterial, remineralizing ions, and hardness of human dentin for the first time. It was hypothesized that: (1) Incorporating NAg, DMAHDM and NACP would not compromise the paste flow and film thickness properties of the root canal sealer; (2) NAg and DMAHDM in the new sealer would inhibit the *E. faecalis* impregnated inside the dentin; (3) NACP in the new sealer would release high levels of Ca and P ions, neutralize the acidic pH, and enhance the dentin hardness.

2. Materials and methods

2.1. Formulation of an experimental endodontic sealer

The experimental sealer was a two-part dual-cured system. The major components of both parts were bisphenol A glycidyl dimethacrylate (BisGMA, Esstech, Essington, PA, USA) at 35.475%, triethylene glycol dimethacrylate (TEGDMA, Esstech) at 35.475%, 2-hydroxyethyl methacrylate (HEMA, Esstech) at 24%, and methacryloyloxy ethyl phthalate (MEP, Esstech) at 3%. Chemical-cure and photo-cure initiators were added, which consisted of 2% benzoyl peroxide (BPO, Sigma-Aldrich, St. Louis, MO, USA), 1% N,N-dihydroxyethyl-p-toluidine (DHEPT, Sigma-Aldrich) and 1% bisacylphosphine oxides (BAPO, Sigma-Aldrich). This resin was denoted "BTH". BTH had a chemical and a photo cure component to provide an immediate coronal seal upon light exposure to prevent microleakage from the oral cavity, while allowing the sealer to chemically cure in the deeper portions of the root canal [39,40].

2.2. Synthesis of NACP

NACP was synthesized using a spray-drying technique. Calcium carbonate and dicalcium phosphate anhydrous were dissolved in acetic acid to produce Ca and P ion concentrations of 8 and 5.333 mmol/L, respectively [41]. The solution was sprayed into a heated chamber, producing dried particles with a mean size of 116 nm, which were collected using an electrostatic precipitator [41]. Silanized barium boroaluminosilicate glass particles with a mean particle size of 1.4 μm (Caulk/Dentsply, Milford, DE, USA) were added as co-filler in the resin to mechanically reinforce the sealer. NACP were incorporated into the BTH resin at 10%, 20%, and 30% mass fractions, to determine the highest filler level of NACP to be incorporated without adversely influencing the physical properties of the sealer.

Therefore, four root canal sealers were formulated with the following fillers: (1) 40% glass; (2) 10% NACP + 30% glass; (3) 20% NACP + 20% glass; and (3) 30% NACP + 10% glass. The glass filler level was gradually reduced when NACP filler level was increased to maintain a similar working viscosity.

2.3. Synthesis of NAg

NAg was synthesized by dissolving silver 2-ethylhexanoate (Strem, Newburyport, MA, USA) of 0.1 g into 0.9 g of 2-(*tert*-butylamino) ethyl meth-acrylate (TBAEMA, Sigma- Aldrich) [13]. TBAEMA was used to allow the silver to fully dissolve by creating Ag-N coordination bonds [13]. In addition, TBAEMA contains reactive methacrylate groups that facilitate its chemical incorporation into the BTH resin. This method allowed the *in situ* formation of NAg with an approximate particle diameter of 2.7 nm, by using the polymeric matrix as the stabilizing agent for silver [13]. The NAg was incorporated into the BTH resin at 0.15% mass fraction, following the results of preliminary experiments that showed no adverse effects on the physical and sealing properties of the sealer.

2.4. Synthesis of DMAHDM

DMAHDM was synthesized based on a modified Menschutkin reaction as described previously [29]. Briefly, a tertiary amine group was reacted with an organo-halide by combining 10 mmol of 2-(dimethylamino) ethyl methacrylate (DMAEMA, Sigma-Aldrich), and 10 mmol of 1-bromohexadecane (BHD; TCI America, Portland, OR, USA) with 3 g of ethanol in a 20 mL scintillation vial. After the vial was stirred at 70 °C for 24 h, the solvent was removed via evaporation [29]. This produced DMAHDM as a clear and viscous liquid. DMAHDM was incorporated at 5% mass fraction into the sealer, based on previous studies [30] A commercial root canal sealer (AH Plus, Dentsply DeTrey, Konstanz, Germany) was included as comparative control. The following five root canal sealers were tested for flow, film thickness, solution pH and Ca and P ion release properties:

(1) AH Plus (referred to as commercial control);
(2) BTH + 40% glass + 5% DMAHDM + 0.15% NAg (referred to as DMAHDM + NAg);
(3) BTH + 30% glass + 5% DMAHDM + 0.15% NAg + 10% NACP (DMAHDM + NAg + 10NACP);
(4) BTH + % glass + 5% DMAHDM + 0.15% NAg + 20% NACP (DMAHDM + NAg+20NACP);
(5) BTH + 10% glass + 5% DMAHDM + 0.15% NAg + 30% NACP (DMAHDM + NAg + 30NACP).

2.5. Flow properties of the sealers

The paste flow ability of the sealer along the root canal wall and penetrating its complex anatomy is very important. The sealer should have a flow rate of ≥20 mm based on the International Standards Organization (ISO) 6876/2012 standards for root canal sealing materials [42]. The flow properties were tested based on the method of the ISO 6876/2012 [42]. A volume of 0.1 mL of the mixed sealer paste was placed on a glass slab (40 × 40 × 5 mm) using a graduated 1-mL syringe. Another glass slab weighing approximately 20 g was then placed on top of the sealer, followed by weight of approximately 100 g. This yielded a total mass of 120 ± 2 g. After 10 min, the weight was removed, and the diameter of the sealer was measured using a digital caliper (Mitutoyo, Tokyo, Japan) as the flow rate of the sealer.

2.6. Film thickness properties of the sealers

Root canal sealers are considered the weak link in root canal filling systems; mainly due to their susceptibility to degradation [39]. For that reason, their application is preferred in thin films. The incorporation of fillers and bioactive agents can increase the film thickness of sealers. Based on the ISO 6876/2012 standards for root canal sealing materials, root canal sealers should have a film thickness of ≤50 μm [42]. To measure the film thickness, two glass plates were combined together, and their combined thickness was measured. In the center of one plate, a portion of the mixed sealer was placed, and the other glass plate was placed on the top. With the aid of a loading device, a load of 150 N was applied on the top glass plate. Ten minutes after the start of mixing, the combined thickness of the two glass plates and the film of sealer placed in between were measured using a micrometer (iGaging, Los Angeles, CA, USA) [42].

2.7. pH analysis of the sealers

During secondary endodontic infections, the metabolic byproducts of anaerobic bacteria can reduce the pH of the canal environment to as low as 5 [43]. In addition, the application of acidic solutions, such as citric acid during endodontic therapy can add to the acidity of the root canal environment [44]. These acidic conditions can lead to root dentin demineralization and adversely affect the setting and sealing ability of some endodontic materials [43]. To evaluate the pH effects of the sealers, a sodium chloride (NaCl) solution (133 mmol/L) was buffered to pH 5 with 50 mmol/L acetic acid. Three specimens with dimensions of 2 × 2 × 12 mm were immersed in vials filled with 1 ml of the pH 5 NaCl solution, yielding a sealer volume/acid ratio of 0.14/1, following a previous study [45]. The pH of the solution was observed with a pH electrode (Orion, Cambridge, MA) for 60 min.

2.8. Ca and P ion release analysis of sealers

A sodium chloride solution (133 mmol/L) buffered to pH 5 with 50 mmol/L acetic acid was used [36–38]. A 50 mL of the pH 5 NaCl solution was used to immerse three specimens of approximately 2 × 2 × 12 mm to yield a specimen volume/solution of 2.9 mm^3/mL, following previous studies [36–38]. The concentrations of Ca and P ions released from the specimens were measured at 1, 3, 5, 7, 14, 21, and 28 days. At each time point, aliquots of 0.5 mL were taken and analyzed for Ca and P ions via a spectrophotometric method (SpectraMax M5) using known standards and calibration curves, following previous studies [36–38].

2.9. Dentin hardness analysis

Based on the results of the flow, film thickness, pH analysis, and Ca and P ion release experiments, the highest NACP mass fraction that did not compromise the physical properties of the sealers, while releasing highest levels of Ca and P ions, was used for the subsequent experiments. Therefore, the following groups were used in the dentin hardness test:

(1) Sound Dentin
(2) NaOCL and EDTA-treated Dentin
(3) AH plus-treated dentin
(4) BTH + 40% glass + 5% DMAHDM + 0.15% NAg (referred to as DMAHDM + NAg-treated dentin)
(5) BTH + 40% glass + 5% DMAHDM + 0.15% NAg + 30% NACP (referred to as DMAHDM + NAg + 30%NACP-treated dentin)

Dentin hardness serves as an indirect measurement of mineral gain or loss [46,47]. Teeth collection was approved by the University of Maryland Institutional Review Board. Fifty single rooted human teeth were collected (n = 10 for each of the 5 groups). The crowns of the teeth were sectioned off with a water-cooled diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) and the lengths of the roots were adjusted to 14 mm. All

roots were prepared and instrumented following a step-back technique. Teeth were then immersed in 5 mL of 5.25% NaOCl under constant shaking for one hour. Next, the teeth were irrigated with 2 mL of 17% EDTA solution for 3 min and received a final flush with distilled water [46]. Ten teeth were left unprepared and rinsed only with distilled water to serve as controls. Root canals were then primed with pyromellitic dianhydride glycerol dimethacrylate (PMGDM, Esstech) and 2-hydroxyethyl methacrylate (HEMA, Esstech) at a 10:3 mass ratio, with 50% acetone solvent, and then obturated with gutta-percha and one of the sealers. Ten teeth were left unobturated to show the effects of NaOCL and EDTA on root dentin. Teeth were restored with Cavit (3 M ESPE, Germany) and stored in distilled water for 1 month at 37 °C and 100% relative humidity [47].

After the evaluation period, teeth were sectioned horizontally at the roots' middle third with a water-cooled diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) to obtain sections of a thickness of approximately 1.5 mm. The dentin specimens were polished with abrasive papers at 320, 600 and 1200 grits consecutively [47]. The hardness measurements were conducted using a Vickers hardness tester (Shimadzu Micro Hardness Testers HMV-G, Shimadzu Corporation, Kyoto, Japan) with a 300 g load and 20 s dwell time. All measurements were taken at a 500 μm depth from the canal lumen [47].

2.10. *Preparation of E. Faecalis-impregnated dentin blocks for antibiofilm testing*

Single rooted maxillary anterior teeth were sectioned transversely with a water-cooled diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) at approximately 3 mm below the cement-enamel junction to obtain 1.5 ± 0.1 mm thick dentin sections (n = 4) [48]. The canal lumens were enlarged with a large water-cooled round bur (2.5 mm in diameter) [48]. All dentin sections were immersed in 5.25% NaOCL solution and followed by 17% EDTA, to remove the smear layer. They were then sterilized with ethylene oxide (Anprolene AN 74i, Andersen, Haw River, NC, USA) and de-gassed for 7 days.

The dentin samples were placed in micro-centrifuge tubes containing 500 μL of *E. faecalis* (ATCC 29,212) suspension in brain heart infusion broth (BHI, Sigma-Aldrich) which was adjusted to 10^7 colony-forming unit counts CFU/mL, based on the standard curve of OD_{600} nm versus the CFU/mL [33,48,49]. All tubes were centrifuged at 1400 *g*, 2000 *g*, 3600 *g*, and 5600 *g* in a sequence twice each for 5 min, based on a previous study [33]. After each centrifugation cycle, the solution was replaced with a fresh bacterial solution. The dentin samples were then placed in a 24-well plate filled with 1 mL BHI broth for 7 days to allow for biofilm formation [49]. The broth was renewed every other day. After 7 days, the dentin samples were placed in a new 24-well plate and rinsed with sterile phosphate-buffered saline (PBS, Sigma-Aldrich) [49]. The following groups were used in the antibiofilm testing to show the antibacterial effects of DMAHDM and NAg in comparison to control groups:

(1) Bacteria-Impregnated Dentin;
(2) AH Plus-treated Dentin;
(3) 30%NACP-treated Dentin;
(4) 30%NACP + DMAHDM + NAg-treated Dentin.

All root canal sealers were mixed and spatulated into the root canal space of the bacteria- impregnated dentin samples [49]. The sealers were allowed to set in the canal space for 7 days at 37 °C and 100% relative humidity [49]. A wet cotton pallet was placed on top of the dentin samples to prevent excessive drying of the dentin. Four dentin samples were treated with distilled water without any sealer to serve as control.

2.11. *Harvesting the E. Faecalis bacteria from the dentin blocks*

All dentin samples were placed in micro-centrifuge tubes filled with 1 mL PBS and sonicated for 5 min. (3510R-MTH; Branson Ultrasonics, Danbury, CT, USA) at a frequency of 40 kHz. This was followed by vortexing at maximum speed for 20 s using a vortex mixer (Fisher, Pittsburgh, PA, USA) to harvest the bacteria from dentin samples, following a previous study [50]. The harvested bacteria were serially diluted, plated on BHI agar, and incubated for 48 h at 37 °C with 5% CO_2 to count the number of colony-forming units (CFU) (n = 4).

2.12. *Live/dead bacterial staining of E. Faecalis-impregnated dentin blocks*

After preparing *E. faecalis*-impregnated dentin blocks and treating them with the different sealers as described previously, all samples were stained with the BacLight live/dead bacterial viability kit (Molecular Probes, Eugene, OR, USA). Green fluorescence was emitted from live bacteria stained with SYTO 9, red fluorescence was emitted from bacteria with compromised membranes stained with propidium iodide. All stained dentin samples were placed on an epifluorescence microscope (Eclipse TE2000-S, Nikon, Melville, NY) and images were obtained (n = 3) [50].

2.13. *Statistical analysis*

Statistical analyses were performed by SPSS 19.0 (SPSS, Chicago, IL, USA) at an alpha of 0.05. One-way analysis of variance was performed to detect significant effects of the variables. Tukey's multiple comparison test was performed to compare the data.

3. Results

The flow properties of the sealers are plotted in Fig. 1 (mean ± sd; n = 3). All experimental sealers had flow rates higher than AH Plus ($p < 0.05$). Increasing the NACP filler level to 20% and 30% reduced the flow rate when compared to that without NACP, but the flow was within the requirements of the ISO standards ($p < 0.05$).

The film thickness properties of the sealers are presented in Fig. 2 (mean ± sd; n = 3). Incorporating NACP did not significantly affect the film thickness of the sealer when compared to AH Plus and that without NACP ($p > 0.05$). All sealers had film thickness values that met the ISO requirement.

The effects of incorporating NACP on NaCl solution pH are plotted in Fig. 3 (mean ± sd; n = 4). The pH curve for (1)

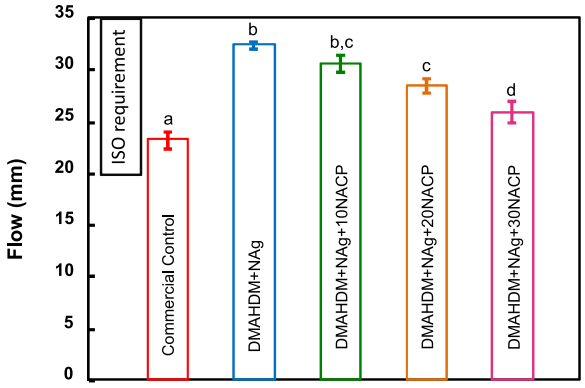

Fig. 1 – Sealer paste flow property (mean ± sd; n = 3). Incorporating 10% NACP did not significantly affect the flow when compared to that without NACP ($p > 0.05$). However, when 20% NACP was added, the flow was reduced ($p < 0.05$). It was still similar to that of AH Plus control ($p > 0.05$). Incorporating 30% NACP reduced the flow when compared to group without NACP but was higher than AH Plus and in accordance with ISO specifications. Dissimilar letters indicate values that are significantly different from each other ($p < 0.05$).

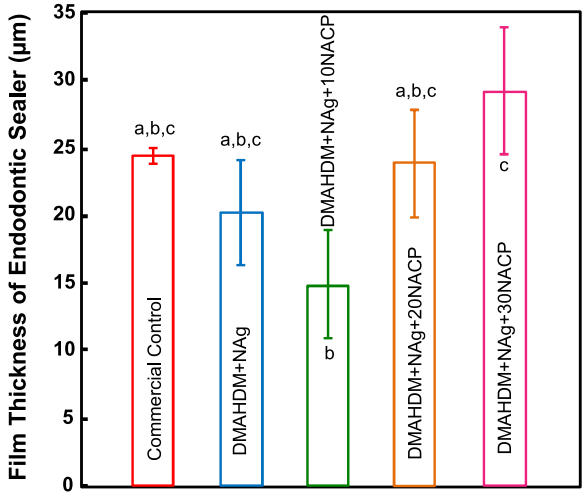

Fig. 2 – Sealer paste film thickness (mean ± sd; n = 3). Incorporating NACP at 10%, 20% and 30% mass fractions did not significantly affect the film thickness when compared to control and showed values in accordance with ISO specifications. Dissimilar letters indicate values that are significantly different from each other ($p > 0.05$).

Commercial control and (2) DMAHDM + NAg achieved minimal increase in pH and were similar to each other ($p > 0.05$). In contrast, due to NACP neutralizing the acids, sealers with 20 and 30% NACP significantly increased the pH ($p < 0.05$). The DMAHDM + NAg + 30NACP group achieved the greatest increase in pH.

The Ca and P ion releases from sealers are plotted in Fig. 4: (A) Ca ion release, and (B) P ion release (mean ± sd; n = 4). The released ion concentrations increased with time, indicating a continuous ion release within the tested time period. The ion concentrations increased with increasing NACP filler level,

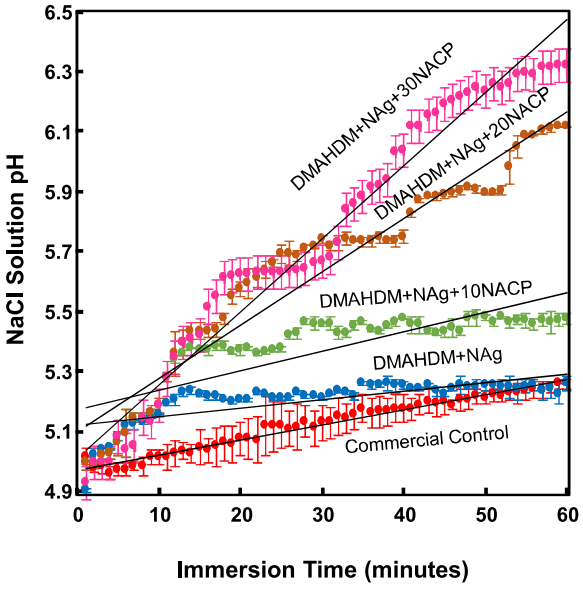

Fig. 3 – Effects of NACP acid-neutralization capability on NaCl pH 5 solution (mean ± sd; n = 4). Increasing the NACP mass fraction in the endodontic sealers increased the solution pH. Incorporating 30% NACP increased the solution pH to above 5.5 in 18 min, and further to pH 6.32 in 60 min.

with DMAHDM + NAg + 30NACP having the highest release of Ca and P ions.

The dentin hardness results are plotted in Fig. 5 (mean ± sd; n = 10). Treating dentin with 5.25% NaOCl and 17% EDTA reduced the hardness to 0.369 GPa. Group with DMAHDM + NAg+30NACP with remineralization effect was able to regenerate dentin minerals, increasing the dentin hardness to 0.516 GPa. It was not significantly different from the 0.519 GPa of sound, untreated dentin ($p > 0.05$).

Representative live/dead images of *E. faecalis*-impregnated dentin blocks treated with different sealers are shown in Fig. 6. The green color represents live bacteria. The red color represents bacteria with compromised membranes. An orange color represents live and dead bacteria on the top of each other. All groups were primarily covered by live bacteria, except for the group treated with DMAHDM + NAg, which was mainly covered by dead bacteria.

The biofilm CFU counts harvested from the *E. faecalis*-impregnated dentin blocks are plotted in Fig. 7 (mean ± sd; n = 6). The dentin blocks treated with commercial control had approximately the same CFU as control dentin without sealers ($p > 0.1$). The dentin blocks treated with the 30NACP sealer reduced the biofilm CFU by about 1 log, compared to control ($p < 0.05$). In sharp contrast, dentin treated with DMAHDM + NAg + 30NACP reduced the biofilm CFU by approximately 3 logs, compared to control ($p < 0.05$).

4. Discussion

This study developed a novel root canal sealer containing triple bioactive agents of NAg, DMAHDM, and NACP to inhibit root canal bacteria through the release of silver ions from NAg

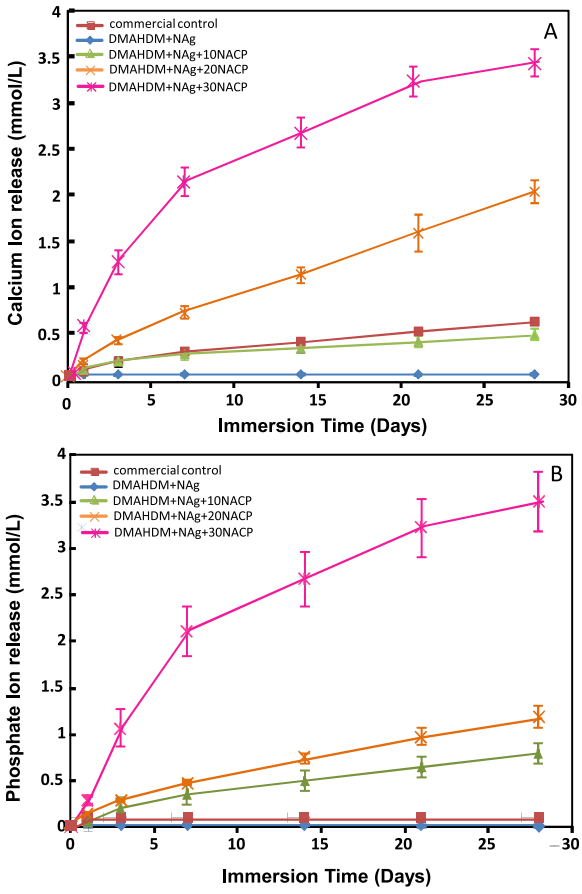

Fig. 4 – Calcium (Ca) ion release from endodontic sealers (mean ± sd; n = 4) in plot (A). Phosphate (P) ion release from endodontic sealers (mean ± sd; n = 4) in plot (B). Increasing the NACP mass fraction significantly increased Ca and P ion release ($p < 0.05$). Incorporating 30% NACP into the sealer resulted in the highest Ca and P ion release.

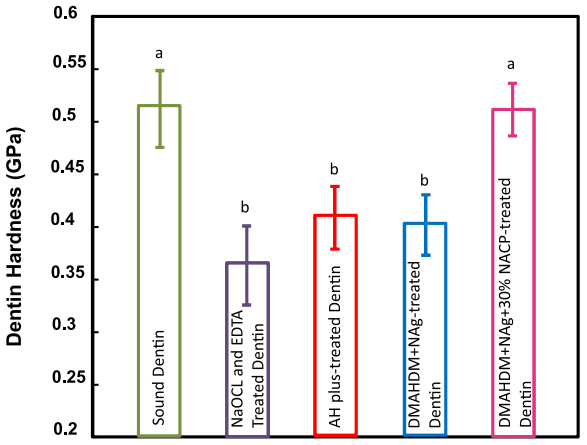

Fig. 5 – Hardness of dentin after various treatments (mean ± sd; n = 10). Treating dentin with NaOCl and EDTA significantly reduced the hardness when compared to sound, untreated dentin ($p < 0.05$). Treating dentin with sealer containing 30% NACP significantly increased the dentin hardness to match that of sound dentin. Dissimilar letters indicate values that are significantly different from each other ($p > 0.05$).

and through the contact-killing mechanisms of DMAHDM. In addition, the sealer could remineralize root dentin through the release of Ca and P ions to strengthen the tooth root structure and prevent potential root fractures. The novel root canal sealer containing 5% DMAHDM, 0.15% NAg, and 30% NACP neutralized the acid and increased the solution pH from 5 to 6.32. It released high levels of Ca and P ions without compromising the flow and film thickness properties of the sealer. Incorporating 30% NACP into the sealer regenerated dentin minerals lost due to adverse alterations caused by the applications of NaOCL and EDTA to root canal dentin. As a result, the dentin hardness was increased from 0.369 GPa to 0.516 GPa, matching that of sound dentin. The novel antibacterial root canal sealer containing NAg and DMAHDM inhibited *E. faecalis* bacteria impregnated inside dentin blocks and reduced the biofilm CFU by nearly 3 logs.

Clinical studies indicate that root canal bacteria can often tolerate the biomechanical procedures performed during root canal therapy, thus causing root canal treatment failure [1,2]. *E. faecalis*, a gram-postive facultative anaerobe, has been frequently isolated from canals of failed treatment cases [8,9]. This bacterium possesses several virulence factors and has shown its ability to adhere to dentin collagen, survive nutritional deprivation and suppress the effects of host lymphocytes, all of which contribute to root canal treatment failure [51–54]. Furthermore, *E. faecalis* organized in biofilms have been shown to resist calcium hydroxide intracanal dressings and NaOCL solutions by becoming 1000 times more resistant than their planktonic counterparts [51–54]. Efforts have been made to incorporate antibacterial agents into root canal filling systems. They either rely on the release of the antibacterial components or are otherwise immobilized in polymeric matrices [55–57]. One disadvantage of releasing antibacterial agents is the depletion of the antibacterial properties overtime, due to the burst release of these materials into the surrounding environment [55–57]. Contact-killing antibacterial agents immobilized within the material do not leach out, and can therefore produce long-term antibacterial effects [57]. However, these materials require bacteria to come in contact with their surfaces to exert their effects. A more desirable approach would be to combine the releasing agent and non-releasing agent together, which can overcome the disadvantages of a single agent. However, there has been no report of a root canal sealer with both contact-killing and release-killing capabilities.

Silver can kill more than 650 organisms, including highly drug-resistant strains [58]. Recently, NAg were synthesized in situ by forming nanoparticles in the resin matrix, thus avoiding the agglomeration associated with the direct incorporation of nanoparticles [13]. In a previous study, when NAg was incorporated into a bonding agent, it was able to inhibit bacteria in contact with its surface and bacteria away from its surface through the release of silver ions, while having no adverse effects on fibroblast cytotoxicity and micro-tensile bond strength [59].

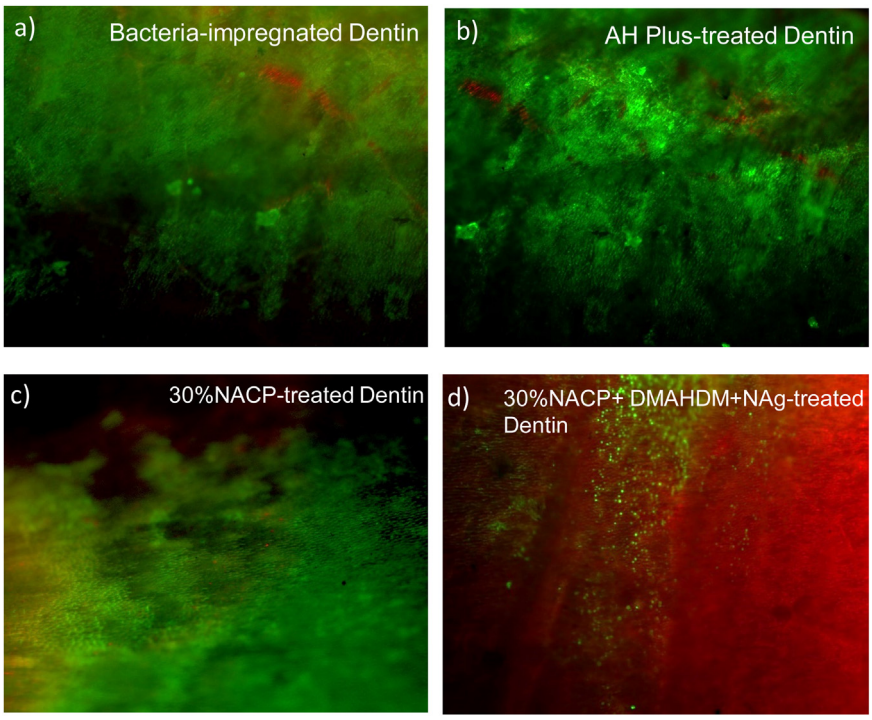

Fig. 6 – Live/dead staining images of biofilms in dentin blocks. Live bacteria were stained green. Dead bacteria were stained red. Yellow/orange colors represent live and dead bacteria on top of each other. Dentin blocks of (A) bacteria-impregnated dentin control, (B) AH Plus-treated dentin, and (C) 30%NACP-treated dentin were mostly covered with live bacteria. Dentin blocks of (D) 30%NACP + DMAHDM + NAg were mostly covered with compromised bacteria. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

QAMs with various chemical compositions were incorporated into resins to produce contact-killing and long-term antibacterial effects [21–28]. When the alkyl chain length of the QAMs was increased to 16, the antibacterial properties were improved [29]. This was mainly due to the increase in their hydrophobicity and positive charge that caused lysis of the negatively charged bacterial membrane [29]. The long cationic chains could penetrate the bacterial membrane resulting in the release of its cellular content [29]. The present study complemented the contact-killing properties of DMAHDM with the releasing effects of NAg to target remote bacteria that would be difficult to kill via contact-inhibition, thereby further enhancing the antibacterial potency of the sealer.

Previous studies showed potent antibacterial effects of DMAHDM or NAg against biofilms on composites and adhesives [29,30,36–38,50]. However, the effects of incorporating DMAHDM and NAg into a root canal sealer against bacteria impregnated into dentin sections have never been reported. The present study tested bacteria-impregnated human dentin blocks to better predict the effects of DMAHDM and NAg in clinical settings. Human radicular dentin was used because it represents the natural substrate to which root canal bacteria adhere and infiltrate [60]. In addition, dentin could produce inhibitory effects against common antibacterial agents such as chlorhexidine, potassium iodide, and calcium hydroxide [61,62].

The dentin infection model used in the present study was shown to effectively impregnate the bacteria into dentinal tubules [33]. In addition, the sonication/vortexing method used in this study was shown to successfully harvest all the bacteria from inside the dentin block [50]. The present study used 5% DMAHDM and 0.15% NAg, based on the results of previous studies that showed no negative effects on the physical and mechanical properties of the resin, while exerting potent antibacterial effects [30,36–38,50]. The DMAHDM- and NAg-containing sealer achieved a 3 log biofilm CFU reduction in viable *E. faecalis* impregnating the dentin block when compared to control groups. It is well known that *E. faecalis* bacteria is one of the more difficult bacterial strains to kill. A previous study showed a higher susceptibility of gram-negative bacteria to DMAHDM [63]. *E. faecalis* is a gram-positive bacterium, and therefore even higher bacterial killing efficacy is expected against gram-negative endodontic pathogens [63]. Future studies should utilize a multispecies root canal biofilm model to better investigate the effects of DMAHDM and NAg root canal sealer on a more complex biofilm structure.

Besides killing the bacteria, it is also important to protect the root structures, especially because of the adverse effects of common irrigation solutions (NaOCL and EDTA) on the mechanical properties of root dentin. These irrigants are usually used in large volumes and high concentrations in the clinical setting. Their effects on dentin were intensively studied [64–66]. Previous studies showed changes in dentin hardness and permeability due to these irrigants, which could weaken the tooth structure and lead to tooth root fractures

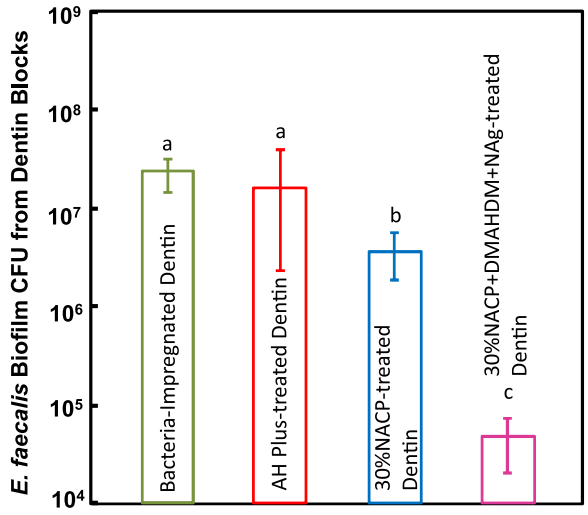

Fig. 7 – Colony-forming unit (CFU) counts of bacteria impregnated in dentin (mean ± sd; n = 6). Incorporating 5% DMAHDM + 0.15% NAg reduced the CFU counts by 3 orders of magnitude when compared to control groups. Dissimilar letters indicate values that are significantly different from each other ($p < 0.05$).

[64–66]. However, NACP could release high levels of Ca and P ions as shown in previous studies [26–38]. Therefore, the sealer containing NACP could potentially reverse the effects of irrigants applied to root dentin, regenerate the lost minerals, and strengthen and increase its hardness.

Furthermore, flow and film thickness of the sealers are also important properties. One of the most important functions of root canal sealers is to fill spaces difficult to access with instruments to prevent leakage and ensure a tight apical seal [39]. The sealer should have good flow properties to be able to flow along the entire canal wall. In addition, it is desirable for sealers to be placed in thin films, as they are more susceptible to polymeric degradation than the core materials. In the present study, incorporating DMAHDM, NAg, and NACP at the tested mass fractions did not adversely influence the flow and film thickness properties.

Another important property of this new sealer is its acid-neutralization ability. During secondary endodontic infections, bacteria can produce metabolites that can result in fluctuation of the root canal pH, lowering the pH to as low as 5 [43,44]. In addition, chelating solutions such as citric acid and lactic acid applied to the canal wall to remove the smear layer can further contribute to the acidity of the root canal [44]. The acidic root canal environment favors the growth of anaerobic bacteria and can also cause dentin demineralization [43]. The present study demonstrated that AH Plus and the sealer without NACP failed to raise the pH above 5.5, the critical pH at which demineralization occurs. However, the sealer with 30% NACP neutralized the acid, raised the solution pH to 5.5 in 18 min, and raised the pH to 6.3 in 60 min. This acid-neutralization effect is meritorious in protecting the root structures.

Indeed, the dentin hardness was substantially increased via the new sealer containing NACP. Treating the root canal dentin with 5.25% NaOCL and 17% EDTA significantly reduced the dentin hardness, consistent with previous studies [64–67]. However, when treated with the sealer with 30% NACP and water aged for 1 month, the minerals were regenerated and the dentin hardness was increased dramatically to match that of sound dentin, indicating a complete remineralization of the pre-dimineralized root dentin. Therefore, incorporating NACP as a remineralizing agent in the sealer is promising to improve the mechanical properties of the dentin and prevent tooth root fractures, which warrants further investigation.

5. Conclusion

This study developed a novel therapeutic root canal sealer with triple bioactive agents of DMAHDM for its contact-killing, NAg for distant-killing, and NACP for dentin remineralization and strengthening properties. The sealer with NACP neutralized acidic solution, raised the pH, and released Ca and P ions, without compromising the flow and film thickness properties. The sealer with NACP, DMAHDM and NAg regenerated the dentin minerals lost due to NaOCL and EDTA treatment, and increased the dentin hardness to match that of sound dentin. When tested against *E. faecalis*-impregnated dentin blocks, the novel sealer with DMAHDM and NAg reduced the biofilm CFU by nearly 3 logs, compared to control. This new sealer has the potential to ensure the success of primary endodontic therapy by targeting difficult-to-reach residual bacteria and preventing secondary endodontic infections in the case of future micro-leakage, while releasing high levels of Ca and P ions to strengthen and protect the tooth root structures.

Acknowledgments

We thank Drs. Nancy J. Lin, Pei Feng, Suping Wang and Joseph M. Antonucci for discussions and experimental help. This work was supported by University of Maryland School of Dentistry bridging fund (HX), and University of Maryland Seed Grant (HX).

REFERENCES

[1] de Paz L, Sedgley C, Kishen A. The root canal biofilm. Springer; 2015.
[2] Fouad AF. Endodontic microbiology and pathobiology: current state of knowledge. Dent Clin North Am 2017;61:1–15, http://dx.doi.org/10.1016/J.CDEN.2016.08.001.
[3] Lynch CD, Burke FM. Quality of root canal fillings performed by undergraduate dental students on single-rooted teeth. Eur J Dent Educ 2006;10:67–72, http://dx.doi.org/10.1111/j.1600-0579.2006.00397.x.
[4] Ahmed HMA, Neelakantan P, Dummer PMH. A new system for classifying accessory canal morphology. Int Endod J 2018;51:164–76, http://dx.doi.org/10.1111/iej.12800.
[5] Ricucci D, Siqueira JF, Bate AL, Pitt Ford TR. Histologic investigation of root canal–treated teeth with apical periodontitis: a retrospective study from twenty-four patients. J Endod 2009;35:493–502, http://dx.doi.org/10.1016/J.JOEN.2008.12.014.
[6] Sakko M, Tjäderhane L, Rautemaa-Richardson R. Microbiology of root canal infections. Prim Dent J 2016;5:84–9, http://dx.doi.org/10.1308/205016816819304231.

[7] Fan W, Wu D, Tay FR, Ma T, Wu Y, Fan B. Effects of adsorbed and templated nanosilver in mesoporous calcium-silicate nanoparticles on inhibition of bacteria colonization of dentin. Int J Nanomedicine 2014;9:5217–30, http://dx.doi.org/10.2147/IJN.S73144.

[8] Fouad AF, Zerella J, Barry J, Spångberg LS. Molecular detection of Enterococcus species in root canals of therapy-resistant endodontic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:112–8, http://dx.doi.org/10.1016/j.tripleo.2004.06.064.

[9] Rôças IN, Siqueira JF, Santos KRN. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod 2004;30:315–20, http://dx.doi.org/10.1097/00004770-200405000-00004.

[10] AlShwaimi E, Bogari D, Ajaj R, Al-Shahrani S, Almas K, Majeed A. In vitro antimicrobial effectiveness of root canal sealers against Enterococcus faecalis: a systematic review. J Endod 2016;42:1588–97, http://dx.doi.org/10.1016/J.JOEN.2016.08.001.

[11] Kayaoglu G, Erten H, Alaçam T, Ørstavik D. Short-term antibacterial activity of root canal sealers towards Enterococcus faecalis. Int Endod J 2005;38:483–8, http://dx.doi.org/10.1111/j.1365-2591.2005.00981.x.

[12] Miguel SP, Sequeira RS, Moreira AF, Cabral CSD, Mendonça AG, Ferreira P, et al. An overview of electrospun membranes loaded with bioactive molecules for improving the wound healing process. Eur J Pharm Biopharm 2019;139:1–22, http://dx.doi.org/10.1016/J.EJPB.2019.03.010.

[13] Cheng Y-J, Zeiger DN, Howarter JA, Zhang X, Lin NJ, Antonucci JM, et al. In situ formation of silver nanoparticles in photocrosslinking polymers. J Biomed Mater Res B Appl Biomater 2011;97B:124–31, http://dx.doi.org/10.1002/jbm.b.31793.

[14] Corrêa JM, Mori M, Sanches HL, Cruz da AD, Poiate E, Poiate IAVP. Silver nanoparticles in dental biomaterials. Int J Biomater 2015;2015:1–9, http://dx.doi.org/10.1155/2015/485275.

[15] Zhang K, Cheng L, Imazato S, Antonucci JM, Lin NJ, Lin-Gibson S, et al. Effects of dual antibacterial agents MDPB and nano-silver in primer on microcosm biofilm, cytotoxicity and dentine bond properties. J Dent 2013;41:464–74, http://dx.doi.org/10.1016/j.jdent.2013.02.001.

[16] Ferracane JL. Resin composite—state of the art. Dent Mater 2011;27:29–38, http://dx.doi.org/10.1016/J.DENTAL.2010.10.020.

[17] Ferracane JL. Resin-based composite performance: Are there some things we can't predict? Dent Mater 2013;29:51–8, http://dx.doi.org/10.1016/j.dental.2012.06.013.

[18] Ferracane JL, Hilton TJ. Polymerization stress – Is it clinically meaningful? Dent Mater 2016;32:1–10, http://dx.doi.org/10.1016/j.dental.2015.06.020.

[19] Vallittu PK, Boccaccini AR, Hupa L, Watts DC. Bioactive dental materials—do they exist and what does bioactivity mean? Dent Mater 2018;34:693–4, http://dx.doi.org/10.1016/J.DENTAL.2018.03.001.

[20] Watts DC. Let there be more light! Dent Mater 2015;31:315–6, http://dx.doi.org/10.1016/J.DENTAL.2015.03.001.

[21] Ferracane JL, Giannobile WV. Novel biomaterials and technologies for the dental, oral, and craniofacial structures. J Dent Res 2014;93:1185–6, http://dx.doi.org/10.1177/0022034514556537.

[22] Beyth N, Yudovin-Farber I, Bahir R, Domb AJ, Weiss EI. Antibacterial activity of dental composites containing quaternary ammonium polyethylenimine nanoparticles against Streptococcus mutans. Biomaterials 2006;27:3995–4002, http://dx.doi.org/10.1016/j.biomaterials.2006.03.003.

[23] Imazato S, Tay FR, Kaneshiro AV, Takahashi Y, Ebisu S. An in vivo evaluation of bonding ability of comprehensive antibacterial adhesive system incorporating MDPB. Dent Mater 2006, http://dx.doi.org/10.1016/j.dental.2006.01.005.

[24] Imazato S, Kinomoto Y, Tarumi H, Ebisu S, Tay FR. Antibacterial activity and bonding characteristics of an adhesive resin containing antibacterial monomer MDPB. Dent Mater 2003;19:313–9.

[25] Beyth N, Houri-Haddad Y, Domb A, Khan W, Hazan R. Alternative antimicrobial approach: nano-antimicrobial materials. Evidence-based complement. Altern Med 2015;2015:1–16, http://dx.doi.org/10.1155/2015/246012.

[26] Beyth N, Kesler Shvero D, Zaltsman N, Houri-Haddad Y, Abramovitz I, Davidi MP, et al. Rapid kill—novel endodontic sealer and Enterococcus faecalis. PLoS One 2013;8:e78586, http://dx.doi.org/10.1371/journal.pone.0078586.

[27] Beyth S, Polak D, Milgrom C, Weiss EI, Matanis S, Beyth N. Antibacterial activity of bone cement containing quaternary ammonium polyethyleneimine nanoparticles. J Antimicrob Chemother 2014;69:854–5, http://dx.doi.org/10.1093/jac/dkt441.

[28] Antonucci JM, Zeiger DN, Tang K, Lin-Gibson S, Fowler BO, Lin NJ. Synthesis and characterization of dimethacrylates containing quaternary ammonium functionalities for dental applications. Dent Mater 2012;28:219–28, http://dx.doi.org/10.1016/j.dental.2011.10.004.

[29] Li F, Weir MD, Xu HHK. Effects of quaternary ammonium chain length on antibacterial bonding agents. J Dent Res 2013;92:932–8, http://dx.doi.org/10.1177/0022034513502053.

[30] Baras BH, Wang S, Melo MAS, Tay F, Fouad AF, Arola DD, et al. Novel bioactive root canal sealer with antibiofilm and remineralization properties. J Dent 2019;83:67–76, http://dx.doi.org/10.1016/j.jdent.2019.02.006.

[31] Morgental RD, Singh A, Sappal H, Kopper PMP, Vier-Pelisser FV, Peters OA. Dentin inhibits the antibacterial effect of new and conventional endodontic irrigants. J Endod 2013;39:406–10, http://dx.doi.org/10.1016/J.JOEN.2012.10.028.

[32] Saleh IM, Ruyter IE, Haapasalo M, Ørstavik D. Survival of Enterococcus faecalis in infected dentinal tubules after root canal filling with different root canal sealers in vitro. Int Endod J 2004;37:193–8, http://dx.doi.org/10.1111/j.0143-2885.2004.00785.x.

[33] Ma J, Wang Z, Shen Y, Haapasalo M. A New noninvasive model to study the effectiveness of dentin disinfection by using confocal laser scanning microscopy. J Endod 2011;37:1380–5, http://dx.doi.org/10.1016/J.JOEN.2011.06.018.

[34] Lynch CD, Burke FM. Incomplete tooth fracture following root-canal treatment: a case report. Int Endod J 2002;35:642–6, http://dx.doi.org/10.1046/j.1365-2591.2002.00563.x.

[35] Saha SG, Sharma V, Bharadwaj A, Shrivastava P, Saha MK, Dubey S, et al. Effectiveness of various endodontic irrigants on the micro-hardness of the root canal dentin: an in vitro Study. J Clin Diagn Res 2017;11:ZC01–4, http://dx.doi.org/10.7860/JCDR/2017/24018.9472.

[36] Al-Dulaijan YA, Weir MD, Melo MAS, Sun J, Oates TW, Zhang K, et al. Protein-repellent nanocomposite with rechargeable calcium and phosphate for long-term ion release. Dent Mater 2018;34:1735–47, http://dx.doi.org/10.1016/J.DENTAL.2018.09.005.

[37] Al-Dulaijan YA, Cheng L, Weir MD, Melo MAS, Liu H, Oates TW, et al. Novel rechargeable calcium phosphate nanocomposite with antibacterial activity to suppress biofilm acids and dental caries. J Dent 2018;72:44–52, http://dx.doi.org/10.1016/J.JDENT.2018.03.003.

[38] al-Qarni FD, Tay F, Weir MD, Melo MAS, Sun J, Oates TW, et al. Protein-repelling adhesive resin containing calcium

phosphate nanoparticles with repeated ion-recharge and re-releases. J Dent 2018;78:91–9, http://dx.doi.org/10.1016/J.JDENT.2018.08.011.
[39] Kim YK, Grandini S, Ames JM, Gu L, Kim SK, Pashley DH, et al. Critical review on methacrylate resin–based root canal sealers. J Endod 2010;36:383–99, http://dx.doi.org/10.1016/j.joen.2009.10.023.
[40] Tay FR, Loushine RJ, Lambrechts P, Weller RN, Pashley DH. Geometric factors affecting dentin bonding in root canals: a theoretical modeling approach. J Endod 2005;31: 584–9.
[41] Xu HHK, Moreau JL, Sun L, Chow LC. Nanocomposite containing amorphous calcium phosphate nanoparticles for caries inhibition. Dent Mater 2011;27:762–9, http://dx.doi.org/10.1016/j.dental.2011.03.016.
[42] International Organization for Standardization. ISO 6876 dentistry: root canal sealing materials. Geneva, Switzerland: International Organization for Standardization; 2012.
[43] Mirhadi H, Moazzami F, Safarzade S. The effect of acidic ph on microleakage of mineral trioxide aggregate and calcium-enriched mixture apical plugs. Iran Endod J 2014;9:257–60.
[44] Wang L, Xie X, Li C, Liu H, Zhang K, Zhou Y, et al. Novel bioactive root canal sealer to inhibit endodontic multispecies biofilms with remineralizing calcium phosphate ions. J Dent 2017;60:25–35, http://dx.doi.org/10.1016/j.jdent.2017.02.011.
[45] Moreau JL, Sun L, Chow LC, Xu HHK. Mechanical and acid neutralizing properties and bacteria inhibition of amorphous calcium phosphate dental nanocomposite. J Biomed Mater Res B Appl Biomater 2011;98:80–8, http://dx.doi.org/10.1002/jbm.b.31834.
[46] Marending M, Luder HU, Brunner TJ, Knecht S, Stark WJ, Zehnder M. Effect of sodium hypochlorite on human root dentine – mechanical, chemical and structural evaluation. Int Endod J 2007;40:786–93, http://dx.doi.org/10.1111/j.1365-2591.2007.01287.x.
[47] Khallaf ME. Effect of two contemporary root canal sealers on root canal dentin microhardness. J Clin Exp Dent 2017;9, http://dx.doi.org/10.4317/jced.53052, e67–70.
[48] Albuquerque MTP, Nagata J, Bottino MC. Antimicrobial efficacy of triple antibiotic–eluting polymer nanofibers against multispecies biofilm. J Endod 2017;43:S51–6, http://dx.doi.org/10.1016/j.joen.2017.06.009.
[49] Wang Z, Shen Y, Haapasalo M. Dentin extends the antibacterial effect of endodontic sealers against Enterococcus faecalis biofilms. J Endod 2014;40:505–8, http://dx.doi.org/10.1016/J.JOEN.2013.10.042.
[50] Cheng L, Zhang K, Weir MD, Liu H, Zhou X, Xu HHK. Effects of antibacterial primers with quaternary ammonium and nano-silver on Streptococcus mutans impregnated in human dentin blocks. Dent Mater 2013;29:462–72, http://dx.doi.org/10.1016/j.dental.2013.01.011.
[51] Fouad AF, Barry J, Caimano M, Clawson M, Zhu Q, Carver R, et al. PCR-based identification of bacteria associated with endodontic infections. J Clin Microbiol 2002;40:3223–31, http://dx.doi.org/10.1128/jcm.40.9.3223-3231.2002.
[52] Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32:93–8, http://dx.doi.org/10.1016/j.joen.2005.10.049.
[53] Love RM. Enterococcus faecalis—a mechanism for its role in endodontic failure. Int Endod J 2001;34:399–405.
[54] Distel JW, Hatton JF, Gillespie MJ. Biofilm formation in medicated root canals. J Endod 2002;28:689–93, http://dx.doi.org/10.1097/00004770-200210000-00003, 10.1111/j.1600-9657.1990.tb00409.x.
[55] Shrestha A, Shi Z, Neoh KG, Kishen A. Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. J Endod 2010;36:1030–5, http://dx.doi.org/10.1016/j.joen.2010.02.008.
[56] Imazato S. Bio-active restorative materials with antibacterial effects: new dimension of innovation in restorative dentistry. Dent Mater J 2009;28:11–9.
[57] Balhaddad AA, Kansara AA, Hidan D, Weir MD, Xu HHK, Melo MAS. Toward dental caries: exploring nanoparticle-based platforms and calcium phosphate compounds for dental restorative materials. Bioact Mater 2019;4:43–55, http://dx.doi.org/10.1016/J.BIOACTMAT.2018.12.002.
[58] Bapat RA, Chaubal TV, Joshi CP, Bapat PR, Choudhury H, Pandey M, et al. An overview of application of silver nanoparticles for biomaterials in dentistry. Mater Sci Eng C 2018;91:881–98, http://dx.doi.org/10.1016/j.msec.2018.05.069.
[59] Li F, Weir MD, Chen J, Xu HHK. Comparison of quaternary ammonium-containing with nano-silver-containing adhesive in antibacterial properties and cytotoxicity. Dent Mater 2013;29:450–61, http://dx.doi.org/10.1016/j.dental.2013.01.012.
[60] Swimberghe RCD, Coenye T, De Moor RJG, Meire MA. Biofilm model systems for root canal disinfection: a literature review. Int Endod J 2019;52:604–28, http://dx.doi.org/10.1111/iej.13050.
[61] Portenier I, Haapasalo H, Ørstavik D, Yamauchi M, Haapasalo M. Inactivation of the antibacterial activity of iodine potassium iodide and chlorhexidine digluconate against Enterococcus faecalis by dentin, dentin matrix, Type-I collagen, and heat-killed microbial whole cells. J Endod 2002;28:634–7, http://dx.doi.org/10.1097/00004770-200209000-00002.
[62] Shrestha A, Kishen A. The effect of tissue inhibitors on the antibacterial activity of chitosan nanoparticles and photodynamic therapy. J Endod 2012;38:1275–8, http://dx.doi.org/10.1016/J.JOEN.2012.05.006.
[63] Wang L, Xie X, Weir MD, Fouad AF, Zhao L, Xu HHK. Effect of bioactive dental adhesive on periodontal and endodontic pathogens. J Mater Sci Mater Med 2016;27:168, http://dx.doi.org/10.1007/s10856-016-5778-2.
[64] Oliveira LD, Carvalho CAT, Nunes W, Valera MC, Camargo CHR, Jorge AOC. Effects of chlorhexidine and sodium hypochlorite on the microhardness of root canal dentin. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology 2007;104, http://dx.doi.org/10.1016/J.TRIPLEO.2007.04.019, e125–8.
[65] Aslantas EE, Buzoglu HD, Altundasar E, Serper A. Effect of EDTA, Sodium Hypochlorite, and Chlorhexidine Gluconate with or without surface modifiers on dentin microhardness. J Endod 2014;40:876–9, http://dx.doi.org/10.1016/j.joen.2013.10.041.
[66] Tartari T, Bachmann L, Zancan RF, Vivan RR, Duarte MAH, Bramante CM. Analysis of the effects of several decalcifying agents alone and in combination with sodium hypochlorite on the chemical composition of dentine. Int Endod J 2018;51, http://dx.doi.org/10.1111/iej.12764, e42–54.
[67] Mohammadi Z. Sodium hypochlorite in endodontics: an update review. Int Dent J 2008;58:329–41, http://dx.doi.org/10.1111/j.1875-595X.2008.tb00354.x.