Antibacterial Properties Associated with Chitosan Nanoparticle Treatment on Root Dentin and 2 Types of Endodontic Sealers

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Abstract

Introduction: The aim of this study was to evaluate the efficacy of carboxymethyl chitosan (CMCS) and chitosan nanoparticles (CNps) to inactivate bacteria and prevent biofilm formation at sealer-dentin interfaces. **Methods:** The study was divided into 3 stages: first stage, the experiment was conducted to analyze the antibacterial properties of CMCS in different formulations against biofilms; second stage, direct-contact and membrane-restricted methods were used to evaluate the antibacterial properties of an epoxy resin (ThermaSeal Plus; Dentsply Tulsa Dental, Tulsa, OK) and calcium silicate (MTA Fillapex; Angelus SA, Londrina, PR, Brazil) based-sealers with or without CNps; and third stage, biofilm formation at the sealer dentin interfaces of root dentin treated with CMCS and filled with gutta-percha and CNp incorporated sealer were analyzed after 1- and 4-week aging periods. The samples were treated and filled as follows: (1) distilled water: unaltered sealer (control group), (2) CMCS: sealer+CNps (CMCS group), and (3) CMCS/rose bengal: sealer+CNps (CMCS/RB group). *Enterococcus faecalis* was used to infect all the samples. Microbiological and microscopic analyses were used to assess the antibacterial characteristics. **Results:** CMCS-based treatments effectively killed bacteria adherent on root dentin ($P < .05$). The addition of CNps to ThermaSeal enhanced its antibacterial ability by direct-contact and membrane-restricted tests ($P < .05$). The CNp incorporation significantly increased the antibacterial efficacy of root canal sealers even after a 4-week aging time ($P < .05$). **Conclusions:** This study highlighted the ability of CMCS to disinfect root canal dentin and inhibit bacterial adhesion. CNps in root canal sealers are capable of maintaining their antibacterial activity even after prolonged aging. *(J Endod 2015;41:1353–1358)*

Key Words

Biofilm, carboxymethyl chitosan, chitosan, dentin, nanoparticles, sealer

Several chitosan-based treatments were proposed to eliminate the remaining bacteria after irrigation, promote dentin remineralization, and increase resistance to collagen degradation (1, 2). Chitosan is a nontoxic cationic, natural biopolymer that is usually obtained by the alkaline deacetylation of chitin, which is the main component of the exoskeleton of crustaceans (3). Chitosan has been established to possess antibacterial activity against a wide variety of fungi and bacteria (4, 5). The immobilization of phosphorylated chitosan on dentinal collagen was proposed to induce remineralization of demineralized dentin because its phosphate groups can bind to calcium ions to form a favorable surface for crystal nucleation. Consequently, these nucleating sites would induce the formation of a calcium phosphate layer on dentin (1), which can inhibit bacterial adherence (6). Chitosan after specific functionalization is also able to induce hydroxyapatite deposition (7).

Photodynamic therapy (PDT) uses a specific wavelength of light to activate a photosensitizer to produce singlet oxygen, which acts on multiple targets within the bacterial cell, resulting in cell death (8). Rose bengal (RB) is a photosensitizer used in PDT; RB may be immobilized on chitosan molecules by covalent interaction or can be used simultaneously with chitosan (9). This combination of PDT and chitosan produced high antibacterial properties and tissue stabilization characteristics during the treatment of infected dentin (2, 10).

Endodontic sealers are used to seal the space between core filling material and root canal walls to obtain a fluid impervious seal (11, 12). The antibacterial properties of endodontic sealer could provide added benefit of eliminating bacteria persisting within the root canals after cleaning and shaping procedures. The antimicrobial components of the endodontic sealers are released from their matrix into the dentinal tubules (11–13). However, with time, endodontic sealers can disintegrate and subsequently compromise the sealer-dentin interface (14). Previous studies have shown that the antibacterial effect of the zinc oxide–eugenol (ZOE) sealer was enhanced by the addition of chitosan nanoparticles (CNps) (13, 15). Similarly, the addition of CNps to a primer of methacrylate-based sealer also resulted in high antibacterial activity without compromising its adhesion force (16). Currently, there is no literature available on the possible advantages of incorporating CNps in 2 of the commonly used endodontic sealers: epoxy resin and calcium silicate–based sealers. The present study explores the potential antibacterial effects of CNps when used to condition root canal dentin and when added to an

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epoxy resin and a calcium silicate–based root canal sealer. Specifically, this study aimed to evaluate the efficacy of carboxymethyl chitosan (CMCS) and CNps to inactivate bacteria and prevent biofilm formation at sealer-dentin interfaces.

Materials and Methods

All of the chemicals used in this study were of analytic grade and were purchased from Sigma-Aldrich (St Louis, MO) unless otherwise stated. Chitosan was obtained from CarboMer Inc (San Diego, CA). CMCS and CNps were synthesized according to the protocols published by Laudenslager et al (17) and Kishen et al (13), respectively. The institutional ethical committee of the university approved this study.

First Stage: Antibacterial Effect of CMCS

Sample Sectioning. Bovine root canals were sectioned horizontally to obtain 30 discs with thicknesses of 5 mm. The 3 mm below the cervical third and above the apical third were discarded. The samples were instrumented using K-file size 45-80 (Dentsply Tulsa Dental, Tulsa, OK) and irrigated with 5% sodium hypochlorite (2 mL between each file), 17% EDTA for 3 minutes, and distilled water. The specimens were autoclaved, and the external surfaces were sealed with 2 coats of nail varnish.

Dentin Infection with *Enterococcus faecalis*. From a subculture of *E. faecalis* (American Type Culture Collection 29212), a standard suspension was prepared in brain-heart infusion (BHI) to obtain 1×10^7 colony-forming units (CFUs). The bacterial concentration was adjusted to an optical density of 0.1 (at 600 nm) per mL. Each sample was transferred to a 1.5-mL centrifuge tube and inoculated with 500 μL *E. faecalis* suspension. The tubes were centrifuged 4 times for 5 minutes at 5000*g* to facilitate bacterial penetration. A fresh cell suspension was added between each centrifugation. Then, each sample was incubated individually under continuous agitation at 37°C in 1 mL BHI for 21 days. BHI was changed every 48 hours. After the infection period, the samples were washed with 1 mL distilled water to remove nonadherent bacteria.

Treatment of Root Canal Surfaces. The specimens were divided into 3 groups (n = 10/group) according to the root canal treatment: G1, the samples were immersed in distilled water for 30 minutes (n = 10) (control); G2, the samples were inundated with 200 μL 1% CMCS for 15 minutes (n = 10); and G3, the samples were inundated with 500 μL 1 mg/mL CMCS/RB for 15 minutes and then filled with 1 mL perfluorodecahydronaphthalene to be irradiated with PDT (540 nm; LumaCare Inc, Newport Beach, CA) for 4 minutes (2 min/side) (n = 10). Finally, the specimens were filled with 500 μL calcium hydroxide solution (40.5 mmol/L) for 15 minutes at 37°C to induce biomineralization (15).

Microbiological Analysis. Largo Peeso Reamers ISO size 150 (Dentsply Maillefer, Ballaigues, Switzerland) were used to remove dentin powder (approximately 300 μm into the dentin) (18). Dentinal shavings were transferred to vials containing 1 mL BHI, vortexed for 10 seconds, and diluted to a concentration of 10^{-4}. Then, 100 μL of the solution was inoculated onto BHI agar and incubated for 48 hours at 37°C. Visible colonies were counted, and the CFUs were calculated (log CFU/mL). The experiment was performed in triplicate.

The data were evaluated for statistical significance using 1-way analysis of variance followed by the Tukey test for multiples comparisons. The significance level was fixed at $P < .05$. Prisma 5.0 (GraphPad Software Inc, La Jolla, CA) was used as the analytic software.

Second Stage: Antibacterial Effect of Sealers Associated with CNps

Sealer Preparation. Epoxy resin- and calcium silicate–based sealers were used in this study. ThermaSeal (TS) (Dentsply Tulsa Dental) and MTA Fillapex (MTA) (Angelus SA, Londrina, PR, Brazil) were prepared according to the manufacturer. Four groups were formed according to the addition of CNps to the sealer. In the G1 (n = 5) and G2 (n = 5) groups, the sealers were used in their original formulation (control). CNps were added to the sealers (1 g/150 mg) in the G3 (n = 5) and G4 (n = 5) groups. Portions of approximately 80 mg were placed in 96-well microplates for 7 days at 37°C and 100% humidity to allow the sealer to set.

Antibacterial Activity of the Sealers with and without CNps. The methodologies developed by Kayaoglu et al (14) and Kishen et al (13) with modifications were used for the direct-contact and membrane-restricted antibacterial experiments. Filter paper discs (Whatman no. 2, 4-mm diameter; Whatman Inc, Maidstone, UK) were immersed in an *E. faecalis* suspension (1×10^7 CFU) for 10 minutes, and then they were removed and drained of excess liquid. The contaminated paper discs were placed on the set sealers for 30 minutes at 37°C. Then, each paper disc (5/group) was transferred to a vial containing 1 mL BHI and vortexed for 30 seconds followed by mild ultrasonication for 5 minutes. The solution was diluted and incubated to allow for bacterial colony growth. The dilution, inoculation, incubation, CFU count, and statistical analysis were the same as described in the first stage. A filter membrane (Whatman, 0.22-μm pore size) was placed between the sealer and infected paper disc to perform the membrane-restricted antibacterial experiment. Experiments were performed in triplicate.

Third Stage: Microscopic Analysis of Bacterial Growth into Sealer-Dentin Interfaces

The aforementioned sectioning, treatment, and infection procedures were repeated, but the infection was performed after root canal filling. The preparation of the sealer with or without CNps was repeated also.

Root Canal Filling and Aging. Forty-eight sterile bovine dentin segments were irrigated according to groups described in the first stage and then filled with gutta-percha (GP) and sealers with or without the addition of CNps (Table 1, n = 8/group).

The sealers were placed manually to the root canal walls using an F5 ProTaper GP point (Dentsply Tulsa Dental). The specimens were obturated using 23-G injection needles with thermoplasticized GP (Calamus System, Dentsply Tulsa Dental). The warm gutta-percha was vertically condensed using a Paiva No. 2 plugger (Dentsply Industria e Comercio Ltda, Petropolis, Brazil). The filled root canals were stored for 7 days at 37°C and 100% humidity.

The specimens were subjected to aging at 37°C in a buffered solution containing 20 mmol/L 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid buffer solution (pH = 7), 1.5 mmol/L calcium as $CaCl_2$, and 0.9 mmol/L phosphate as KH_2PO_4 (15, 19). Samples were

TABLE 1. Sterile Bovine Dentin Segments Irrigated According to Groups Described in the First Stage and Then Filled with Gutta-percha and Sealers with or without the Addition of CNps (n = 8/group)

Groups	Treatment	*n*	Filling
Control	Distilled water	8/8	MTA/TS
CMCS	CMCS	8/8	MTA + CNps/TS + CNp
CMCS/RB	CMCS/RB	8/8	MTA + CNps/TS + CNp

CMCS, carboxymethyl-chitosan; CNps, chitosan nanoparticles; MTA, mineral trioxide aggregate; RB, rose bengal; TS, ThermaSeal Plus.