Infection of Apical Dentin and Root-end Cavity Disinfection

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Abstract

Introduction: The purpose of this study was to assess *Enterococcus faecalis* penetration into the dentin of the apical 3 mm and bacterial death after the application of either chlorhexidine or laser to root-end cavities. **Methods:** Root canals of 60 single-rooted teeth were prepared. In part 1, cementum was removed semicircumferentially from 21 roots, and the smear layer was removed from 15 roots using 17% EDTA/cetrimide. Teeth were inoculated and incubated with *E. faecalis* for 10 days, rinsed, and live/dead stained. The effect of cementum and smear on bacterial penetration was assessed by confocal laser scanning microscopy (CLSM). In part 2, 39 teeth had root ends resected and cavities ultrasonically prepared. Inoculated roots were assigned to 1 of the following 3 groups: (1) root-end cavities irrigated with 0.2 % chlorhexidine, (2) root-end cavities irradiated with a laser for 20 seconds at 1.5 W, or (3) root-end cavities that received no treatment. Roots were live/dead stained, sectioned, and examined by CLSM. The depth of the bacterial penetration and bacterial survival were compared using the Mann-Whitney *U* test. **Results:** The presence of a smear layer and/or cementum did not significantly affect bacterial penetration. In root-end cavities, chlorhexidine was more effective than laser ($P < .001$), reducing bacterial viability by 93% versus 70% with a laser. **Conclusions:** *E. faecalis* invaded the entire width of dentin in the apical 3 mm irrespective of the smear layer and/or cementum. Chlorhexidine was more effective than laser in disinfecting root-end cavities. *(J Endod 2012;38:1387–1390)*

Key Words

Bacterial viability study, chlorhexidine, dentin, endodontics, laser, root-end cavity

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Despite the clinician's best efforts, some root treatments fail (1). A persistent infection may result if any remaining bacteria are not isolated from nutrients. Bacteria must also be prevented from entering the root canal system where they can cause a secondary infection, which occurs mainly through coronal leakage and because of poor-quality root canal treatment (2). Nonsurgical retreatment is the option of choice, with surgery reserved for those few teeth in which retreatment is not possible (3). In such cases, the apical 3 mm of the root tip is resected, in part to remove anatomic and other variations that can harbor bacteria (4). The removal of all infected tissue in a root canal system is difficult, particularly at the root apex (5), and root resection opens communications in the form of potentially infected dentinal tubules even in the absence of a bevel (6). Efforts to kill microorganisms in the dentin of the cut root face and within the root-end cavity should be considered, and these might involve disinfectants such as chlorhexidine or laser irradiation. Disinfection could be facilitated by alternative methods of root face isolation (7).

Enterococcus faecalis is present in many failed root canal treatments (8) and is the most commonly reported bacterium present within the canals of nonhealing cases and asymptomatic infected roots (9, 10). The aims of the current study were to determine the extent of bacterial penetration of dentinal tubules in the apical region in an *ex vivo* infection model involving *E. faecalis* and to compare the antimicrobial effects of chlorhexidine irrigation and laser irradiation in an infected root-end cavity model.

Methods

Ethical approval was obtained from the University of Otago, Dunedin, New Zealand, to use extracted human teeth. Sixty unrestored single-rooted premolars with straight roots were collected and stored in 10% neutral-buffered formalin. The teeth were rinsed in 4% sodium hypochlorite (NaOCl) for 10 minutes to remove organic deposits and washed in running tap water in an ultrasonic bath for 2 days. Teeth were then decoronated with a water-cooled diamond saw.

The patency of the roots was confirmed using #10 K-files (Dentsply Maillefer, Ballaigues, Switzerland), and the working length was set by subtracting 0.5 mm from the length of the files when their tips were just visible at the apical foramina. Teeth were prepared to this length, finishing with an ISO 35 0.06 taper ProFile (Dentsply Maillefer) and 4% NaOCl irrigation, and then stored in 0.9% saline at 4°C until required.

Part 1 involved 21 prepared roots (Fig. 1). Cementum was removed semicircumferentially at the apex using contouring and polishing discs (Sof-Lex; 3M, St Paul, MN). The smear layer was removed from 15 of the 21 roots by immersion in 20 mL 17% EDTA/cetrimide (Endosure EDTAC; Dentalife Pty, Croydon, Australia) for 5 minutes followed by 5 minutes in 4% NaOCl and a thorough saline rinse.

E. faecalis V583 (Department of Microbiology, University of Otago) was maintained on brain-heart infusion (BHI) (Difco Laboratories, Detroit, MI) agar supplemented with 5% defibrinated horse blood. Cultures were incubated at 37°C for 24 hours under anaerobic conditions. Roots were placed in BHI (10 mL) and sterilized by autoclaving. Preparations were inoculated with 200 μL BHI culture of *E. faecalis* overnight (optical density, A_{600} >1.0, Ultrospec 6300 Pro; GE Healthcare, Piscataway, NJ) and incubated for 10 days with medium replacement every second day. The optical density of the sequential cultures confirmed substantial bacterial growth (A_{600} >1.0), and samples from final cultures were plated onto BHI blood agar to assess culture purity. The pH of uninoculated BHI was determined (pH 211 Meter; Hanna

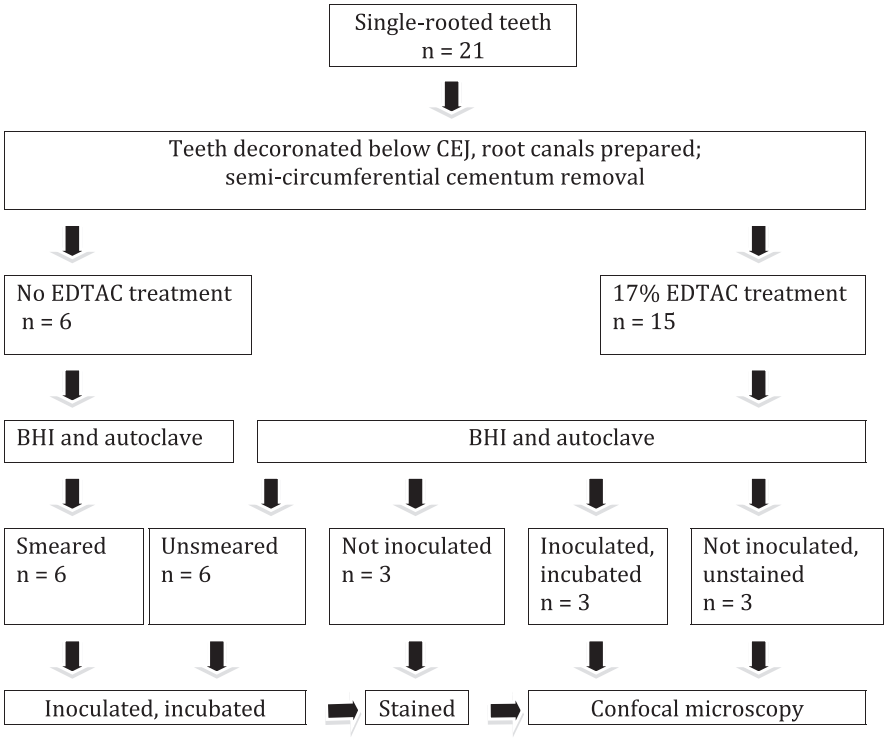

Figure 1. A flowchart for part 1.

Instruments, Woonsocket, RI), and after every 2-day growth of *E. faecalis*, culture supernatants were filtered (Millex 22 μm; Millipore, Carrigtwohill, County Cork, Ireland) and the pH measured. This was to assess changes that could potentially influence the smear layer.

Part 2 involved 39 roots (Fig. 2). Three millimeters of each apex was measured with a ruler, marked, and resected using a high-speed tungsten carbide surgical bur (H162; Komet, Brasseler, Germany). A root-end cavity was prepared in each root with an ultrasonic retrotip at power setting 8 (ProUltra SURG 2, Dentsply Maillefer). Cementum was removed with Sof-Lex discs, and the smear layer was removed by immersion in EDTAC for 5 minutes followed by 4% NaOCl and a thorough saline rinse.

The roots were infected as described previously and randomly allocated to 1 of 3 groups. In the chlorhexidine group (18 roots), the root-end cavities were irrigated with 10 mL 0.2% chlorhexidine solution (Multichem, Auckland, New Zealand) for 2 minutes using an irrigation needle (Monoject 27 $\times$ 1-1/4 slot; Covidien, Dublin, Ireland).

In the laser group (18 roots), the cavities were treated for 20 seconds at 1.5 W, 150 mJ, and 10 Hz with a Waterlase Er,Cr:YSGG laser fitted with an end-firing laser tip (AZ3, 320 μm, 25 mm; BIOLASE Technology, Irvine, CA). A 34% air and 24% water mix was used as coolant as recommended by the manufacturer, and the laser output was validated with an Ophir power and energy meter (Ophir Photonics, Jerusalem, Israel). The tip was introduced into the root-end cavity and fired with the tip moving in a circular motion to expose the cavity walls. A control group of 3 roots received no antimicrobial treatment.

Staining, Sectioning, and Measurement of Viable Bacteria

Viability staining, root sectioning, and microscopy were performed according to procedures described by Parmar et al (11). The roots were exposed to LIVE/DEAD stain (Baclight Bacterial Viability Test; Invitrogen, Eugene, OR) prepared according to the manufacturer's instructions. Three roots were placed in each tube of prepared

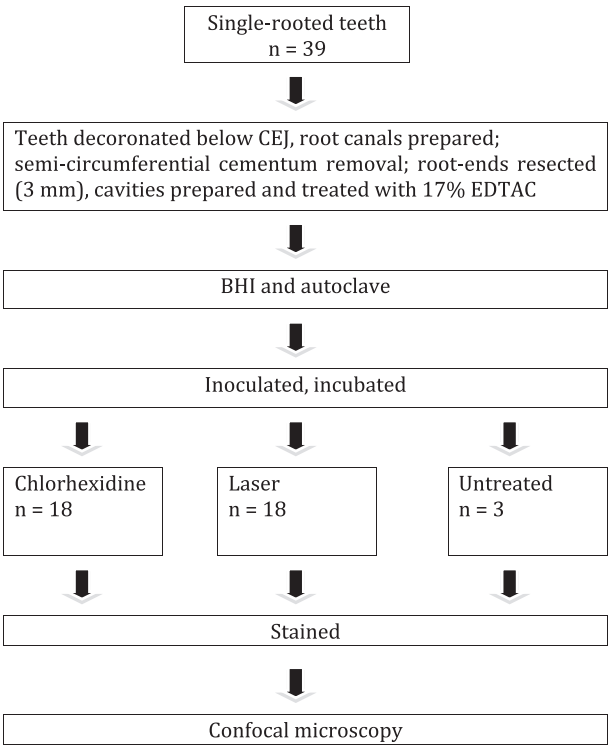

Figure 2. A flowchart for part 2.