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4 **Effect of a benzalkonium chloride surfactant-sodium hypochlorite combination on**
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6 **elimination of *Enterococcus faecalis***
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Abstract

Introduction: The purpose of this investigation was to determine the effect of a sodium hypochlorite-surfactant combination on the removal of *Enterococcus faecalis* from infected teeth.

Methods: Sixty-four extracted human single canal anterior teeth were prepared with rotary instrumentation and sterilized. Teeth were divided into four groups; N=16. Three experimental groups were inoculated with *E. faecalis* and cultured for 21 days before use: Positive Control Group: no irrigation; NaOCl Group: irrigated with 5mL of 6% NaOCl; and NaOCl/BAK Group: irrigated with 5mL of 6% NaOCl/0.008% benzalkonium chloride (BAK). The Negative Control Group received media only, no inoculate. Paper point sampling of the canals was obtained pre-irrigation (S1) for all four groups, and for two groups post-irrigation (S2) to determine remaining colony forming units (CFUs). After sampling, all teeth were split in half and evaluated for bacterial viability CFUs and penetration of dentinal tubules using fluorescent vital dye staining and confocal laser scanning microscopy (CLSM).

Results: Comparison of pre- and post-irrigation paper point samples from the two irrigated groups showed a significant reduction in bacterial canal load ($p < 0.001$, Kruskal-Wallis), with a significantly lower load in the NaOCl/BAK Group than in the NaOCl Group ($p = 0.001$, Mann-Whitney U); 68.8% of the NaOCl/BAK samples gave no recoverable counts. In contrast, no significant difference between these groups was found for counts recovered from dentin. CLSM showed no differences in tubule penetration.

Conclusions: The addition of BAK to NaOCl significantly reduced the number of remaining bacteria within the canal after irrigation compared to NaOCl alone.

INTRODUCTION

Endodontic success relies largely on chemo-mechanical removal of bacteria, bacterial endotoxin, and debris from the root canal system (1,2). The chemical portion is often facilitated by common endodontic irrigants such as sodium hypochlorite (NaOCl) or 2% Chlorhexidine (CHX). NaOCl has reported advantages over CHX such as tissue dissolution and disruption of a biofilm (3). However, research has demonstrated that during instrumentation and irrigation with 5% NaOCl, nearly 1/3 to 1/2 of the root canal remains contaminated (4). *Enterococcus faecalis* is a commonly isolated species that may play a role in persistent endodontic infections (5). It has the ability to form a biofilm in monoculture (6), can adapt to harsh environmental changes and invade the dentinal tubules, thereby resisting endodontic medicaments and becoming difficult to eliminate (5).

Bacterial biofilms are the most common cause of persistent infection (7) and apical periodontitis is referred to as 'an intraradicular biofilm-induced chronic disease' (8). Microorganisms in biofilms are far better protected, and can be greater than one thousand times more resistant to antimicrobial agents as the same organisms in planktonic form (8,9), and from 2-1000-fold more resistant to antimicrobial agents than corresponding planktonic organisms (9,10). Bacterial biofilms show extreme hydrophobic properties, greatly surpassing Teflon for repelling water and lower surface tension liquids (11). High surface tension (12) could affect the ability of NaOCl to penetrate into dentine and thus reduce its antibacterial effectiveness within dentinal tubules.

Externally added surfactants often affect the stability of biofilm structures negatively (13), disrupting hydrophobic interactions in biofilm cohesion (14). BAK is a surfactant commonly used to improve wetting properties and was found to exhibit an overall 70-fold reduction in biofilm accumulation (15). BAK combined with NaOCl could reduce the bacterial load to a greater extent than NaOCl alone, and allow further dentinal tubular diffusion of NaOCl. Bukiet *et al* (16)

demonstrated that combining BAK and NaOCl reduced the contact angle of NaOCl by over 50%, but left the antibacterial efficiency unchanged. However, this study did not evaluate the effect on diffusion of the modified NaOCl into dentinal tubules or its antibacterial effect on a biofilm *ex vivo*. The aims of this study were: 1) to measure the effect of BAK and NaOCl on removal of a biofilm of *E. faecalis* in a human tooth model and 2) to compare the diffusion of the irrigant into the dentinal tubules with and without the surfactant using confocal laser scanning microscopy (CLSM).

MATERIALS AND METHODS

Specimen Preparation

This study was approved by the Dwight D. Eisenhower Army Medical Center Research Regulatory Compliance Office, and teeth collected in accordance with the USA Fort Gordon Dental Activity "Collection of Extracted Teeth" standard operating procedure. Extracted human anterior maxillary and mandibular teeth were stored at 4°C in 0.9% sodium chloride containing 1% sodium azide to retard bacterial growth. Seventy-six teeth were radiographed in a mesio-distal direction to screen for the presence of a single canal, resulting in a total sample size of N=64. The tooth length was standardized to 17mm by decoronating with an Isomet 5000 saw (Buehler, Lake Bluff, IL). The working length was determined as the point in which a #10 file was just visible with 20X magnification in the apical foramen. Each canal was instrumented to a size 50/.04 using Profile Vortex rotary nickel-titanium instruments (Dentsply, York, PA). The canal was flooded with 6% NaOCl (Vista Dental Products, Racine, WI) and replenished after each file using a 30 gauge side-vented needle (Max-I-Probe, Dentsply, Elgin, IL). The smear layer was removed with 17% EDTA irrigation for one minute, followed by a final rinse of 5mL of 6% NaOCl. Five milliliters of 5% sodium thiosulfate were used to inactivate the NaOCl followed by 5mL of sterile saline. The apical foramen was sealed using cyanoacrylate and the root surface was sealed with varnish. Teeth were placed individually in 1.5mL microtubes (Sarstedt,

Numbrecht, Germany), and steam autoclaved at 121°C for 30 minutes, after which the samples were handled under strict aseptic measures. The samples were randomly assigned to four groups of N=16. Positive Control Group: no irrigation (positive control); NaOCl Group: irrigated with 5mL of 6% NaOCl; and NaOCl/BAK Group: irrigated with 5mL of 6% NaOCl/0.008% benzalkonium chloride (BAK) prepared per Bukiet *et al* (16). The Negative Control Group received media only, no inoculate.

Infection with *E. faecalis*

The Negative Control samples were filled with sterile brain-heart infusion (BHI) broth (Becton Dickinson and Company, Sparks, MD). The other three groups were infected using a 24-hour pure culture suspension of *E. faecalis* (ATCC 19434) cultivated in BHI and then inoculated with 15µL of a 1×10^8 CFU/mL suspension of *E. faecalis* (determined by serial dilution and plating). Teeth were incubated at 37°C and 95% humidity for 21 days, during which the BHI was removed from the canal by gentle aspiration and replenished every 72 hours.

Determination of Bacterial Viability

After 21 days, the media was removed from all of the specimens. A sterile paper point was used at working length to sample the canals from all four groups (S1). After paper point sampling, the NaOCl and NaOCl/BAK Groups were irrigated with 5mL of the respective experimental irrigant as described above. All irrigants were delivered at a flow rate of 0.04mL/s using a side-vented 30G needle (Max-I-Probe, Dentsply, Elgin, IL). A second post-irrigation paper point sample (S2) was obtained from each canal in the NaOCl and NaOCl/BAK Groups as described above. The positive and negative controls received no irrigation and were only sampled pre-irrigation.

The paper points were placed individually in 1.5mL microtubes containing 0.5mL of BHI broth and vortexed at the highest speed for three consecutive intervals of 15 seconds each. One hundred microliters of media was plated on BHI agar in triplicate and the remainder stored at

4°C. All plates were incubated at 37°C and 95% humidity for 24 hours after which the colonies were enumerated using a colony counter (Protocol, Synbiosis, Frederick, MD). If any sample was too numerous to count, it was diluted, plated and counted.

Pulverization and CLSM Analysis

Teeth were prepared for the bacterial count determination and bacterial viability staining within the dentin tubules using CLSM as follows⁽¹⁷⁾: A shallow groove was made longitudinally in the tooth using a diamond coated disc and split using a single-edged surgical blade and a surgical mallet. One-half of the tooth was placed in a microtube and stored at -80°C. Subsequently, this tooth half was placed in liquid nitrogen, ground to a fine powder, and weighed. One milliliter of media was added, and 100µL plated for CFU determination in triplicate. The surface of the remaining sample half was stained with the fluorescent LIVE/DEAD® BacLight™ Bacterial Viability stain (Molecular Probes, Eugene, OR) according to the methods outlined in Ma *et al* (18) and analyzed with CLSM (Carl Zeiss Microimaging, Thornwood, NY).

STATISTICS

Descriptive statistics evaluation of bacterial counts and non-parametric tests (as the data were not normally distributed) were performed using Prism 6.0 software (alpha=0.05). Normality of distributions were assessed using the D'Agostino and Pearson omnibus and the Shapiro-Wilk normality tests. Group values are shown as median and interquartile range.

RESULTS

All teeth in the Negative Control Group showed no bacterial growth from S1 paperpoint samples (Fig. 1A). S1 values for the three inoculated groups had medians (interquartile ranges) of 2.46 (2.27-2.59) x10⁵, 2.36 (2.24-2.61) 0.06 x10⁵, and 2.31 (1.90-2.62) x10⁵ CFUs/point respectively, with no significant difference between these values (p=0.6, Kruskal-Wallis

test). Both experimental irrigated groups (NaOCl and NaOCl/BAK) indicated efficacy with regards to bacterial load reduction seen in S2 sampling (Fig. 1A, $p < 0.001$, Kruskal-Wallis test). In the NaOCl Group, all samples gave viable counts, with a median of 6.50×10^4 (4.13 - 8.63×10^4) CFUs/point, but in the NaOCl/BAK Group, viable bacteria were only recovered in 5/16 samples, resulting in a median of 0 (0 - 1×10^4) CFUs/ point. That is, 68.8% of the NaOCl/BAK Group samples showed complete elimination of bacteria from the canal. The reduction in canal bacterial load determined by paperpoint sampling showed a significant difference between the two treatments, with the NaOCl/BAK combination being more effective than NaOCl alone ($p = 0.001$, Mann-Whitney U test). The average load reduction in the NaOCl Group was 71.8%, compared to 87.8% in the NaOCl/BAK Group. Considering only the five positive samples in the NaOCl/BAK Group, there was a 60.8% reduction.

Consistent with the results from paperpoint testing, the Negative Control Group showed no viable counts in the pulverized tooth, while in the Positive Control Group all samples showed a substantial bacterial load with a median (interquartile range) of 8.90 (6.76 - 12.37) $\times 10^2$ CFU/mg (Fig. 1B). Significantly lower values of viable bacteria were found in the irrigated groups ($p < 0.001$, Kruskal-Wallis test). In the NaOCl Group, all samples gave viable counts, with a median (interquartile range) of 85 (72 - 108) CFU/mg, representing a 91.0% reduction from the positive control. In the NaOCl/BAK Group one sample gave no viable counts, with a median value of 85 CFU/mg (75 - 111), a 92% reduction from the positive control. There was no significant difference between the NaOCl and NaOCl/BAK Groups dentin in CFU/mg ($p = 0.8$, Mann-Whitney U test).

When the distribution of dead bacteria in dentin tubules was examined by fluorescent dye staining, a modest increase in clearance of bacteria over a few tens of micrometers was seen near the canal wall surface, evidenced by the lack of any staining, but there was no large

increase in the depth of killing of bacteria within the tubules, with propidium iodide staining limited to about 50-150 μm from the surface (Fig 2).

DISCUSSION

Research has elucidated the complex interactions, mainly hydrophobic, which allow for resiliency within an established biofilm of *E. faecalis* (19). Several studies (12,16,20) have reported a significant reduction in surface tension of NaOCl when combined with surfactants, and this reduced surface tension may allow for greater dissolution of these hydrophobic interactions. Previous research demonstrated that addition of a surfactant to NaOCl has little to no effect on its ability to enhance tissue dissolution (21,22). The identities of surfactants added to NaOCl to enhance the eradication of a monoculture biofilm found within the dentinal tubules are proprietary and undisclosed. This is the first study to evaluate the ability of BAK, combined with NaOCl, to disrupt an ex-vivo biofilm of *E. faecalis*. We found the addition of BAK to NaOCl resulted in a significant difference in the elimination of *E. faecalis* within the canals as determined by paper point sampling of the canal. The NaOCl/BAK Group showed a substantial difference from NaOCl Group in the pattern of clearance of bacteria from the canal. However, the addition of BAK to NaOCl resulted in no significant difference in bacterial reduction within the dentinal tubules of the experimental teeth, as measured by the CFU/mg of powdered dentin (Fig. 1B). Consistent with these findings, examination of bacterial distribution by fluorescent staining and CLSM demonstrated equal penetration into the dentinal tubules in both experimental groups, (Fig. 2) with penetration of NaOCl or NaOCl plus surfactant not exceeding more than 150 μm . That is, representative CLSM images (Fig 2) show no added benefit of adding the surfactant BAK to enhance dentinal tubular penetration. This could solely be attributed to time; Zou *et al* (23) showed that 20 minutes of 6% NaOCl was necessary for maximum

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4 169 penetration of up to 300µm in dentinal tubules. Our irrigation was completed in 5 minutes with
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6 170 only 5mL of irrigant consistent with studies showing inhibition of *E. faecalis* with NaOCl
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8 171 exposure ranging from 30 seconds to 2 minutes (24-26).Consequently, both irrigant groups
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10 172 killed a significant amount of bacteria within the tubules near the canal wall surface, which may
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12 173 have created an organic barrier that prevented further irrigant progression. Thissupports the
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14 174 findings of Wang *et al*(27) who determined that older biofilms within dentinal tubules were more
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21 176 One limitation of thisstudy was the use of a monoculture biofilm, which provided a
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25 178 root canals and form a complex biofilm comprised of several species (9, 10). We chose to focus
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27 179 on *E. faecalis* as it is a species commonly found in persistent infections (5),consistent with its
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29 180 ability to form a biofilm even in monoculture (6,19).Examination of the positive control vital dye
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31 181 stained canal surfaces and tubules (Fig 2C) revealed dense masses of bacteria consistent with
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33 182 biofilm formation under our growth conditions.Additionally, a pilot study showed that incubating
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35 183 the teeth for 3 weeks with *E. faecalis*provided adequate CFUs for the investigation(data not
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37 184 shown).The effect of surfactant on a complex biofilm remains to be investigated.An additional
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39 185 potential limitation is that although *E. faecalis* has been well-documented in infected root canal
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43 187 incorporated in this study. However, the extensive literature employing this organism in
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45 188 clearance studies supports its use in initial monoculture studies without prior endodontic
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47 189 treatment, as here, where adhesion to mineral surfacesand invasion of tubules would be
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55 191 The use of paper point sampling may be a limitation. The paper point sampling method has
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paper point method was used to confirm the bacterial status of the canal before pulverization of the tooth, which has been shown to dramatically improve the sensitivity of culturing (32-34). Our results are in agreement with other studies (18, 23, 35), which report a reduction in bacterial load but not complete elimination when current irrigation techniques combined with surfactants are employed. Research evaluating what effect this may have on success of outcomes in endodontics is yet to be realized, as no study has shown the critical level of bioburden that tips the scale towards chronic inflammation in periapical periodontitis. It remains essential that research focuses on improving irrigation strategies and obturation of the root canal system. In conclusion, our results demonstrate that the addition of a surfactant, benzalkonium chloride to NaOCl, can significantly improve microbial clearance of heavily infected straight canals. It is important to note that BAK is an FDA category 3 antiseptic, and the application of surfactants in patient treatment will require appropriate testing for safety. Supplementation of irrigation with adjunct agitation has been shown to enhance bacterial clearance (36). Future research could incorporate agitation to potentially aid in diffusion of a surfactant-modified irrigant while encouraging removal of cellular debris.

REFERENCES

1. Sjögren U, Figdor D, Persson S, *et al.* Influence of infection at the time of root filling on the outcome of Eradication of endodontic infection endodontic treatment of teeth with apical periodontitis. *Int Endod J* 1997;30:297-306.
2. Katebzadeh N, Sigurdsson A, Trope M. Radiographic evaluation of periapical healing after obturation of infected root canals: an in vivo study. *Int Endod J* 2000;33:60-66.
3. Clegg MS, Vertucci FJ, Walker C, *et al.* The effect of exposure to irrigant solutions on

- apical dentin biofilms in vitro. J Endod 2006;32:434-7.
4. Byström A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35-40.
5. Portenier I, Waltimo TM, Haapasalo M. *Enterococcus faecalis*—the root canal survivor and ‘star’ in post-treatment disease. Endod Topics 2003;6:135-59.
6. Chavez de Paz LE, Bergenholtz G, Svensäter G. The effects of antimicrobials on endodontic biofilm bacteria. J Endod 2010;36:70-77.
7. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Science 1999;284:1318-22.
8. Nair PNR, Henry S, Cano V, *et al.* Microbial status of apical root canal system of human mandibular first molars with primary apical periodontitis after “one-visit” endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2005;99:231-52.
9. Nair PNR. On the causes of persistent apical periodontitis: a review. Int Endod J 2006;39:249-81.
10. Svensäter G, Bergenholtz G. Biofilms in endodontic infections. Endod Topics 2004;9:27-36.
11. Epstein AK, Pokroy B, Seminara A, *et al.* Bacterial biofilms show persistent resistance to liquid wetting and gas penetration. Proc Natl Acad Sci U S A 2011;108:995-1000.
12. Giardino L, Ambu E, Becce C, *et al.* Surface tension comparison of four common root canal irrigants and two new irrigants containing antibiotic. J Endod 2006;32:1091-93.
13. Schreiberová O, Hedbávná P, Čejková A, *et al.* Effect of surfactants on the biofilm of *Rhodococcus erythropolis*, a potent degrader of aromatic pollutants. N Biotechnol 2012;30:62-68.

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2
3
4 238 14. Chen X, Stewart PS. Biofilm removal caused by chemical treatments. Water Res
5
6 239 2000;34:4229-33.
7
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9
10 240 15. Jaramillo DE, Arriola A, Safavi K, *et al.* Decreased bacterial adherence and biofilm growth
11
12 241 on surfaces coated with a solution of benzalkonium chloride. J Endod 2012;38:821-25.
13
14
15 242 16. Bukiet F, CoudercG, Camps J, *et al.* Wetting properties and critical micellar concentration of
16
17 243 benzalkonium chloride mixed in sodium hypochlorite. JEndod 2012;38:1525-29.
18
19
20 244 17. Tran KT, Torabinejad M, Shabahang S, *et al.* Comparison of efficacy of pulverization and
21
22 245 sterile paper point techniques for sampling root canals. J Endod 2013;39:1057-59.
23
24
25 246 18. Ma J, Wang Z, Shen Y, *et al.* A new noninvasive model to study the effectiveness of dentin
26
27 247 disinfection by using confocal laser scanning microscopy. J Endod 2011;37:1380-85.
28
29
30
31 248 19. George S, Kishen A, Song P. The role of environmental changes on monospecies biofilm
32
33 249 formation on root canal wall by *Enterococcusfaecalis*. J Endod2005;31:867-72.
34
35
36 250 20. Palazzi F, Morra M, Mohammadi Z, *et al.* Comparison of the surface tension of 5.25%
37
38 251 sodium hypochlorite solution with three new sodium hypochlorite-based endodontic irrigants. Int
39
40 252 Endod J 2012;45:129-135.
41
42
43
44 253 21. Stojicic S, Zivkovic S, Qian W, *et al.* Tissue dissolution by sodium hypochlorite: effect of
45
46 254 concentration, temperature, agitation, and surfactant. J Endod 2010;36:1558-1562.
47
48
49 255 22. Clarkson RM. Kidd B, Evans GE, *et al.* The Effect of Surfactant on the Dissolution of Porcine
50
51 256 Pulpal Tissue by Sodium Hypochlorite Solutions. J Endod2012;38:1257-60.
52
53
54
55 257 23. Zou L, Shen Y, Li W, *et al.* Penetration of sodium hypochlorite into dentin. J Endod
56
57 258 2010;36:793-96.
58
59
60
61
62
63
64
65

24. Baumgartner JC, Johal S, Marshall JG. Comparison of the antimicrobial efficacy of 1.3% NaOCl/BioPure MTAD to 5.25% NaOCl/15% EDTA for root canal irrigation. J Endod 2007;33:48-51.
25. Vianna ME, Gomes BP. Efficacy of sodium hypochlorite combined with chlorhexidine against *Enterococcus faecalis* in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;107:585-9.
26. Gomes BP, Ferraz CC, Vianna ME, et al. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. Int Endod J 2001;34:424-8.
27. Wang Z, Shen Y, Haapasalo M. Effectiveness of endodontic disinfecting solutions against young and old *Enterococcus faecalis* biofilms in dentin canals. J Endod 2012;38:1376-79.
28. Sundqvist G, Figdor D, Persson S, et al. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85:86-93.
29. Sunde PT, Olsen I, Debelian GJ, et al. Microbiota of periapical lesions refractory to endodontic therapy. J Endod 2002;28:304-10.
30. Chavez de Paz LE. Redefining the persistent infection in root canals: possible role of biofilm communities. J Endod 2007;33:652-62.
31. Sathorn C, Parashos P, Messer HH. How useful is root canal culturing in predicting treatment outcome? J Endod 2007;33:220-25.

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279 32.Alves FR, Siqueira JF Jr, Carmo FL, *et al.* Bacterial community profiling of cryogenically
280 ground samples from the apical and coronal root segments of teeth with apical periodontitis. J
281 Endod 2009;35:486-92.

282 33.Rocas IN, Alves FR, Santos AL, *et al.* Apical root canal microbiota as determined by reverse-
283 capture checkerboard analysis of cryogenically ground root samples fromteeth with apical
284 periodontitis. J Endod 2010;36:1617-21.

285 34.Miller TA, Baumgartner JC. Comparison of the antimicrobial efficacy of irrigation using the
286 EndoVac to endodontic needle delivery. J Endod 2010;36:509-11.

287 35. Wang Z, Shen Y, Ma J, *et al.* The effect of detergents on the antibacterial activity of
288 disinfecting solutions in dentin. J Endod 2012;38:948-53.

289 36.Gu LS, Kim JR, Ling J, *et al.* Review of contemporary irrigant agitation techniques and
290 devices. J Endod 2009;35:791-804.

Figure 1. (A) The antibacterial efficacy of NaOCl modified with BAK in teeth infected with *E. faecalis* determined from paper point sampling of the canal space. S1 values for Negative Control and Positive Control Groups are compared to S2 (post-irrigation) values of NaOCl and NaOCl/BAK Groups. The reduction of canal bacteria for each tooth determined by paper point sampling was calculated as $100 \times (S1 - S2) / S1$; where S1 = Positive Control Group and S2 = either NaOCl or NaOCl/BAK Groups. (B) Total bacteria/mg (Y-Axis) obtained from pulverization of tooth halves.

Figure 2. Representative CLSM images of BacLight™ stained tooth sections. (A) Tooth from NaOCl Group. The red fluorescence of propidium iodide is indicative of dead bacterial cells. The black area to the right represents the empty canal space, with very few living cells on the root canal wall. (B) Tooth from NaOCl/BAK Group. The canal is to the right. There has been a modest increase in clearance of bacteria adjacent to the canal wall (shown by the dark band of dentin without staining). The green fluorescence of SYTO9 represents live bacteria in the dentin tubules. (C) Representative CLSM image for Positive Control Group. A thick band of green fluorescent live cells (biofilm) is seen on the canal wall.

Figure 1A

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Figure 2A

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Figure 2C

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