Novel Endodontic Disinfection Approach Using Catalytic Nanoparticles

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Abstract

Introduction: The aim of this study was to test a new disinfection technology using biomimetic iron oxide nanoparticles (IO-NPs) with peroxidaselike activity to enhance antibacterial activity on root canal surfaces and in dentinal tubules. **Methods:** The canal surfaces and dentinal tubules of single-rooted intact extracted teeth were infected by growing *Enterococcus faecalis* biofilms for 3 weeks. The samples were divided into 6 treatment groups: (1) phosphate-buffered saline (PBS) (negative control), (2) 3% hydrogen peroxide (H_2O_2) (test control), (3) IO-NPs (0.5 mg/mL) (test control), (4) IO-NPs (0.5 mg/mL) + 3% H_2O_2, (5) 3% sodium hypochlorite (positive control), and (6) 2% chlorhexidine (positive control). Environmental scanning electron microscopy coupled with energy-dispersive spectroscopy was used to confirm IO-NPs binding to the canal surface after a single treatment. Specimens were labeled with fluorescent staining for live/dead cells, and confocal laser scanning microscopy was used for the quantification of dead bacteria relative to the negative control (PBS). **Results:** Both biofilm formation and dentinal tubule infection were successfully recapitulated using the *in vitro* model. IO-NPs were capable of binding to the infected canal surfaces despite a single, short-term (5-minute) treatment. IO-NP activation of H_2O_2 killed significantly more *E. faecalis* present on the canal surfaces and at different depths of dentinal tubules when compared with all other experimental groups ($P < .05–.0005$). **Conclusions:** The results reveal the potential to exploit nanocatalysts with enzymelike activity as a potent alternative approach for the treatment of endodontic infections. *(J Endod 2017;■:1–7)*

Key Words

Biofilms, confocal microscopy, dentin disinfection, *E. faecalis*, nanocatalysts

Significance

A new disinfection technology using biomimetic iron oxide nanoparticles with peroxidaselike activity enhances antibacterial activity on root canal surfaces and in dentinal tubules.

The role of microorganisms as the primary cause of apical periodontitis has been well established (1), and thereby efforts have been directed toward eliminating them for higher success in endodontics (2). The disinfection process is challenging because of the complexity of the root canal system and the presence of isthmuses, accessory canals, and dentinal tubules, all of which can harbor bacteria and biofilms (3). Several studies have shown the presence of biofilm inside the root canal (4, 5) with bacterial penetration of dentinal tubules at varying depths (6, 7).

Mechanical preparation can physically remove tissue remnants, biofilms, and infected dentin (8). However, large portions of the root canal system after mechanical preparation may remain uninstrumented (9, 10). Although current chemical irrigants, such as chlorhexidine (CHX) and sodium hypochlorite (NaOCl), are effective antimicrobials, they are still incapable of eradicating bacterial infection with limited efficacy to completely disinfect dentinal tubules (11, 12).

Advances in nanotechnology have provided new and promising opportunities to kill bacteria, disrupt biofilm, and control dentinal tubule infection (13, 14). A wide range of nanoparticles (NPs) with antimicrobial activity have been developed including inorganic (particularly silver) and chitosan-based NPs. Although these NPs are a potentially effective technology for endodontic disinfection, the prolonged contact time required to achieve effective bacterial killing and the toxicity issues in silver NPs impose significant drawbacks (13). Nevertheless, newer NP formulations as well as other technologies (such as photodynamic therapy) have been reported to enhance biofilm elimination (13, 15).

Iron oxide nanoparticles (IO-NPs) have been widely used as contrast agents in magnetic resonance imaging because of their high biocompatibility and ability to penetrate tumor and atherosclerotic plaque, resulting in many Food and Drug Administration–approved formulations. Recently, biocompatible IO-NPs (Fe_3O_4) have been shown to have potent antibiofilm properties without deleterious effects on oral tissues *in vivo* (16). These NPs possess an intrinsic peroxidaselike activity, which enables them to catalyze hydrogen peroxide (H_2O_2) to generate free radicals in a pH-dependent manner. H_2O_2 is a commonly used disinfectant that displays antibacterial activity via free radical generation, but the process is slow with limited antibiofilm effects when used alone (17). Iron oxide nanocatalysts can potentiate the antibiofilm efficacy of H_2O_2. The IO-NPs retained within the biofilm after topical treatment can rapidly catalyze

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H_2O_2 at an acidic pH generating free radicals and killing the embedded bacteria within minutes, resulting in an effective and biocompatible treatment *in vivo* (16, 18).

Considering the complex endodontic environment and the difficulty of penetrating dentinal tubules, there is a potential opportunity to exploit nanocatalysts as a feasible therapeutic approach against biofilm infection of the root canal. Therefore, the aim of the study was to test whether catalytic IO-NPs can be used as a new endodontic disinfection technology to enhance antibacterial activity on root canal surfaces and in dentinal tubules.

Materials and Methods

Single-rooted intact extracted teeth were used in this study. Each tooth was horizontally sectioned at 1 mm below the cementoenamel junction to produce 4-mm dentin blocks. The canals were enlarged to a size 6 Gates Glidden drill (1.5 mm in diameter) (Tulsa Dentsply, Tulsa, OK). Each dentin block was split into 2 semicylindrical halves. The outer surfaces of each half were ground to achieve a standard thickness of 2 mm and to remove the root surface cement. Each specimen was treated with 5.25% NaOCl followed by 17% EDTA for 4 minutes using an ultrasonic bath for smear layer removal. Samples were rinsed in sterile water for 10 minutes thereafter to eliminate any residual chemicals. Specimens were then sterilized in an autoclave for 20 minutes at 121°C (Fig. 1*A–E*).

The clinical strain *Enterococcus faecalis* OG1RF isolated from retreatment cases (a gift from Dr. Brenda Gomes, State University of Campinas, São Paulo, Brazil) was used as a test organism for this study. Isolated colonies of pure *E. faecalis* culture grown in blood agar were suspended in brain-heart infusion (BHI) (Dot Scientific, Inc,

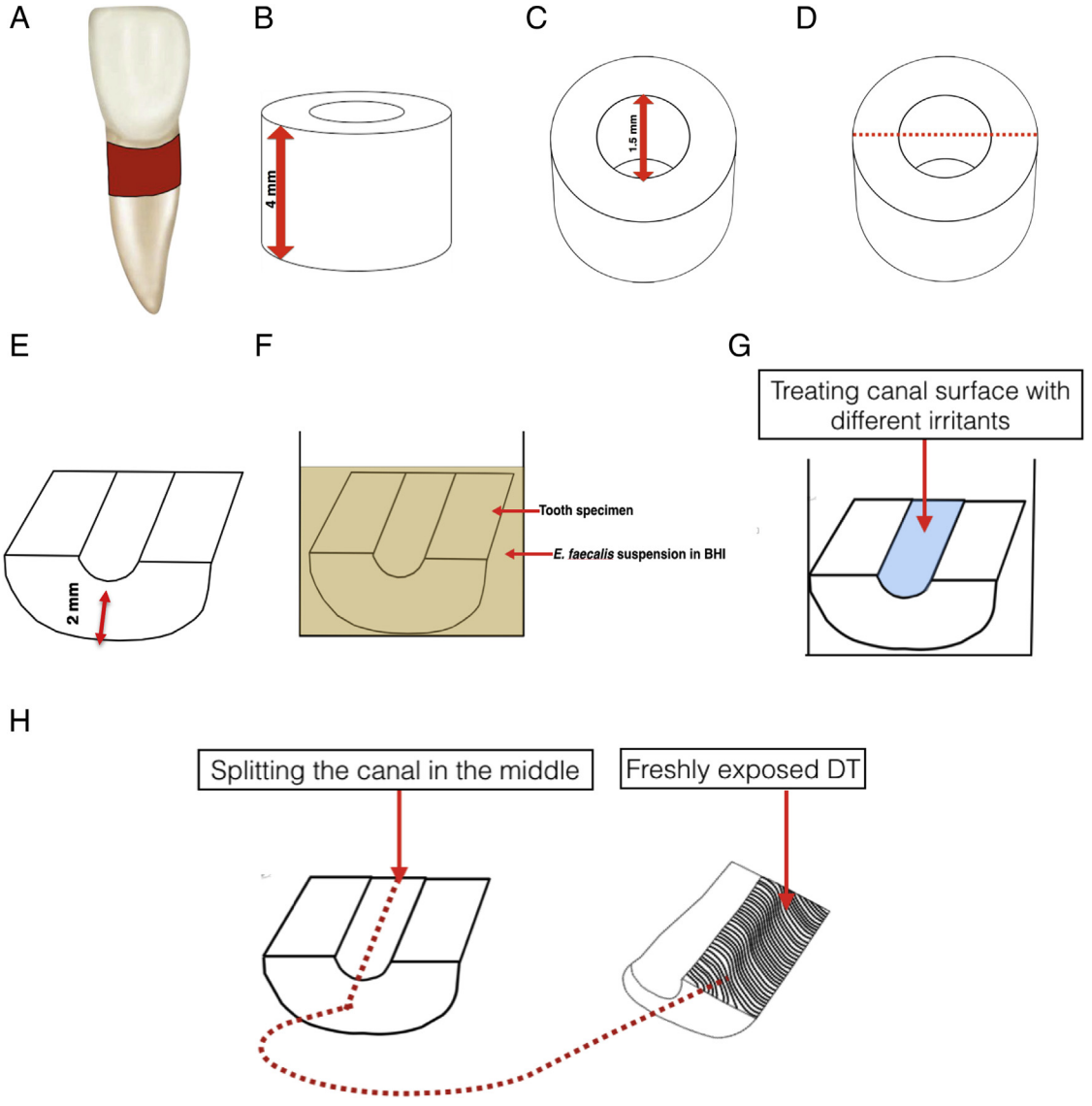

Figure 1. A schematic diagram of root canal sample preparation. (*A*) Single-rooted teeth; the area from which the dentin block is prepared marked with *red*. (*B*) A standardized 4-mm root dentin block. (*C*) Standardized root canal diameter preparation with size 6 Gates Glidden (1.5-mm diameter). (*D*) The vertical direction of block splitting into 2 semicylindrical halves. (*E*) The outer surfaces of the semicylindrical halves (the cemental side) were ground to achieve a standard thickness of 2 mm and to remove the root surface cement. (*F*) The specimen was inoculated with an overnight suspension of *E. faecalis* in BHI (2 mL) adjusted spectrophotometrically to OD600 of 0.5, which was grown for 3 weeks. (*G*) Disinfecting the sample with different control and test irrigants. (*H*) Splitting the canal surface to label freshly exposed dentinal tubules with LIVE/DEAD BacLight staining and examine them under confocal laser scanning microscopy.