Efficacy of 4 Irrigation Protocols in Killing Bacteria Colonized in Dentinal Tubules Examined by a Novel Confocal Laser Scanning Microscope Analysis

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Abstract

Introduction: The aim of this study was to determine the efficiency of 4 irrigation systems in eliminating bacteria in root canals, particularly in dentinal tubules. **Methods:** Roots of human teeth were prepared to 25/04, autoclaved, and inoculated with *Enterococcus faecalis* for 3 weeks. Canals were then disinfected by (1) standard needle irrigation, (2) sonically agitating with EndoActivator, (3) XP Endo finisher, or (4) erbium:yttrium aluminum garnet laser (PIPS) (15 roots/group). The bacterial reduction in the canal was determined by MTT assays. For measuring live versus dead bacteria in the dentinal tubules (4 teeth/group), teeth were split open and stained with LIVE/DEAD BackLight. Coronal, middle, and apical thirds of the canal dentin were scanned by using a confocal laser scanning microscope (CLSM) to determine the ratio of dead/total bacteria in the dentinal tubules at various depths. **Results:** All 4 irrigation protocols significantly eliminated bacteria in the canal, ranging from 89.6% to 98.2% reduction ($P < .001$). XP Endo had the greatest bacterial reduction compared with other 3 techniques ($P < .05$). CLSM analysis showed that XP Endo had the highest level of dead bacteria in the coronal, middle, and apical segments at 50-μm depth. On the other hand, PIPS had the greatest bacterial killing efficiency at the 150-μm depth in all 3 root segments. **Conclusions:** XP Endo appears to be more efficient than other 3 techniques in disinfecting the main canal space and up to 50 μm deep into the dentinal tubules. PIPS appears to be most effective in killing the bacteria deep in the dentinal tubules. *(J Endod 2016;42:928–934)*

Key Words

CLSM, dentinal tubules, EndoActivator, MTT assay, PIPS, root canal disinfection, XP Endo

The main goal of chemomechanical treatment of the root canal system is to eliminate or reduce bacterial populations in the canal to a level that can allow periradicular tissue healing with a positive treatment outcome (1–3). To render the canal bacteria-free is challenging. Mechanical debridement alone is limited in reaching all the root canal spaces (4, 5). Previous studies have demonstrated that only 40%–60% of the cases can have a negative culture after cleaning and shaping of the canals (6–10). Recent endeavors on advancing regenerative endodontics further underscore the importance of effective root canal disinfection (11). Thus, strategies for root canal disinfection should be directed to use more effective irrigation activation techniques that may maximize root canal disinfection. Multiple activation methods have been proposed to improve the efficacy of irrigants, including sonic, ultrasonic, negative apical pressure irrigation, as well as laser activation (12).

EndoActivator (EA) (Dentsply, York, PA) is a battery-operated sonic handpiece that uses plastic tips to agitate irrigant solutions vigorously. The activator tips are available in 3 different sizes and produce 2000–10,000 cycles/min. It is recommended to use after cleaning and shaping of the root canal system to activate the irrigation solution (13). Photon-induced photoacoustic streaming (PIPS) has been recently introduced and gained attention because of its properties that appear to enhance the disinfection of the root canal system (14–16). PIPS operates by transferring the energy to the irrigation molecules, resulting in rapid and powerful shock waves, forcing the irrigant throughout the entire root canal system (17). XP Endo Finisher (FKG Dentaire, Switzerland) is also a new file that has been recently introduced to be used as a final disinfection step to disturb the bacterial biofilm. It is claimed by the manufacturer to provide an optimal cleaning of the root canal system while preserving dentin.

The effectiveness of multiple irrigation techniques on reducing the bacterial count in the main root canal space has been previously investigated (18, 19). More recent studies have visualized bacteria in dentinal tubules and determined its status (live or dead) by confocal laser scanning microscopy (CLSM) (14, 20). Zou et al (21) have shown that NaOCl can penetrate into the dentinal tubules

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at a range between 77 and 300 μm, depending on time, concentration, and temperature. There is a lack of studies providing quantitative assessment for the level of bacterial reduction in the dentinal tubules after disinfection. Therefore, the aim of this study was to quantify and compare the bacterial viability in root canals treated by 4 different irrigation systems: standard needle irrigation (SNI), EA, XP Endo, and PIPS. We measured the level of bacterial reduction in the main canal by using a chemical method and in the dentinal tubules by using CLSM analysis.

Materials and Methods

Specimen Preparation

Intact mandibular premolars and molars with no apical resorption were collected from the clinic in the Department of Oral and Maxillofacial Surgery and placed in phosphate-buffered solution (PBS). The tooth sample collection in this study conformed to exempt protocols approved by the Institutional Review Board of University of Tennessee Health Science Center (12-01937-XM). Molars were vertically split into mesial and distal roots by using a water-cooled high-speed bur. Only distal roots with single canals were used in this study. The configuration of the single canal was confirmed through high magnification and buccolingual and mesiodistal radiographs. Composite resin was used to build the remaining walls of the coronal portion of the tooth crown to provide a reservoir for the irrigant. The crowns of the teeth were adjusted to a standardized working length of 18 mm. Canals were instrumented by using rotary files up to 25/04 (Endo Sequence; Brasseler) while maintaining apical patency. Teeth were then autoclaved in PBS at 121°C for 20 minutes.

Canal Inoculation with *Enterococcus faecalis*

A standard suspension (1×10^8 cells/mL) of *E. faecalis* (ATCC 47077; Rockville, MD) was prepared from a 24-hour culture of bacteria grown in brain heart infusion (BHI; Difco). Each canal was filled to the orifice level with *E. faecalis* suspension by using sterile 1-mL insulin syringes with a 30-gauge needle. The root was then placed in a 15-mL tube containing 10 mL BHI broth and incubated at 37°C for 21 days in 100% humidity to allow colonization of the bacteria on the canal wall and into the dentinal tubules. Aliquots of culture medium (5.0 mL) were replaced with fresh medium every 3 days.

Disinfection Procedures

After 21 days, specimens were removed from the inoculation tubes, and the root apexes were sealed with composite resin in a clean environment laminar flow cabinet to prevent sample contamination. The canals were disinfected by using 4 different irrigation systems/groups described below (15 teeth/group). In each procedure, the canals were irrigated with 2 mL 17% EDTA for 1 minute by using a 30-gauge side-vented needle, followed by 3 mL 6% NaOCl at a flow rate of 2 mL/min with the following cycles: 30 seconds of 6% NaOCl irrigation (1 mL/30 sec) followed by 30 seconds of no irrigation. Procedures were performed by one board-certified endodontist except for the PIPS group. Below are the different treatment protocols.

Group 1: SNI. A 30-gauge side-vented needle was placed within 2 mm from the working length and moved in a vertical motion to avoid the needle being locked in the canal. To ensure length control, a stopper was placed on the needle at the required length.

Group 2: EA. The canal was first passively filled with irrigant. The irrigation needle was then placed at the pulp chamber level, and under constant irrigation, a yellow EA tip was placed in the canal 1 mm short of the working length, and irrigant was activated by following the method described above.

Group 3: XP Endo. In a manner similar to EA, the file was placed 1 mm short of the working length and operated by using a slow-speed motor at 900 RPM in a vertical motion. Similar to SNI, a stopper was adjusted at the required length for length control.

Group 4: PIPS. Fotona LightWalker Er:YAG (Fotona LLC, Dallas, TX) was set at the recommended settings (20 mJ, 15 Hz, 0/0 air/water). For this part of the study, a clinician with experience in using PIPS operated the instrument. The canal was first passively filled with irrigant as described above, and under constant irrigation, the PIPS tip was placed in the pulp chamber and was submerged in irrigant as described previously (14). The tip was left stationary and activated for the cycles described under constant irrigation, while ensuring the canal and pulp chamber remained passively filled with irrigant throughout irrigation. If the pulp chamber was seen without irrigant, the cycle was stopped and then continued after replenishing the pulp chamber with NaOCl.

E. faecalis Sampling

After the 21-day bacterial incubation, *E. faecalis* in the root canal of each tooth was sampled before (S1) and after (S2) disinfection. All materials and instruments used in the following sample collection were sterile. For collection of S1 samples, the BHI broth in the canal space was first aspirated and then filled with PBS. A Hedström instrument #25 was used to file the dentinal walls vigorously (20 strokes). Canal content was then aspirated by using 1-mL insulin syringes with a 25-gauge needle and transferred to a microcentrifuge tube. This procedure was repeated 3 times/canal, and the final volume of collected bacteria in PBS was 100 μL for each tooth/canal. After S1 sample collection, each tooth/canal was disinfected by one of the disinfection methods described above, followed by flushing the root canal with 1 mL 10% sodium thiosulfate to neutralize the NaOCl. The canal was then ready for S2 sample collection. Each canal was treated and bacterial samples were collected with the same manner as that for S1 sample collection.

MTT Assay

Collected bacterial samples (S1 and S2) from the canals were subjected to a standard MTT assay to detect the viable bacteria. Ten microliters of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) agent (Sigma-Aldrich, St Louis, MO) was placed in each of the microcentrifuge tubes containing the bacteria samples. Samples were vortexed and then incubated at 37°C for 4 hours. Then 110 μL isopropanol/HCl was added to each tube to solubilize the formazan dye. Microcentrifuge tubes were then spun for 5 minutes at 6000 RPM, and 190 μL supernatant was placed in a 96-well plate. The optical density was read at 570 nm by using a SpectraStar Nano spectrometer (BMGLabTech, Ortenberg, Germany). Two blanks (PBS only) were included for each group as a negative control. Two samples in the PIPS group were excluded because of errors during sampling.

CLSM

To study the ability of each disinfection method to eliminate/kill bacteria in the dentinal tubules, we used CLSM to directly visualize the live/dead bacteria in the tubules. Eighteen intact mandibular premolars were used for this part of the study. Teeth were instrumented, autoclaved, and then inoculated with *E. faecalis* for 21 days and disinfected with the above-mentioned procedures