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Efficacy evaluation of a cordless ultrasonic unit in achieving reduction of bacterial load within a root canal system as compared to a conventional ultrasonic unit and negative pressure irrigation

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Abstract

Introduction: Increase in efficacy during root canal irrigation may contribute to better treatment outcomes. This study investigated the efficacy of ultrasonic and negative pressure irrigation systems using sodium hypochlorite (NaOCl) in the reduction of bacterial load in human teeth.

One hundred thirty-one single-rooted teeth were cleaned and shaped, autoclaved, and incubated with *E. faecalis*. Teeth were randomly assigned to three experimental groups ($n = 40$) and treated in the presence of 1% sodium hypochlorite using EndoUltra cordless ultrasonic, conventional ultrasonic, and Endovac negative pressure irrigation. Syringe irrigation controls were treated with 1% sodium hypochlorite and phosphate-buffered saline via side-vented needle irrigation. All groups with NaOCl received 5% sodium thiosulfate neutralization for 5 min after treatment. Samples of root canal fluid and dentin chips were acquired from canals before and after treatment, incubated on BHI agar, and colony forming units categorized according to quantity. Wilcoxon rank-sum and Bonferroni tests were used for statistical analysis. p values less than 0.05 were considered significant.

Results: Endovac group was significantly better in eliminating bacteria from the root canals than 1% NaOCl ($p = 0.006$) and PBS syringe irrigation ($p = 0.015$). However, it was not significantly different from the two ultrasonic groups ($p > 0.05$). Both ultrasonic groups showed better performance than 1% NaOCl and PBS syringe irrigation, however, not statistically significant ($p < 0.03$). There was no significant difference between the two ultrasonic devices ($p > 0.05$).

Conclusion: EndoVac may be an important tool for bacterial load reduction in oval canals.

Keywords: *E. faecalis*, EndoVac, Ultrasonic, EndoUltra

Introduction

Bacterial persistence at the time of root canal obturation in endodontically treated teeth is a risk factor for post-treatment apical periodontitis (Siqueira Jr. and Rocas 2008). Intracanal biofilms and smear layer residues harboring bacterial pathogens contribute to apical periodontitis development (Kakehashi et al. 1965; Ricucci

and Siqueira Jr 2010; Siqueira Jr 2001). Biofilms and smear layer are often incompletely removed by mechanical instrumentation techniques; conventional hand and rotary instruments may miss root canal surfaces located in ovals, fins, accessory canals, isthmus, and associated intricacies (Paque et al. 2010; Dalton et al. 1998; Hubscher et al. 2003; Peters 2004).

Irrigants can bridge the gap of disinfection and tissue removal by reaching into these difficult to access areas (Park et al. 2012). Therefore, improvement of irrigant effectiveness in reducing bacterial loads should be an

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important goal in endodontic treatment (Siqueira Jr. and Rocas 2008; Chrepa et al. 2014).

Sodium hypochlorite (NaOCl) is the most commonly used endodontic irrigant (Mohammadi 2008). This solution has the advantage of functioning as intracanal disinfection while enabling the dissolution of residual tissue. However, a major disadvantage it carries is the toxicity caused to tissues when the high concentrations are extruded beyond the canal space (Guivarc'h et al. 2017; Kleier et al. 2008). Traditionally, manual syringe needle irrigation of the canal space has been used to deliver NaOCl into the root canal. This method is not efficient in the removal of bacterial biofilm and debris from the apical part of the canal (Ordinola-Zapata et al. 2014).

Enterococcus faecalis, a Gram-positive pathogen, plays a major role in the etiology of persistent periradicular lesions after root canal treatment and is commonly found in a high percentage of root canal failures (Siqueira Jr. and Rocas 2008; Portenier et al. 2003; Barbosa-Ribeiro et al. 2016; Stuart et al. 2006; Evans et al. 2002). Therefore, the *E. faecalis* root canal infection is considered an appropriate model for testing novel antimicrobial treatments (Rosen et al. 2016).

Various techniques were reported to improve the NaOCl efficacy to reduce bacterial loads within the root canal system, such as ultrasonic activation, temperature increase of the NaOCl solution, and negative pressure volume replenishment (Park 2013; Basrani and Haapasalo 2012; Zehnder 2006). Ultrasonic activation is defined as device operation at vibration frequencies of 20 kHz or more, with dental devices ranging from 25 to 42 kHz (Ahmad et al. 1987). Ultrasonic irrigation activation methods involve the two main principles of action: acoustic streaming and cavitation (Macedo et al. 2014; Ahmad et al. 1987; Ahmad et al. 1987b).

The EndoUltra™ (Vista US, Racine, WI) is a new ultrasonic device that has been developed with miniaturized piezoelectric technology that enables an operating frequency of 40 kHz. As the EndoUltra™ is a compact handheld cordless design, it may offer a convenient alternative to conventional ultrasonic activators (Lloyd et al. 2016).

Another irrigation technique is the negative pressure system. This procedure involves replenishing, exchange, and circulation of a low concentration of NaOCl. The method may increase the irrigation effectiveness and compensate for lower concentrations of the solution, potentially reducing solution toxicity (Moorer and Wessellink 1982). In the negative pressure technique, a fresh solution is delivered and the spent solution and debris are removed in a circulatory manner via an apically directed current. An example of negative pressure replenishment circulation technology is the EndoVac system (Kerr, Orange CA).

The aim of this study is to evaluate the efficacy of the EndoUltra™ cordless ultrasonic system and the negative pressure irrigation (EndoVac) in comparison to the conventional ultrasonic irrigation by determining the reduction of bacterial loads in human teeth.

Materials and methods

Tooth specimens

One hundred thirty-six previously extracted human single-rooted teeth with a single canal were collected from the University of Texas School of Dentistry Oral and Maxillofacial Surgery and Periodontics Clinics. The teeth were extracted due to periodontal or restorative reasons. The study (HSC-DB-16-0871) was reviewed and approved by the Committee for the Protection of Human Subjects (CPHS) of the institution.

All teeth were inspected using a dental microscope (Global Dental Microscope .5× with Led illumination St. Louis, MO). Two periapical radiographs were taken for each tooth from buccal-lingual and mesial-distal projections. Only single-rooted anterior teeth with a single-root canal were used in this study.

The following exclusion criteria were applied: teeth with multiple roots, multiple canals, immature roots, teeth with open apices, fractured roots, internal or external resorption, calcified canals, dental cracks, or fracture lines, root caries. All teeth were stored in phosphate-buffered saline (PBS) until used.

Specimen preparation

The crowns of the teeth were removed by a diamond disk (Kerr Orange, CA) to acquire roots 16 mm in length

A 15 K file (Kerr, Orange, CA.) was fitted inside the canal until the file tip was just visible at the apical foramen. One millimeter was subtracted to confirm the working length of 15 mm. The canals were instrumented with a TF Adaptive NiTi Endo File System (Kerr, Orange, CA) following the manufacturer's instructions to a 35/.04 apical preparation; 6% NaOCl was used as an irrigant solution.

Smear layer removal and sterilization

Upon completion of instrumentation, NaOCl was inactivated with 5% sodium thiosulfate for 5 min followed by irrigation with 5 mL of 17% EDTA for one minute. A closed canal system was adopted according to Tay (Tay et al. 2010).

A random sample of five teeth was selected as a control to verify the cleanness of the root canal and smear layer removal. The teeth were prepared as following: the roots were splitted longitudinally using a diamond disk (Kerr Orange, CA) without passing the disk through the root canal lumen under constant cooling with water to

prevent accumulation of dentinal debris. A chisel and small hammer were used to split the root into two halves. Once the roots were separated, they were dehydrated using an ascending series of ethanol concentrations and t-butanol. The roots were allowed to dry in a desiccator and subsequently sputter-coated (Cressington 208HR) with a 7 nm of gold. The root canal surfaces were examined using a scanning electron microscopy (FEI Nova Nano SEM 230, Austin, TX) in order to evaluate smear layer removal. The criterion for complete smear layer removal was to be able to observe complete opening of dentinal tubules in the apical middle and coronal portion of the root at a magnification of $\times 1000$.

All the experimental specimen teeth were then autoclaved for 20 min at 121 °C.

Bacterial load of the prepared root canal specimens

E. faecalis (strain OG1RF) was cultured in 1 mL of brain heart infusion (BHI) for 16 h and inoculated into 5 mL of fresh BHI with 2% glucose solution. The ability to colonize the root canal was verified by observing the root canal surfaces via SEM ($n = 5$). The specimen root canals were inoculated with 100 μ L of the suspension and incubated at 37° for 30 days. The medium was exchanged every 7 days.

Sampling of bacterial growth (S1)

A sterile #35 Hedstrom© file (Maillefer, Tulsa, OK) was inserted to the working length in order to create dentin shavings and sterile Autofit paper points (Kerr Orange, CA) were used to collect the remaining fluid from the infected canals (S1). The threaded file portion and Autofit paper points were collected and placed into Eppendorf microtubes (Fisher Scientific, Pittsburg, PA.) containing 1 mL of PBS. The samples were vortexed for 10 s and 30 μ L aliquot samples were spread on BHI agar plates and incubated at 37° for 24 h. Thereafter, the number of colony forming units (CFU) was counted to determine the presence of viable bacteria in the tooth samples.

Experimental groups

Out of 136 roots, 131 prepared specimens infected with *E. faecalis* were randomly assigned into 5 groups. Five random teeth were selected as a control to verify the cleanness of the root canal and smear layer.

Group 1: (EndoUltra™ cordless ultrasonic device (Vista US, Racine, WI), $n = 40$)

Canals were filled with 1% NaOCl (Zehnder 2006) and the ultrasonic irrigation protocol was performed using the supplied irrigation tips as follows: 3 cycles of 20 s, at the end of each cycle, a syringe replenished the solution

(total volume = 3 ml). EndoUltra ultrasonic tip was placed 1 mm from the working length.

Group 2: (Satelec P5 Booster® and Irrisafe™ (Acteon, Mount Laurel, NJ), $n = 40$)

Canals were filled with 1% NaOCl and the ultrasonic irrigation protocol was performed using #20, 0.02 taper activator as following: 3 cycles of 20 s, at the end of each cycle, a syringe replenished the solution (total volume = 3 ml). Irrisafe Ultrasonic tip was placed 1 mm from the working length.

Group 3: (EndoVac (Kerr, Orange, CA), negative pressure irrigation, $n = 40$)

Canals were filled with 1% NaOCl and the irrigation was performed in two steps as the following:

- a. Macro-cannula step:
One percent NaOCl was constantly delivery for 15 seconds and a macro-cannula was placed approximately to the mid-canal level with up and down (pecking) motion.
- b. Micro-cannula step:
The deliver solution was carried into the root canals and pulp chamber using a micro-cannula. Thereafter, the solution was withdrawn by suction and delivered all the way to the working length. The micro-cannula was removed rapidly to prevent the evacuation of the solution from inside of the root canal.
b1. The root canal was flooded with NaOCl for 10 s and subsequently, it was evacuated using a micro-cannula. (Purge in Endovac language means, evacuate root canal content (solution) completely). Then the root canal was again flooded with NaOCl for 10 s and the micro-cannula was positioned all the way to the working length (total volume = 3 ml; irrigation time = 1 min).

Group 4: (control)—1% NaOCl, $n = 9$)

Canals were irrigated with 1% NaOCl (total volume = 3 ml, irrigation time = 1 min) using a syringe side-vented needle (Dentsply, Tulsa, OK) with no agitation. The needle was placed 1 mm short of the working length.

Group 5: (negative control, $n = 2$)

Canals were irrigated with PBS using a syringe side-vented needle (Dentsply, Tulsa, OK). Total volume = 3 ml; irrigation time = 1 min.

For groups 1, 2, 3, and 4, subsequent to the NaOCl treatment, the root canals were flushed with 5 ml 5% sodium thiosulfate for 5 min to inactivate the NaOCl.

Thereafter, for all groups, the canals were filled with PBS and a sterile #35 Hedstrom© file was used to create

dentine debris. Samples were collected using a sterile paper point as described previously for bacterial growth and CFU counting (S2).

Statistical analysis

A grading score was assigned to the CFU count for each sample before (S1) and after irrigation procedures (S2) yielding a basis for rank comparison. The CFU counts were quantified by category as very heavy (score = 5), heavy (score = 4), moderate (score = 3), sparse (score = 2), and very sparse (score = 1), and none (no growth, score = 0) on agar plates, corresponding to > 10000, 1001–10000, 101–1000, 1–10, and 0 colony forming units per 0.1 ml transport medium, respectively (Dahlen et al. 1982) (Table 1).

Descriptive statistics (frequency and percentage) were provided for the score values of CFU in the 5 groups (Table 2). Wilcoxon rank-sum test was used to compare the grading scores after treatment between the three treatment groups and controls and Bonferroni adjustment was used to adjust for multiple testing. *p* values less than 0.05 were considered significant. Statistical analysis was performed with SAS 9.4 software (Cary, NC).

Results

SEM evaluation of the prepared root canals before bacterial inoculation confirmed smear layer removal and the absence of bacterial growth.

In all the experimental and control groups, there was a heavy presence of bacteria prior to treatment—S1 all scored 5 (CFU > 10000).

Following the treatment, none of the groups had a very heavy bacterial load (CFUs > 10000, score 5). Only groups 4 (1% NaOCl) and 5 (negative control) exhibited heavy bacterial presence (CFUs 1001–10000, score 4). All experimental groups showed moderate bacterial presence (CFUs 101–1000, score 3) or less after treatment. Although certain individual samples from all experimental groups showed no growth, no group as a whole exhibited complete eradication of bacteria.

Table 1 The CFU counts quantified by category of growth on agar plates, corresponding to colony forming units per 0.1 ml transport medium

Grading score	Growth descriptor	CFU
0, blue	None	0
1, dark blue	Very sparse	1–10
2, orange	Sparse	11–100
3, burnt orange	Moderate	101–1000
4, dark orange	Heavy	1001–10000
5, deep blue	Very heavy	> 10000

Table 2 CFU score percentages according to irrigation technique

CFU score	EndoUltra™	Satelec P5 booster	EndoVac	1% NaOCl + standard needle irrigation	PBS + standard needle irrigation
Score 5					
Very heavy > 10000	0.0%	0.0%	0.0%	0.0%	0.0%
Score 4					
Heavy 1001–10000	0.0%	0.0%	0.0%	33.3%	100.0%
Score 3					
Moderate 101–1000	10.0%	5.0%	0.0%	0.0%	0.0%
Score 2					
Sparse 11–100	12.5%	17.5%	12.5%	22.0%	0.0%
Score 1					
Very sparse 1–10	37.5%	35.0%	40/0%	33.3%	0.0%
Score 0					
None 0	40.0%	42.5%	47.5%	11.1%	0.0%

The Endovac group (group 3) was significantly better in eliminating bacteria from the root canals than the 1% NaOCl control group (group 4), ($p = 0.006$), and the negative control group (group 5), ($p = 0.015$). There was no significant difference between the Endovac and two ultrasonic device groups ((group 1, EndoUltra; $p = 0.283$) and (group 2, conventional US; $p = 0.398$)) (Fig. 1).

There was no significant difference between the two ultrasonic devices ($p = 0.806$). Furthermore, there was no significant difference between EndoUltra (group 1) and conventional ultrasonic groups (group 2) as compared to 1% NaOCl (group 4, $p = 0.03$ and $p = 0.02$, respectively) and PBS syringe irrigation groups (group 5, $p = 0.02$ for both).

Discussion

In this study, we examined antibacterial efficacy of three irrigation techniques using an extracted tooth model (Wu et al. 2000) *E. faecalis* is commonly found in cases of persistent apical periodontitis and it was used in the present study due to its ability to form a stable biofilm (Siqueira Jr. and Rocas 2008; Stuart et al. 2006; Duggan and Sedgley 2007; Al-Ahmad et al. 2014).

Cultivable bacteria (CFU) count before and after irrigant treatment has served in previous irrigation studies as an indicator of viable bacteria load reduction (Molander et al. 1998; Shen et al. 2012; Hockett et al. 2008; Spoletti et al. 2003). The volumes and exposure times of the irrigants were

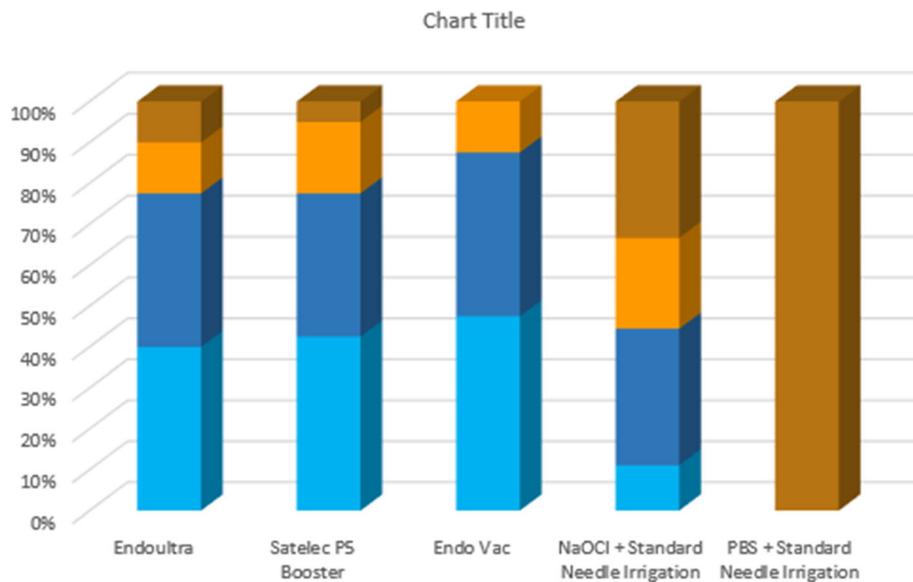


Fig. 1 Post-treatment CFU counts quantified by five categories of growth on agar plates, corresponding to colony forming units per 0.1 ml transport medium, ranging from very heavy (score 5) to none (score 0). Post-treatment bacterial growth from canal samples was significantly less for EndoVac than the syringe 1% NaOCl ($p = 0.006$) and PBS ($p = 0.015$) techniques, but there was no significant difference between the cordless ($p = 0.283$) and conventional ultrasonic devices ($p = 0.398$). There was no difference between the EndoVac negative pressure irrigation and either of the ultrasonic devices ($p = 0.0806$)

equal in all regimens performed to the control for these variables.

The aim of the study was to determine the efficacy of the EndoUltra™ cordless ultrasonic system and the negative pressure irrigation (EndoVac) relative to the conventional ultrasonic irrigation techniques. Our data suggests EndoUltra™ cordless ultrasonic and conventional ultrasonic groups are not significantly different for each other but differ from the Endovac group in terms of reducing bacterial load. The two ultrasonic devices were not statistically different in terms of reducing bacterial load. This result was expected because the two devices are assumed to operate with similar ultrasonic induced flow patterns. The fact that the two devices are supposed to operate at different frequencies (27 to 33 kHz versus 40 kHz) had no effect on the treatment outcome.

In the ultrasonic instrument oscillation, the pressure waves generate acoustic streaming and cavitation. However, the presence and clinical significance of cavitation in ultrasonic irrigation have been debated (Park 2013; Ahmad et al. 1987b; Plotino et al. 2007). Ahmad (Ahmad et al. 1988) did observe cavitation light emissions under sufficient amplitude at the tip of a freely moving file. Nevertheless, the study determined that cavitation was not the important mechanism in debridement. The main flow factor appears to be acoustic streaming. The streaming flow at the end of the instrument can penetrate into the region of spent resident irrigant located at the apical portion of the root canal system. It is this

streaming flow that is likely to play an important role in debriding the biofilm-infected apical anatomy (Gulabivala et al. 2010). If any cavitation was produced with the relatively high EndoUltra™ 40 kHz frequency, it did not produce a substantial effect compared to the conventional ultrasonic range of 27 to 33 kHz. This was confirmed by the absence of the reduction of bacterial loads between the two techniques. Further research is needed to elucidate the contribution of cavitation to the efficacy of ultrasonic irrigation.

An unexpected result was that in spite of a different flow system, the EndoVac negative pressure device was not statistically different from both ultrasonic devices in terms of antimicrobial efficacy. In their study, Townsend (Townsend and Maki 2009) compared several agitation and irrigation devices in terms of antibacterial efficacy using *E. faecalis*. They found that ultrasonic agitation was significantly more effective than needle irrigation or EndoVac irrigation. The differences between results in the current study and the study conducted by Townsend may be explained by differences in the experiment design. The Townsend group irrigated with water instead of NaOCl and they used plastic canals instead of extracted teeth with dentinal tubules as in our study. Although using water may isolate the pure mechanical effect, NaOCl is used in practice and may yield a more realistic model of the synergistic effects of ultrasonication and the irrigant (Cameron 1987). In addition, replenishment of spent solution may be an important

dynamic factor in NaOCl reactions with organic tissue (Moorer and Wesselink 1982; Gulabivala et al. 2010) as opposed to water as an irrigant alone.

In this study, we observed that EndoVac significantly reduced the bacterial loads compared to the needle irrigation controls. This finding was in agreement with previous antibacterial studies (Hockett et al. 2008; Pawar et al. 2012; Cohenca et al. 2010) with limitations due to the differences in the study design.

Hockett et al. (Molander et al. 1998) found significantly better antibacterial efficacy with EndoVac negative pressure irrigation treatment compared to standard positive pressure needle irrigation in extracted mandibular molars with 6% NaOCl in 45/.02 and 35/.02 tapered canals. The present study differed in terms of NaOCl concentration (1%), canal shape (tapered 35/.04), and volume (3.0 ml total). Consistently refreshing with 1% NaOCl solution should suffice to dissolve the entire pulp tissue in the course of an endodontic treatment reducing the need for concentrated solutions with higher toxicity (Zehnder 2006; Moorer and Wesselink 1982).

A study conducted in patients that underwent endodontic treatment showed no difference in antimicrobial efficacy between EndoVac and standard needle irrigation with 0.5% NaOCl (Townsend and Maki 2009). The EndoVac manufacturer's clinical protocol calls for the use of at least 5.25% NaOCl with one macro-cannula cycle (30 s) and 3 micro-cannula cycles (90 s each) for a total of 5 min of irrigant exposure. The time, concentration, and volume in our study were modified to allow for equal exposure of NaOCl between the device techniques.

There was no significant difference in bacterial load reduction between ultrasonic devices and the needle irrigation controls (EndoUltra vs 1% NaOCl control, $p = 0.025$; EndoUltra vs negative control, $p = 0.018$; conventional ultrasonic vs NaOCl control, $p = 0.019$;

conventional ultrasonic vs negative control, $p = 0.018$). However, the p values were very close to significant. The small number of controls may possibly explain the discrepancy since there were only two negative controls with phosphate-buffered saline irrigant (Figs. 2 and 3).

We also discovered variability in the bacterial counts using the syringe irrigation method. This observation may be explained by the inherent inconsistency in the manual syringe technique (Nielsen and Craig Baumgartner 2007). The advantage of the traditional side needle technique is the low cost and simplicity. In terms of disadvantages, manual operation of the positive pressure syringe may not be conducive due to inconsistent operator technique, potentially resulting in varying flow rate, pressure, and turbulence. In addition, a traditional needle has a side-facing vent, even when rotated may not consistently affect the sides of the canal evenly while projecting very limited turbulence (approximately 1.5 mm) and hydrodynamic shear stress to effect cleaning (Chen et al. 2014).

Some disadvantages posed by ultrasonic instrumentation include ledging (Boutsoukis and Tzimpoulas 2016) with loss of working length and instrument breakage (Pedrazzi et al. 2010). A disadvantage of the original EndoVac system is that it is relatively complicated to use in practice, requiring two hands or operators, one for introduction of irrigant with the delivery tip and one for cannula vacuum. Clogging of the very small micro-cannula vents if larger debris has not been removed adequately with larger macro-cannula vacuum efforts is another disadvantage of the EndoVac. However, this situation may be remedied with positive pressure flush or changing the cannula (Nielsen and Craig Baumgartner 2007). In spite of these disadvantages, the EndoVac may present an advantage in breaking vapor lock. Vapor lock occurs when a gas bubble remains entrapped in the apical portion of the root canal, precluding fluid entry

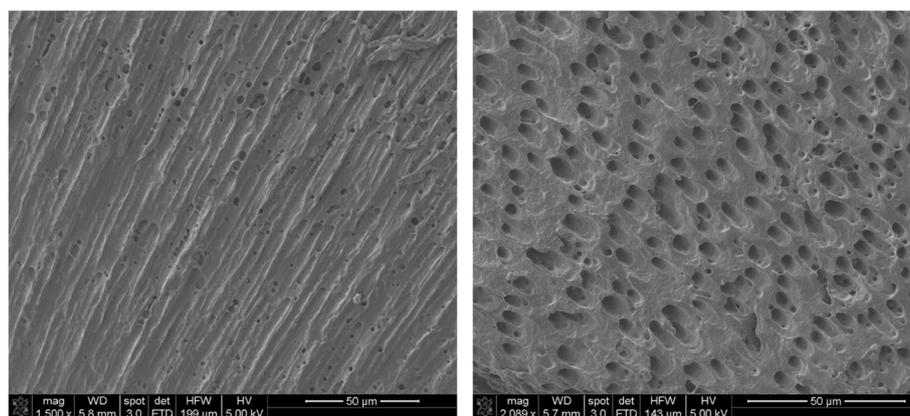


Fig. 2 Negative control. Upon completion of instrumentation, NaOCl was inactivated with 5% sodium thiosulfate for 5 min followed by irrigation with 5 mL of 17% EDTA for 1 min to ensure the complete removal of smear layer

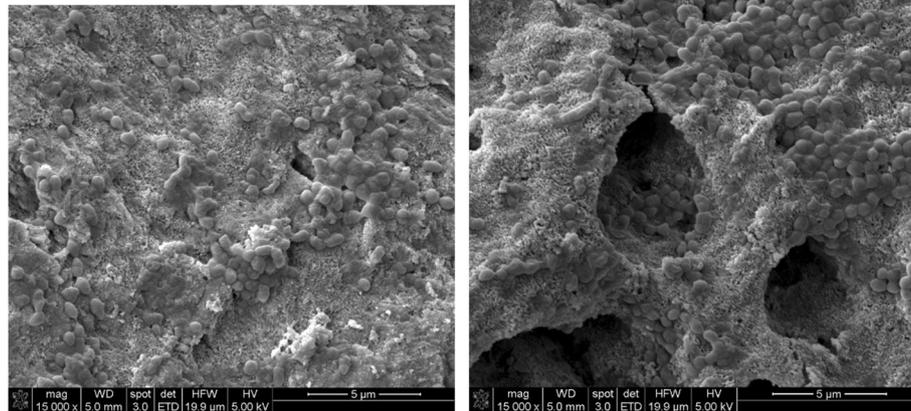


Fig. 3 Positive control. *Enterococcus faecalis* (strain OG1RF) was cultured in 1 ml of brain-heart infusion (BHI) for 24 h and inoculated into 5 ml of fresh BHI with 2% glucose solution. The specimen root canals were inoculated with 100 µL of the suspension and incubated at 37° for 30 days with weekly medium change, ×15000

flow (Gu et al. 2009). Acoustic micro-streaming and cavitation, the hydrodynamic phenomena of ultrasonic, cannot occur effectively in the absence of liquids, leading to vapor lock (Gu et al. 2009), which was not a judge factor during the present experiment. In a recent study by Parente et al. (Parente et al. 2010), they showed that a combination of needle syringe irrigation and the manual dynamic agitation method was less effective than the EndoVac in removing smear layer and debris from closed canal systems. The advantage of the ultrasonic devices is their simplicity of use, especially with the cordless EndoUltra handpiece, while the EndoVac has the benefit of rapid irrigant volume delivery with circulation (Nielsen and Craig Baumgartner 2007) and the tendency against extrusion (Parente et al. 2010).

Conclusion

Overall, the results of the present study showed no difference between the EndoVac negative pressure irrigation system, conventional ultrasonic, and portable EndoUltra systems.

While clinical decisions should be based on high-level evidence, for the irrigation techniques using root canal instrumentation, clinicians may choose to base their decisions upon the mechanical advantages of each system balanced against the practicality of use, cost, and on a case-by-case basis of necessity for the unique advantages of each technique. Future studies are needed on the combined effect of these devices.

Abbreviations

CA: California; CFU: Colony forming units; *E. faecalis*: *Enterococcus faecalis*; EDTA: Ethylene diamine tetra-acetic acid; kHz: Kilohertz; ml: Milliliter; mm: Millimeter; MO: Missouri; N: Number; NaOCl: Sodium hypochlorite; NC: North Carolina; NiTi: Nickel titanium; NJ: New Jersey; OK: Oklahoma; PA: Pennsylvania; PBS: Phosphate-buffered solution; S1, S2: Sample one, sample two; TX: Texas; WI: Wisconsin; µL: Microliters

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Authors' contributions

RM was in charge of collecting and preparing teeth, helped in laboratory work and data collection, reviewed the literature. AN and RvdH were responsible for microbiology lab; helped in the preparation of broth, cultivation of bacteria, and BHI plates; performed the CFU's counting; and reviewed the manuscript. IT and ER helped in the manuscript preparation and final reviews. DEJ designed the study, mentored Dr. Mikulik, made laboratory work, and plating of samples and helped in data collection, DEJ performed SEM imaging and final review of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Please contact corresponding author for data request.

Ethics approval and consent to participate

The following study received approval (HSC-DB-16-0871) qualified for exempt status according to 45 CFR 46.101(b) (waiver of consent granted) from the Committee for the protection of human subjects of the University of Texas Health Science Center at Houston Institutional Review Board (IRB) office.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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