Published in final edited form as:

J Endod. 2018 April ; 44(4): 609–613. doi:10.1016/j.joen.2017.12.014.

Enterococcus faecalis Hydrolyzes Dental Resin Composites and Adhesives

Muna Q Marashdeh, B.D.S., Ph.D., Russel Gitalis, B.Sc., Celine Levesque, Ph.D., and Yoav Finer, D.M.D., Ph.D., FRCD(C)

Abstract

Background—Post root canal treatment, the dentin-sealer interface undergoes degradation, allowing for interfacial microbial biofilm proliferation and treatment failure. Saliva and cariogenic bacteria showed esterase-like activities; cholesterol esterase-like (CE) and/or pseudocholine esterase-like (PCE) 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* esterase-like, CE-like and PCE-like activities, and to assess the ability of the bacterium to degrade methacrylate-based resin composite, 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 (RC), total-etch (TE), and self-etch (SE) adhesives was examined by quantifying the release of a universal resin degradation byproduct, bis-hydroxy-propoxy-phenyl-propane (BisHPPP), using high performance liquid chromatography.

Results—*E. faecalis* showed CE-like (1.23 ± 0.13 Units/ μ g 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 vs. media for TE and RC but not SE ($p < 0.05$). The amount of BisHPPP released after 30 days of incubation with bacteria was highest for TE (23.69 ± 1.72 μ g/ cm^2), followed by RC (3.43 ± 1.20 μ g/ cm^2), and lowest for SE (0.86 ± 0.44 μ g/ cm^2) ($p < 0.05$).

Conclusions—*E. faecalis* possesses esterase-like degradative activity towards 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.

Keywords

Enterococcus faecalis; esterases; biodegradation; methacrylate resins; dental resin composite; dental adhesives

Corresponding author: Dr. Yoav Finer, D.M.D., Ph.D., FRCD(C), University of Toronto, yoav.finer@dentistry.utoronto.ca.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Methacrylate-based endodontic sealers were developed since they could bond to the root canal walls (1). However, no added advantages were noticed over the use of the 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 canal is mainly limited to post cementation (3), and coronal restorations of endodontically treated teeth (4), thus their ability to maintain interfacial seal is still important to the success of the 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: etch and rinse (total etch (TE)) technique that is designed to remove the smear layer and self-etch (SE) technique that 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 the 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* as 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 periapical infections (16). *E. faecalis* has the ability to invade root canals and adhere to dentin, survive and grow in harsh environments, and cause periradicular infections (17). The hypothesis of this study is that *E. faecalis* has esterase-like activity in levels that enables the bacteria to degrade methacrylate resin composites and adhesives.

Methods

Bacterial esterase activity assays

Part of overnight (o/n) culture of *E. faecalis* ATCC 29212 in brain heart infusion (BHI) was heat inactivated (HIN), for 30 min at 80°C. The other part was diluted 1:20 and incubated (37°C) until reaching lag, log, or stationary growth phases. Then bacterial suspension for each growth phase and HIN were centrifuged (13,000 RPM, 10 m), 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 (PBS). One mL of the bacterial suspension and the culture supernatant were incubated with either 0.5 mL of p-nitrophenylbutyrate (p-NPB), or p-nitrophenylacetate (p-NPA) to measure the acetate-like

dependent esterases, or butyrylthiocholine iodide (BTC) substrates to measure the butyrate-like dependent esterases (Sigma, St. Louis, MO). CE-like and PCE-like activities were quantified with a spectrophotometer at 401, 405 nm, respectively as described previously (8). Absorbance values were normalized to bacterial suspensions without substrates absorbance readings.

Biodegradation of composite and adhesive

Photopolymerized cylindrical samples (4×4 mm) (N=3/group) were made from RC (Filtek™ Z250), TE adhesive (Scotchbond™ Multipurpose), and SE adhesive (Adper™ Easybond) and incubated in sterile vials containing 2 mL BHI (control), or 1:20 diluted *E. faecalis* ATCC 29212 o/n in BHI (experimental) for 2, 4, 8, 14, and 30 days. Incubation solutions were collected and replaced every 48 h. When collected, solutions were mixed with equal amounts of methanol (100%) to halt enzymatic activity, then filtered (Amicon Ultra centrifugal filters, 14,000 RPM for 10 m at 4°C) and refrigerated at 4°C in a well-sealed Eppendorf tubes. High performance liquid chromatography (HPLC) was used to quantify bis-hydroxy-propoxy-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 cm²).

Surface morphology

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

Statistical analysis

After testing for normal distribution, data were analyzed by three-way ANOVA followed by Tukey's test for repeated-measurement pair-wise comparison within the growth phase, type of substrate, bacteria or supernatant for their effect on the esterase activity level, and a three-way ANOVA followed by Tukey's test for repeated-measurement pair-wise comparison within the incubation media, time and material for their effect on the amount of BisHPPP release ($p<0.05$). Results Bacterial esterase activity assays (Fig. 1)

Results

Bacterial esterase activity assays (Fig. 1)

All growth phases of *E. faecalis* had activity towards both nitrophenyl esters (p-NPA, and p-NPB) but not toward BTC. At each growth phase, the preference towards the p-NPA substrate was higher than p-NPB with the highest activity observed in the log growth phase ($p<0.05$), at 1.23 ± 0.13 Units/ μ g cell dry weight. For all substrates, the HIN and culture supernatant showed significantly lower activity for all growth phases compared to the bacteria (live samples). *E. faecalis*, HIN, and the culture supernatant did not show activity towards the BTC substrate at all growth phases.

Biodegradation of composite and adhesives (Fig. 2)

For all materials (RC, TE, and SE), a trend of increasing BisHPPP released throughout the incubation period was observed. After 30 days of incubation with resin composite, and 14 and 30 days incubation with TE but not SE, the amount of BisHPPP released was significantly higher in the presence of bacteria compared to control ($p<0.05$). After 30 days of incubation with *E. faecalis* the amount of BisHPPP released from TE ($23.69\pm1.72\text{ }\mu\text{g}/\text{cm}^2$) was 7 and 28 times higher than that released from RC ($3.43\pm1.20\text{ }\mu\text{g}/\text{cm}^2$) and SE ($0.86\pm0.44\text{ }\mu\text{g}/\text{cm}^2$), respectively ($p<0.05$). The total amount of BisHPPP released in the samples incubated with bacteria over the entire incubation period was significantly higher in TE ($97.78\pm9.40\text{ }\mu\text{g}/\text{cm}^2$) compared to RC ($17.28\pm1.53\text{ }\mu\text{g}/\text{cm}^2$) and SE ($5.31\pm0.42\text{ }\mu\text{g}/\text{cm}^2$) ($p<0.05$).

SEM micrographs (Fig. 3) demonstrated that the surfaces of the TE and RC specimens incubated with *E. faecalis* for 30 days appear rougher than BHI-incubated and non-incubated specimens.

Discussion

The results support the hypothesis that *E. faecalis* ATCC29212 has esterase-like activity in levels that enables the bacteria to degrade methacrylate resin composites 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 resin composite, and the least activity was toward self-etch adhesive.

Human saliva (7) and *S. mutans* species (8) have been shown to hydrolyze resin composites and adhesives. Human salivary esterases have been characterized by their activity toward nitrophenyl esters and BTC (7), while *S. mutans* had characteristic activity towards 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 resin composites and adhesives (8). The result of the current study indicates a similar potential for degradation dental restorations by *E. faecalis*.

Kermanshahi et al. (9) showed that exposure of dentin-resin interfaces to salivary esterase-like activity resulted in the formation of gaps that were infiltrated by bacterial biofilms. *E. faecalis* could contribute further to the deterioration of the dentin-resin interface by releasing both protease collagenase enzyme (18) and esterases, affecting the hybrid layer, tooth and methacrylate-resin, increasing the bacterial biofilm proliferation within the root canal system, and contributing to the failure of root canal treatment (10, 17). Increasing the esterase resistance of methacrylate-resins and application of anti-collagenolytic agents could improve the materials' biochemical stability, and extend the longevity of dentin-resin bonds, therefore increasing the lifespan of endodontic treatment and dental resin restorations (6, 19).

Resin composite (RC, Z250, 3M), total etch adhesive (TE, Scotchbond™ Multipurpose, 3M), and self-etch adhesive (SE, Adper™ Easybond, 3M) were selected in this study as they are commonly used materials in many dental practices, and since these materials were extensively investigated, there are good baseline data regarding their biostability (6, 8, 20–22). All three materials are BisGMA-based which makes the analysis of the degradation and comparison feasible and standardized and are good representatives of RC, TE, and SE group of materials so the results of this study could be generalized to similar materials from each group.

BisHPPP is a biodegradation by-product of BisGMA, a universal monomer present in most adhesives and composite materials. BisHPPP was chosen for analyses since it is a good marker of true resin biodegradation due to the hydrophobic nature of its precursor (8, 9, 20). *E. faecalis* significantly increased the amount of BisHPPP released for RC and TE adhesive after 30 and/or 14 days of incubation, while there was significantly lesser effect on the SE material compared to the control. The total amount of BisHPPP released in the samples incubated with *E. faecalis* over 30 days was significantly higher in TE adhesive compared to RC and SE adhesive. The percent composition of BisGMA of total-etch adhesive, self-etch adhesive and composite are 60–70, 15–25, and 5–10% of the total mass of the materials, respectively (MSDS, 3M). Since the amount of BisHPPP released was not correlated with the BisGMA content per material, and since all materials were similar in their surface area, volume, and degree of vinyl group conversion (23), this difference could have arisen only from the inherent variations in chemical composition of the materials and their interaction with the specific enzymatic activities of *E. faecalis*.

Susceptibility of the polymers to microbial enzymatic attack generally depends on enzyme availability, enzyme specificity for a polymer, availability of a site in that polymer for enzyme attack, and the presence of coenzyme if required (24). *S. mutans* UA159 showed different preferences than *E. faecalis* ATCC 29212 toward the nitrophenyl substrates, with preferences toward pNPB compared to pNPA as well as differences in the degradation of the restorative materials, RC, TE and SE adhesives (8). This further suggests that specific enzymatic activities from each species are responsible for the observed activity profiles and degradative activities. Variations in the enzymatic activities are expected due to the difference in the esterase genes between different species (25, 26).

Following 30-days of incubation, SEM analysis showed increased surface roughness for TE and RC specimens incubated with bacteria compared to the non-incubated samples and specimens incubated with media only. This observation corroborates previous studies that showed surface degradation and changes in surface-topography of polymer-based restorative materials by bacteria (8, 27, 28) However, except for Bourbia et al. (8), these studies provided only observation of surface changes without characterization of possible degradative activities from the bacteria. The current study is the first to characterize esterase activities by *E. faecalis* that degrade methacrylate-based restorative materials.

Esterase-like activities were different for each growth phase of the bacteria, with the lag phase having the lowest enzymatic activity followed by the stationary phase and the highest values were measured for the log phase. This could be explained by the physiology of each

phase; in the lag phase the bacterial cells start modifying to avail new environmental conditions, and the cells are metabolically less active in this phase. Log phase is the most metabolically active; cell division proceeds at a constant rate, when conditions become unfavorable the stationary phase starts and the bacteria reduce its metabolism (29, 30). When the cells transfer from log to stationary phases they go over a period of disturbed growth and unbalanced cellular synthesis (31). Consequently, the difference in the chemical composition of those phases could have led to the difference in the activity noticed in the current study.

The heat inactivation process affects the structural and physiological properties of every cellular component of the bacteria (membrane, enzymes and proteins, DNA, and RNA) (32). Therefore, this process is useful to establish a group of inactive bacterial suspensions to act as negative control to which the live bacterial suspensions can be compared (33). In the current study HIN *E. faecalis* showed a significant decrease in the esterase-like activity compared with the untreated bacteria. Since the pH (~ 4.50), and the growth conditions were similar for the two bacterial cultures, it could be hypothesized that the measured activity is due to true enzymatic activity from the bacteria and not due to other factors, such as low pH, or the incubation media.

The supernatant had less esterase activity than the bacterial suspensions under the conditions of the current study. However, the lower enzymatic activity of the supernatant does not exclude the possibility of the enzyme being secreted, as the bacteria utilize several methods to invade mammalian hosts, damage tissue sites, and thwart the immune system from responding (34), including transport of proteins from the cytoplasm across the inner membrane into the bacterial supernatant or onto the surface of the bacterial cell (35). Overall, the fact that in the current study the bacteria hydrolyzed the resinous materials confirms that enzymes were either released or attached to the cell wall, though the exact mechanism and identity of the enzyme require further investigation.

Conclusions

E. faecalis has esterase-like activities that enable the bacteria to hydrolyze resin-based materials. The extent of degradation was material dependent, and material chemistry was the most important factor in determining the material's biochemical stability. When present within the confined space of the restoration-tooth marginal interface *E. faecalis* could contribute to the deterioration of the interface accelerating its proliferation into the root canal and potentially compromising the success of the treatment.

Acknowledgments

The authors thank Dr. Mahmood Abu Ruja, and Ms. Yasaman Delaviz for their technical assistance, Jian Wang for processing the SEM images. This investigation was funded by National Institute of Dental & Craniofacial Research R01DE021385; Canadian Institute of Health Research MOP115113.

References

1. Kim YK, Grandini S, Ames JM, Gu LS, Kim SK, Pashley DH, et al. Critical review on methacrylate resin-based root canal sealers. *J Endod.* 2010; 36:383–99. [PubMed: 20171352]
2. Breschi L, Mazzoni A, Dorigo ED, Ferrari M. Adhesion to Intraradicular Dentin: A Review. *J Adhes Sci Technol.* 2009; 23:1053–83.

3. Monticelli F, Ferrari M, Toledano M. Cement system and surface treatment selection for fiber post luting. *Med Oral Patol Oral Cir Bucal*. 2008; 13:E214–21. [PubMed: 18305446]
4. Slutzky-Goldberg I, Slutzky H, Gorfil C, Smidt A. Restoration of endodontically treated teeth review and treatment recommendations. *Int J Dent*. 2009; 2009:150251. [PubMed: 20309408]
5. Ozer F, Blatz MB. Self-etch and etch-and-rinse adhesive systems in clinical dentistry. *Compend Contin Educ Dent*. 2013; 34(1):12–4. 6, 8. quiz 20, 30.
6. Finer Y, Santerre JP. The influence of resin chemistry on a dental composite's biodegradation. *J Biomed Mater Res A*. 2004; 69:233–46. [PubMed: 15057996]
7. Finer Y, Santerre JP. Salivary esterase activity and its association with the biodegradation of dental composites. *J Dent Res*. 2004; 83:22–6. [PubMed: 14691108]
8. Bourbia M, Ma D, Cvitkovitch DG, Santerre JP, Finer Y. Cariogenic bacteria degrade dental resin composites and adhesives. *J Dent Res*. 2013; 92:989–94. [PubMed: 24026951]
9. Kermanshahi S, Santerre JP, Cvitkovitch DG, Finer Y. Biodegradation of resin-dentin interfaces increases bacterial microleakage. *J Dent Res*. 2010; 89:996–1001. [PubMed: 20505047]
10. Saunders WP, Saunders EM. Coronal leakage as a cause of failure in root-canal therapy: a review. *Endod Dent Traumatol*. 1994; 10:105–8. [PubMed: 7995237]
11. Siqueira JF Jr, Rocas IN. Bacterial pathogenesis and mediators in apical periodontitis. *Braz Dent J*. 2007; 18:267–80. [PubMed: 18278296]
12. Krzysciak W, Jurczak A, Koscielniak D, Bystrowska B, Skalniak A. The virulence of *Streptococcus mutans* and the ability to form biofilms. *Eur J Clin Microbiol Infect Dis*. 2014; 33:499–515. [PubMed: 24154653]
13. Lun S, Bishai WR. Characterization of a novel cell wall-anchored protein with carboxylesterase activity required for virulence in *Mycobacterium tuberculosis*. *J Biol Chem*. 2007; 282:18348–56. [PubMed: 17428787]
14. Zhu H, Liu M, Sumby P, Lei B. The secreted esterase of group A streptococcus is important for invasive skin infection and dissemination in mice. *Infect Immun*. 2009; 77:5225–32. [PubMed: 19805529]
15. Wang QQ, Zhang CF, Chu CH, Zhu XF. Prevalence of *Enterococcus faecalis* in saliva and filled root canals of teeth associated with apical periodontitis. *Int J Oral Sci*. 2012; 4:19–23. [PubMed: 22422085]
16. Zehnder M, Guggenheim B. The mysterious appearance of enterococci in filled root canals. *Int Endod J*. 2009; 42:277–87. [PubMed: 19220511]
17. Kayaoglu G, Orstavik D. Virulence factors of *Enterococcus faecalis*: relationship to endodontic disease. *Crit Rev Oral Biol Med*. 2004; 15:308–20. [PubMed: 15470268]
18. Rolfe MD, Rice CJ, Lucchini S, Pin C, Thompson A, Cameron AD, et al. Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. *J Bacteriol*. 2012; 194:686–701. [PubMed: 22139505]
19. Marashdeh MQ, Gitalis R, Lévesque C, Finer Y. *Enterococcus faecalis* possesses Collagenolytic Activity that Degrades Human Dentin Collagen Matrix. 2017 unpublished manuscript submitted for publication.
20. Delaviz Y, Finer Y, Santerre JP. Biodegradation of resin composites and adhesives by oral bacteria and saliva: A rationale for new material designs that consider the clinical environment and treatment challenges. *Dent Mater*. 2014; 30:16–32. [PubMed: 24113132]
21. Shokati B, Tam LE, Santerre JP, Finer Y. Effect of salivary esterase on the integrity and fracture toughness of the dentin-resin interface. *J Biomed Mater Res B Appl Biomater*. 2010; 94:230–7. [PubMed: 20524199]
22. Serkies KB, Garcha R, Tam LE, De Souza GM, Finer Y. Matrix metalloproteinase inhibitor modulates esterase-catalyzed degradation of resin-dentin interfaces. *Dent Mater*. 2016; 32:1513–23. [PubMed: 27692555]
23. Roth KA, Friedman S, Levesque CM, Basrani BR, Finer Y. Microbial biofilm proliferation within sealer-root dentin interfaces is affected by sealer type and aging period. *J Endod*. 2012; 38:1253–6. [PubMed: 22892745]
24. Siqueira, JF., Moap, JIPT., editors. *Essential endodontology*. Oxford, UK: Blackwell; 2008. p. 135–9.

25. Mittal, V. Nanocomposites with Biodegradable Polymers: Synthesis, Properties, and Future Perspectives. Oxford: Oxford University Press; 2011.
26. Kim EB, Kopit LM, Harris LJ, Marco ML. Draft genome sequence of the quality control strain *Enterococcus faecalis* ATCC 29212. J Bacteriol. 2012; 194:6006–7. [PubMed: 23045510]
27. Ajdic D, McShan WM, McLaughlin RE, Savic G, Chang J, Carson MB, et al. Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. Proc Natl Acad Sci U S A. 2002; 99:14434–9. [PubMed: 12397186]
28. Fucio SB, Carvalho FG, Sobrinho LC, Sinhoreti MA, Puppim-Rontani RM. The influence of 30-day-old *Streptococcus mutans* biofilm on the surface of esthetic restorative materials--an in vitro study. J Dent. 2008; 36:833–9. [PubMed: 18621456]
29. Gregson KS, Shih H, Gregory RL. The impact of three strains of oral bacteria on the surface and mechanical properties of a dental resin material. Clin Oral Investig. 2012; 16:1095–103.
30. Madar D, Dekel E, Bren A, Zimmer A, Porat Z, Alon U. Promoter activity dynamics in the lag phase of *Escherichia coli*. BMC Syst Biol. 2013; 7:136. [PubMed: 24378036]
31. Meneses N, Mendoza-Hernandez G, Encarnacion S. The extracellular proteome of *Rhizobium etli* CE3 in exponential and stationary growth phase. Proteome Sci. 2010; 8:51. [PubMed: 20942974]
32. McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 1999; 12:147–79. [PubMed: 9880479]
33. Fleming WL, Neill JM. Studies on Bacterial Enzymes: V. The Carbohydrases and Lipase of the Welch Bacillus. J Exp Med. 1927; 45:947–59. [PubMed: 19869310]
34. Green, ER., Mecsas, J. Microbiol Spectr. 2016. Bacterial Secretion Systems: An Overview; p. 4
35. Hueck CJ. Type III protein secretion systems in bacterial pathogens of animals and plants. Microbiol Mol Biol Rev. 1998; 62:379–433. [PubMed: 9618447]

Highlights

- *E. faecalis* has esterase-like hydrolase activities
- *E. faecalis* hydrolyzes dental resin composites and adhesives
- *E. faecalis* could compromise the restoration-tooth interface
- *E. faecalis* could compromise the endodontic sealer-tooth interface

Figure 1.

Activity profile of *E. faecalis*, and supernatant at lag, log, stationary phase, and when heat inactivated (HIN), measured using p-nitrophenolacetate (p-NPA), p-nitrophenolbutyrate (p-NPB), and butyrylthiocholine iodide (BTC). Data are shown as mean $\pm$ SD (N=3). Different letters indicate significant difference ($p < 0.05$).

Figure 2.

Cumulative amount of BisHPPP release from resin composite (A), self etch (B), and total-etch (C) adhesives. Data shown as mean $\pm$ SD. Incubation with *E. faecalis* increased the amount of degradation indicated by increased BisHPPP compared to media only for resin composite after 30 days of exposure and for total-etch adhesive after 14, and 30 days of exposure. Different letters indicates significant difference between the same incubation groups at different time points, and media ($P < 0.05$). Note: composite and SE have same Y-axes scale, which is different from TE.

Figure 3.

SEM micrographs (X10K original magnification) of representative images of the surface of RC (resin composite), SE (self-etch adhesive), and TE (total-etch adhesive) samples before incubation (0) and 30 days after incubation in either BHI, or *E. faecalis* in BHI. All three materials degraded over time regardless the incubation media, which was indicted by rougher surface. *E. faecalis* increased the surface roughness of RC, and TE after 30 days of incubation when compared to media only (BHI).