Enterococcus faecalis Hydrolyzes Dental Resin Composites and Adhesives

Muna Q. Marashdeh, BDS, PhD,[*†] *Russel Gitalis, BSc,*[*†] *Celine Levesque, PhD,*[*] *and Yoav Finer, DMD, PhD, FRCD(C)*[*†]

Abstract

Introduction: After root canal treatment, the dentin-sealer interface undergoes degradation, allowing for interfacial microbial biofilm proliferation and treatment failure. Saliva and cariogenic bacteria showed esteraselike activities (ie, cholesterol esterase [CE]-like and/or pseudocholinesterase [PCE]-like) that degrade methacrylate-based resin materials and/or the restoration-tooth interface, increasing microbial interfacial proliferation. *Enterococcus faecalis* is a gram-positive bacterium that is commonly detected in persistent endodontic infections. The aim of this study was to measure *E. faecalis* esteraselike, CE-like, and PCE-like activities and to assess the ability of the bacterium to degrade methacrylate-based resin composite and total-etch and self-etch adhesives. **Methods:** CE-like and PCE-like activities from *E. faecalis* were measured using nitrophenyl and butyrylthiocholine substrates, respectively. The ability of *E. faecalis* to degrade resin composite, total-etch and self-etch adhesives was examined by quantifying the release of a universal resin degradation by-product (ie, Bis[hydroxypropoxy]-phenyl propane [BisHPPP]) using high-performance liquid chromatography. **Results:** *E. faecalis* showed CE-like (1.23 ± 0.13 U/μg dry bacteria) but no PCE-like activity. After 30 days and/or 14 days of incubation, the amount of BisHPPP released was significantly higher in the presence of bacteria versus media for TE and RC but not SE ($P < .05$). The amount of BisHPPP released after 30 days of incubation with bacteria was highest for TE (23.69 ± 1.72 μg/cm^2) followed by RC (3.43 ± 1.20 μg/cm^2) and lowest for SE (0.86 ± 0.44 μg/cm^2) ($P < .05$). **Conclusions:** *E. faecalis* possesses esteraselike degradative activity toward dental methacrylate resin restoration materials, which could accelerate the degradation of the dentin-methacrylate resin interface, increasing bacterial biofilm proliferation and penetration into the root canal system. *(J Endod 2018;■:1–5)*

Key Words

Biodegradation, dental adhesives, dental resin composite, *Enterococcus faecalis*, esterases, methacrylate resins

Significance

E. faecalis has esteraselike hydrolase activities that enable the bacteria to degrade methacrylate-based resin composite and adhesives, potentially compromising the restoration-tooth interface, and could facilitate the migration of the bacteria to periradicular tissues and cause treatment failure.

Methacrylate-based endodontic sealers were developed because they could bond to the root canal walls (1). However, no added advantages were noticed over the use of conventional sealers, and it was difficult to establish a reliable bond between the root canal dentin and the resin material (2). The use of methacrylate resins nowadays in root canals is mainly limited to post cementation (3) and coronal restorations of endodontically treated teeth (4); thus, their ability to maintain an interfacial seal is still important to the success of endodontic treatment. Resin composite (RC) materials require the application of resin adhesive systems in order to bond to dentin. Two main adhesive systems are currently available: the etch-and-rinse (total etch [TE]) technique, which is designed to remove the smear layer, and the self-etch (SE) technique, which modifies the smear layer. Both are dependent on the formation of a resin-impregnated collagen or hybrid layer (5). Methacrylate resin polymers in restorative material and adhesives are prone to hydrolysis due to the presence of unprotected ester linkages (6); salivary (7) and bacterial esterases (8) catalyze this process, increasing the degradation of the resin-dentin interface and bacterial proliferation into the interface (9). Bacterial infection caused by coronal leakage is an important cause of endodontic failure (10).

Bacterial enzymes are considered an important part of bacterial pathogenicity (11) and could cause tissue destruction (12). Bacterial esterases have been linked to the virulence of *Mycobacterium tuberculosis* since they hydrolyzed the free esters in the local environment and accordingly could be a source of fatty acids to be used nutritionally by the bacteria (13). Esterase from group A streptococcus has been found to contribute to severe invasive infections (14). More recently, esterase activity from the cariogenic bacteria *Streptococcus mutans* has been found to hydrolyze dental methacrylate resins, and, therefore, these bacteria have the potential to degrade the restoration-tooth interface and further increase interfacial bacterial biofilm proliferation and restoration failure (8).

E. faecalis is a gram-positive bacterium that enters the root canal through the coronal part (15) and has been linked to persistent root canal infections (16). *E. faecalis* has the ability to invade root canals and adhere to dentin, survive and grow in harsh environments, and cause infections (17). The hypothesis of this study is that *E. faecalis* has esterase-like activity in levels that enables the bacteria to degrade methacrylate RCs and adhesives.

From the *Faculty of Dentistry, and †Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, Ontario, Canada.

Address requests for reprints to Dr Yoav Finer, University of Toronto, 124 Edward Street, Toronto, Ontario, Canada M5G 1G6. E-mail address: yoav.finer@dentistry.utoronto.ca

0099-2399/$ - see front matter

https://doi.org/10.1016/j.joen.2017.12.014

Methods

Bacterial Esterase Activity Assays

Part of overnight culture of *E. faecalis* ATCC 29212 (American Type Culture Collection, Manassas, VA) in brain-heart infusion (BHI) was heat inactivated (HIN) for 30 minutes at 80°C. The other part was diluted 1:20 and incubated (37°C) until reaching lag, log, or stationary growth phases. Then, bacterial suspensions for each growth phase and HIN were centrifuged (13,000 rpm, 10 minutes); the bacterial culture supernatant was collected and filtered through a 0.22-μm pore size filter, and the bacterial cells were resuspended in phosphate-buffered saline. One milliliter of the bacterial suspension and the culture supernatant was incubated with either 0.5 mL p-nitrophenyl butyrate (p-NPB) or p-nitrophenyl acetate (p-NPA) substrates to measure the nitrophenyl-dependent esterase activities or butyrylthiocholine iodide (BTC) substrate to measure the butyrate-dependent esterase (Sigma-Aldrich, St Louis, MO). CE-like and PCE-like activities were quantified with a spectrophotometer at 401 and 405 nm, respectively, as described previously (8). Absorbance values were normalized to bacterial suspensions without substrate absorbance readings.

Biodegradation of Composite and Adhesive

Photopolymerized cylindrical samples (4 × 4 mm) (n = 3/group) were made from RC (Filtek Z250; 3M ESPE, St Paul, MN), TE adhesive (Scotchbond Multipurpose, 3M ESPE), and SE adhesive (Adper Easybond, 3M ESPE) and incubated in sterile vials containing 2 mL BHI (control) or 1:20 diluted *E. faecalis* ATCC 29212 in BHI (experimental) for 2, 4, 8, 14, and 30 days. Incubation solutions were collected and replaced every 48 hours. When collected, solutions were mixed with equal amounts of methanol (100%) to halt enzymatic activity, filtered (Amicon Ultra centrifugal filters [Millipore Corporation, Bedford, MA], 14,000 rpm for 10 minutes at 4°C), and refrigerated at 4°C in well-sealed Eppendorf tubes. High-performance liquid chromatography was used to quantify bis(hydroxypropoxy)phenyl-propane (BisHPPP), a universal degradation by-product of bisphenol-glycidyl dimethacrylate (BisGMA). The amount of BisHPPP released was normalized to the surface area of the specimens (0.754 cm^2).

Surface Morphology

Observation for pre- and postincubation specimens was performed using scanning electron microscopy (Hitachi S-2500 SEM; Hitachi Ltd, Mito City, Japan). Specimens were sonicated before analysis to remove bacterial cells adhering to the surface followed by sample dehydration, gold coating, and then imaging.

Statistical Analysis

After testing for normal distribution, data were analyzed using 3-way analysis of variance followed by the Tukey test for repeated measures pair-wise comparison within the growth phase, type of substrate, bacteria, or supernatant for their effect on the esterase activity level and 3-way analysis of variance followed by the Tukey test for repeated measures pair-wise comparison within the incubation media, time, and material for their effect on the amount of BisHPPP release (P < .05).

Results

Bacterial Esterase Activity Assays

All growth phases of *E. faecalis* had activity toward both nitrophenyl esters (p-NPA and p-NPB) but not toward BTC (Fig. 1). At each growth phase, the preference toward the p-NPA substrate was higher than p-NPB, with the highest activity observed in the log growth phase (P < .05) at 1.23 ± 0.13 U/μg cell dry weight. For all substrates, the HIN and culture supernatant showed significantly lower activity for all growth phases compared with the bacteria (live samples). *E. faecalis*, HIN, and the culture supernatant did not show activity toward the BTC substrate at all growth phases.

Biodegradation of Composite and Adhesives

For all materials (RC, TE, and SE), a trend of increasing BisHPPP released throughout the incubation period was observed (Fig. 2). After 30 days of incubation with RC and 14 and 30 days of incubation with TE but not SE, the amount of BisHPPP released was significantly higher in the presence of bacteria compared with control (P < .05). After 30 days of incubation with *E. faecalis*, the amount of BisHPPP released from TE (23.69 ± 1.72 $\mu g/cm^2$) was 7 and 28 times higher than that released from RC (3.43 ± 1.20) $\mu g/cm^2$ and SE (0.86 ± 0.44 $\mu g/cm^2$), respectively (P < .05). The total amount of BisHPPP released in the samples incubated with bacteria over the entire incubation period was significantly higher in TE (97.78 ± 9.40 $\mu g/cm^2$) compared with RC (17.28 ± 1.53 $\mu g/cm^2$) and SE (5.31 ± 0.42 $\mu g/cm^2$) (P < .05).

Scanning electron microscopic micrographs (Fig. 3) showed that the surfaces of the TE and RC specimens incubated with *E. faecalis* for 30 days appear rougher than BHI-incubated and nonincubated specimens.

Discussion

The results support the hypothesis that *E. faecalis* ATCC29212 has esteraselike activity in levels that enables the bacteria to degrade methacrylate RCs and adhesives. This finding shows a potential ability of *E. faecalis* strains with esterase activity to penetrate the methacrylate-tooth interface, enter the root canal, and cause secondary infections. The bacterial enzymes showed higher activity toward degrading total-etch adhesive followed by RC, and the least activity was toward SE adhesive.

Human saliva (7) and *S. mutans* species (8) have been shown to hydrolyze RCs and adhesives. Human salivary esterases have been characterized by their activity toward nitrophenyl esters and BTC (7), whereas *S. mutans* had characteristic activity toward the nitrophenyl esters only. In the current study, *E. faecalis* expressed minimal activity toward BTC and a higher activity toward nitrophenyl esters, in levels similar to several strains of *S. mutans* that were previously shown to degrade RCs and adhesives (8). The result of the current study indicates a similar potential for degradation of dental restorations by *E. faecalis*.

Kermanshahi et al (9) showed that exposure of dentin-resin interfaces to salivary esteraselike activity resulted in the formation of gaps that were infiltrated by bacterial biofilms. *E. faecalis* could contribute

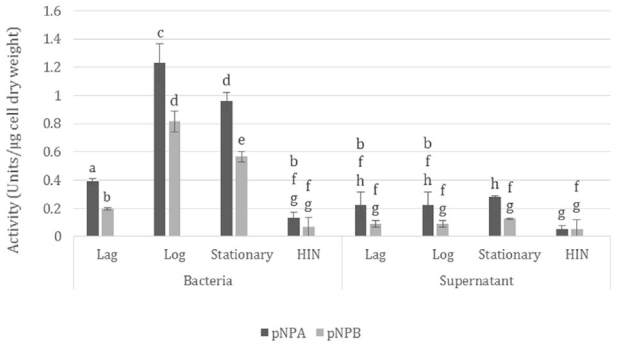

Figure 1. The activity profile of *E. faecalis* and supernatant at the lag, log, and stationary phases and when HIN measured using p-NPA, p-NPB, and BTC. Data are shown as mean ± standard deviation (n = 3). Different letters indicate a significant difference (P < .05).