Microbial Biofilm Proliferation within Sealer–Root Dentin Interfaces Is Affected by Sealer Type and Aging Period

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Abstract

Introduction: Root canal fillings are intended to prevent microbial proliferation over time in the canal after treatment. The objective of this study was to assess biofilm proliferation within the sealer-dentin interfaces of 2 methacrylate resin–based systems, self-etch (SE) and total-etch (TE), and an epoxy resin–based sealer (EP), aged for up to 6 months. **Methods:** Standardized specimens (n = 45) comprising the coronal 5 mm of human roots were filled with the test materials and gutta-percha. Specimens were either not preincubated (control, n = 9) or were incubated in sterile saline for 1 week, 1 month, 3 months, or 6 months (n = 3/group). Monospecies biofilms of *Enterococcus faecalis* were grown on the specimens for 7 days in a chemostat-based biofilm fermentor mimicking pathogenic oral conditions. The extent of *E. faecalis* proliferation within the sealer-dentin interface for each material and incubation period group was assessed by using fluorescence microscopy of dihydroethidium-stained specimens. **Results:** TE had less biofilm proliferation than both EP and SE ($P < .01$). Deeper biofilm proliferation was detected in SE and EP specimens aged for 1 and 3 months than those aged for 1 week or 6 months ($P < .05$). Maximum depth of biofilm penetration was recorded for SE at 1 month ($P < .05$). **Conclusions:** Within the test model used, the SE and EP sealers were more susceptible to interfacial biofilm proliferation than the TE restorative material. This susceptibility diminished after aging the materials' interfaces for 6 months. *(J Endod 2012;38:1253–1256)*

Key Words

Biofilm, *E. faecalis*, endodontic sealer, fluorescence microscopy, resin-composite, resin-dentin interface

Root canal fillings, comprising a core and a flowable sealer, should prevent bacterial ingress into the canal after treatment (1). Sealers that adhere or bond to root dentin (2) are expected to resist bacterial proliferation within the sealer-dentin interface (3). Epoxy resin (ER)–based sealers adhere to dentin and are considered the gold standard (4). Methacrylate resin (MR)–based sealers bond to conditioned dentin (5) by penetrating the tubules (6) and interlocking with dentin collagen, forming a hybrid layer (2, 7, 8). Two main MR-based systems are currently available. Total-etch systems require acid-etching, priming, and bonding to form a hybrid layer (8). Although they are the benchmark for bonded restorative systems (7), they are not available as commercial endodontic sealers. Self-etch systems use 1-step etching, priming, and bonding, incorporating the smear layer into the hybrid layer (9). They are available as endodontic sealers, but concerns have recently emerged about inadequacy of their bond (9, 10).

Bonding MR-based sealers to root dentin is challenging (2, 10, 11). Resin polymerization is inhibited by dentin exposure to sodium hypochlorite (12), shrinkage-related debonding occurs because of unfavorable cavity configuration (13), and interfacial degradation over time, allowing salivary and tissue fluid movement between the hybrid layer and dentin (14), may lead to bacterial proliferation and reinfection of the tooth (15).

Sealer-dentin interfaces have been studied by using *in vitro* models measuring penetration of dyes (16), endotoxins (17), inoculated bacteria (18), or saliva (19) and by assessment of their mechanical (10) and physicochemical (20) properties, with questionable clinical relevance (10, 20, 21). Our group has introduced the use of the chemostat-based biofilm fermentor (CBBF) for assessment of interfacial bacterial biofilm proliferation of the cariogenic biofilm organism *Streptococcus mutans* after aging of MR-dentin specimens (14). The purpose of the present study was to assess biofilm proliferation within the sealer-dentin interface of 2 MR-based systems, self-etch and total-etch, and an ER-based sealer by using the CBBF model.

Materials and Methods

Specimen Preparation and Aging

Intact human teeth with single canals (University of Toronto Human Ethics Protocol #24315) were sterilized by gamma-irradiation (4080 Gy) (22) and decoronated at the cementoenamel junction. Canals were negotiated to the apical foramen with K-files (Lexicon Flex SSK; Dentsply Tulsa Dental Specialties, Tulsa, OK) and cleaned and shaped with ProTaper rotary instruments (Dentsply Tulsa Dental Specialties) up to a size F4 at the foramen, with intermittent 5.25% sodium hypochlorite irrigation.

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The last rinse with 5 mL of 5.25% NaOCl was activated with the EndoActivator (Dentsply Tulsa Dental Specialties) to sonically agitate the irrigation solution. Smear layer was removed with 5 mL of 17% ethylenediaminetetraacetic acid solution (2) (Vista Dental, Racine, WI), followed with 5 mL of 5.25% NaOCl and a final flush with 10 mL distilled water. Canals were then dried with paper points. Roots were randomly divided into 3 sealer-type groups (n = 18/group): EP, an ER-based sealer (AH Plus Dentsply, Konstanz, Switzerland); SE, an MR-based self-etch sealer (RealSeal; SybronEndo, Orange, CA); and TE, an MR-based total-etch restorative material (Adper Scotchbond multi-purpose; 3M, St Paul, MN and Bisfil 2B self-cured resin; Bisco, Schaumburg, IL).

In EP and SE, sealers were applied with a lentulo (Dentsply), canals were filled with injectable gutta-percha (Elements; SybronEndo) compacted with Schilder pluggers (Dentsply), and the coronal end was light-cured (SE only). Canals in TE were etched with 37% phosphoric acid (Bisco) for 15 seconds, rinsed with sterile water for 15 seconds, lightly air-dried, treated with Scotchbond primer and adhesive, light-cured, coated with Bisfil 2B, and filled with RealSeal SE gutta-percha points (SybronEndo) by using passive lateral compaction.

Filled roots were stored for 72 hours at 37°C and 100% humidity (Hera Cell 150; Heraeus, Newton, CT) and sectioned horizontally 5 mm from the coronal end with a slow-speed water-cooled rotary diamond disk (Brasseler, Savannah, GA) under sterile conditions, obtaining standardized 5-mm-long specimens. Peripheral cementum, apical surfaces, and exposed coronal dentin adjacent to root fillings were coated with nail varnish to prevent bacterial access to the sealer-dentin interface through cut dentinal tubules. Specimens were subjected to aging in vials with sterile phosphate-buffered saline (PBS) and incubated (37°C, pH 7.0) for 1 week or 1, 3, or 6 months (n = 3/material group/time).

Biofilm Cultivation

Aged specimens were suspended in CBBF (37°C) to cultivate monospecies biofilms of *Enterococcus faecalis* (ATCC 47077) over interfacial margins (14), under continuous flow of fresh tryptic soy broth (BD Bioscience, Sparks, MD) with 0.25% (wt/vol) glucose, at 0.72 L/d (23) and dilution rate D = 0.075/h, mimicking the resting salivary flow rate (15).

Specimens were aseptically removed after 7 days and gently rinsed with sterile PBS. To assess bacterial viability, a 10-mL sample was collected from each vial, serially diluted, and spot-plated in triplicate onto brain heart infusion agar for bacteria colony-forming unit (CFU) counting after 24 hours of incubation at 37°C. Bacterial counts in the order of 10^9 CFU/mL were obtained for all tested samples.

Outcome Assessment

Specimens were stained with dihydroethidium (Invitrogen, Molecular Probes, Carlsbad, CA) and examined with an epifluorescence microscope (DMIRE2; Leica Microsystems, Wetzlar, Germany). Red fluorescence was visualized with TX2 filter cube (excitation BP560/40, dichroic 595 nm, emission BP645/75).

Biofilm proliferation and individual bacterial cell penetration were analyzed at 4 cardinal points (×100 magnification). Captured images (CCD; Hamamatsu, Shizuoka, Japan) were processed (Openlab 4.0.2; Improvision, Waltham, MA), and Z-stack series were established in a corono-apical direction for each specimen in 5-μm increments up to 500 μm. 3-dimensional (3D) images of biofilm formation were reconstructed from Z-stack series by Image J software (National Institutes of Health, Bethesda, MD) (14).

Analysis

We performed 1-way analysis of variance and least significant difference post hoc analysis ($P < .05$) to determine the effects of material and aging period on biofilm proliferation and bacterial cell penetration along the sealer-dentin interfaces.

Results

Representative 3D image reconstructions from Z-stack series captured from 1-month-aged specimens revealed different patterns of interfacial *E. faecalis* biofilm formation for EP, SE, and TE groups (Fig. 1, *top*). Z-stack images depict individual bacterial cells at different depths within the interfaces of all test materials (Fig. 1, *bottom*).

Mean interfacial biofilm proliferation depth ranged across test materials and aging periods (Fig. 2). When aging periods were lumped together, biofilm proliferation depth differed significantly

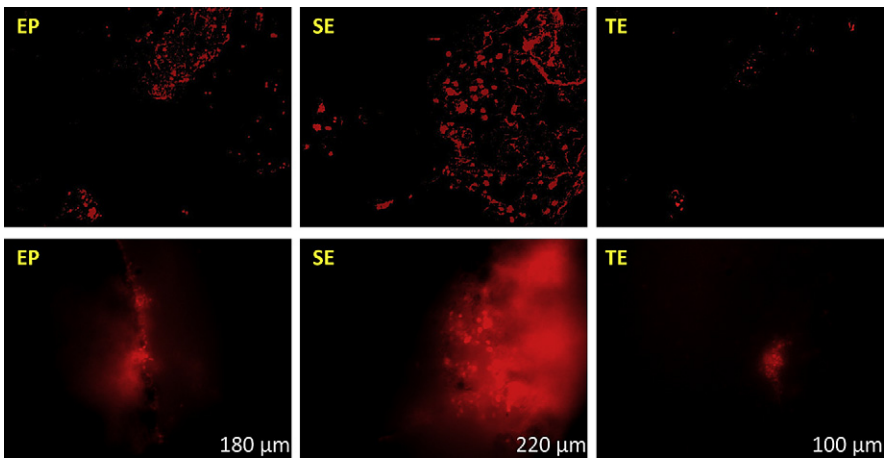

Figure 1. *Top images:* representative 3D reconstruction of select Z-stack series of *E. faecalis* biofilms captured from sealer-dentin interfaces of EP, SE, and TE sealers, aged for 1 month. Specimens were dihydroethidium-stained and examined by using epifluorescence microscopy (excitation in the range of 560/40, dichroic 595 nm, and emission of 645/75 nm, ×100 magnification). 3D images were reconstructed by using Image J software. Levels of luminescence demonstrate different patterns of biofilm formation for different materials. *Bottom images:* representative Z-stack images of *E. faecalis* captured from sealer-dentin interface of specimens aged for 1 month. Images show differences in trends of bacterial cell presence within the interface (μm from the specimen's surface in an apical direction) for each material group.