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ORIGINAL ARTICLE

Comparison of bacterial removal from dentinal tubules with different irrigant agitation techniques: An in vitro study

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KEYWORDS

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EDDY sonic activation;
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Dentinal tubules

Abstract *Aim:* This investigation was conducted to assess the ability of various irrigant agitation devices to eradicate *Enterococcus faecalis* from the dentinal tubules of extracted teeth.

Methodology: Fifty roots of extracted human teeth were instrumented to size 30 k with a 0.04 taper. The roots were autoclaved and then injected with *E. faecalis*. The canals were assigned to one of four intervention groups and disinfected using (A) standard needle irrigation, (B) EndoUltra® Ultrasonic Activator, (C) the EndoActivator system, or (D) EDDY sonic activation and to two control groups that were (E) treated with saline and (F) not inoculated with any bacteria. The roots were split in half, dyed with a LIVE/DEAD Back Light Bacterial Viability Kit, and then scanned with a confocal laser scanning microscope (CLSM) to identify live/dead bacteria in the dentinal tubules.

Results: CLSM images revealed differences among the groups. Both the EndoUltra® Ultrasonic Activator group and the EDDY group had a combination of dead and live bacteria, while the EndoActivator group had mostly dead bacteria, in contrast to single needle irrigation which had mostly live bacteria. Activation of the irrigating solution resulted in more dead bacteria than standard needle irrigation at the coronal, middle, and apical parts of the roots. Overall, the EndoActivator system was superior to all other techniques in reducing live bacteria within the root canal.

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Conclusion: Activation of sodium hypochlorite with sonic and ultrasonic systems dramatically reduced live bacteria contamination in the dentinal tubules of infected root canals.

1. Introduction

Endodontic infection is the main etiologic factor of apical periodontitis (Narayanan and Vaishnavi, 2010). Persistent or re-infected bacteria are the prime reason for post-treatment apical periodontitis in the root canal system following primary endodontic therapy (Zandi et al., 2018). *Enterococcus faecalis* is the primary resistant bacteria present in endodontic infections (Vivacqua-Gomes et al., 2005). The major goal of root canal therapy is to eliminate bacteria and avoid re-infection of the root canal, facilitate healing, and avoid inflammation of the periapical tissues (Wong and Cheung, 2014). The process of endodontic treatment consists of enlarging the root canal by the use of instruments and cleaning the endodontic space with the aid of chemical disinfectants to eliminate residual vital or necrotic tissues and microbes within the root canal system (Zehnder, 2006). Disinfection of this system is challenging due to the intricacy of the root canal, as well as the multi-species nature of biofilms (Neelakantan et al., 2017). In addition, complete debridement with an irrigant is unachievable with a needle and syringe alone. Dispersal of the irrigant can be greatly improved via agitation within the root canal. Agitation of irrigants within the canal can be achieved via manual agitation of the fluid by the filing motion of files or via automated agitation by sonic or ultrasonic instruments (Mohmmed et al., 2017).

With passive ultrasonic irrigation (PUI), a smooth wire or an oscillating file transmits energy by means of ultrasonic waves to the irrigant, which causes cavitation of the irrigant and acoustic streaming. EndoUltra® Ultrasonic Activator (Vista, Racine, Wisconsin, USA) is the only cordless ultrasonic activator device with a tip frequency of 40,000 Hz (Ballal and Rao, 2017). The EndoActivator system (Dentsply Tulsa Dental Specialties, Tulsa, OK, USA) uses sonic waves to activate irrigants and produce strong intracanal fluid agitation that increases the efficacy of irrigation in relation to standard needle irrigation (Mancini et al., 2013). Recently, EDDY (VDW, VDW, Munich, Germany), a sonic powered irrigation system, was introduced. These systems are reported to create a 3D movement that produces "cavitation" and "acoustic streaming," two physical effects that to date have only been achieved via PUI and that have been proved to have improved cleaning efficiency (Urban et al., 2017). Limited information is available regarding the ability of ultrasonic and sonic techniques to enhance elimination of biofilm-infected dentine (Ordinola-Zapata et al., 2014).

The objective of this investigation was to appraise the ability of three dissimilar irrigation devices; the EndoUltra® Ultrasonic Activator, EndoActivator System, and EDDY, to eradicate *E. faecalis* from the dentinal tubules of extracted teeth.

2. Materials and methods

2.1. Specimen preparation

Intact extracted anterior teeth were collected. The tooth sample collection followed the regulations set by the "Institutional Review Board of King Saud University". The teeth were decoronated to a standard working length of 16 mm. Apical patency was established, and canals were instrumented with XP Endo Shaper to size 30 k with a taper of 0.04. The teeth were autoclaved at 121 °C for 1 h and then stored in phosphate-buffered saline (Dominici et al., 2001).

2.2. Bacterial preparation and inoculation

A standard suspension of *E. faecalis* (1×10^8 cells/mL) was made from a bacterial culture grown in brain heart infusion (BHI). A sterile 1 mL insulin syringe was used to fill each canal to the orifice with the culture. The roots were placed separately in 10 mL of BHI broth tubes and incubated at 37 °C and 100% humidity for 21 days to permit bacterial colonization along the wall of the canals and within the dentinal tubules. Every 7 days, culture medium aliquots (5 mL) were changed with fresh medium.

The samples were taken out of the inoculation tubes after 21 days and the canals were then disinfected using four irrigation groups as described below. NaOCl (2.5%) was used for irrigation in each of the groups.

2.3. Intervention

In Group A (NaOCl with a standard needle irrigation syringe), ten roots were irrigated using a side-vented 30-gauge needle inserted to 2 mm from the working length. Length control was ensured by placing a rubber stopper on the needle at the desired length. In Group B (NaOCl activated with the EndoUltra® Ultrasonic Activator), the EndoUltra® Ultrasonic Activator was used to irrigate ten roots for a duration of 1 min each with gradual movement. In Group C (NaOCl activated with the EndoActivator), the EndoActivator® was used to irrigate ten roots for a duration of 1 min each with gradual movement. In Group D (NaOCl activated with EDDY), EDDY was used to irrigate ten roots for a duration of 1 min each with gradual movement. In Group E (Positive Control using saline), five bacteria-inoculated roots were irrigated with saline using a 30-gauge needle tip. In Group F (Negative Control using saline), five roots that were not inoculated with bacteria were irrigated with saline using a 30-gauge needle tip.

2.4. Evaluation

Each tooth was cut in half along its long axis using a slow-speed saw (IsoMet 2000 Precision Saw; IsoMet, Buehler, IL) set at 1000 rpm in a bucco-lingual direction with water cooling. The specimens were dyed using a LIVE/DEAD Back Light Bacterial Viability Kit (Molecular Probes, Inc, Eugene, OR, USA) for 30 min. The dye comprises two nucleic acid-binding stains (SYTO 9 and propidium iodide) that are applied to distinguish dead and live cells. Bacteria with damaged cell membranes are colored red, while cells with intact membranes are colored green. A confocal laser scanning electron microscope (CLSM) was used to identify the live/dead bacteria in the dentinal tubules. Three segments of the root canal were evaluated: apical, middle, and coronal (eight slices/scan). The eight image slices were merged to create one composite image. The software then evaluated the percentages of green (live bacteria) and red fluorescence (dead bacteria) in each segment. The green/red fluorescence concentrations were used to measure the proportion of dead bacteria.

2.5. Statistical analysis

Calculation of the proportion of dead bacteria in relation to the total bacteria was performed for each image. Mean and standard deviation were calculated for each section; coronal, middle and apical.

3. Results

The mean and standard deviation of live and dead bacteria percentage in the apical, coronal, and middle sections of each group are presented in Table 1. In the coronal segment; EndoActivator resulted in the greatest percentage of dead bacteria, followed by EndoUltra® Ultrasonic Activator and then EDDY (Fig. 1). In the middle segment; no significantly different change was found among the irrigant activated groups (Table 1). In the apical segment; EndoActivator resulted in the highest percentage of dead bacteria followed by EDDY and then EndoUltra® Ultrasonic Activator (Fig. 1). Overall, EndoActivator resulted in the greatest percentage of dead bacteria, which was significantly more than the single needle irrigation and controls but was not significantly different from EndoUltra® or EDDY (Table 1). Representative images of

Table 1 Mean live/dead bacteria percentage in each group.

Group Third	A Standard Needle Irrigation	B EndoUltra® Ultrasonic Activator	C Endo Activator	D EDDY	E Positive Control	F Negative Control
Coronal	57.42/42.58 (10.49)	27.9/72.10 (31.25)	16.39/83.61 (47.53)	39.77/60.23 (14.47)	63.09/36.91 (18.52)	19.6/80.4 (42.99)
Middle	54.69/45.31 (6.63)	40.66/59.34 (13.21)	45.60/54.40 (6.23)	37.64/62.36 (17.48)	74.5/25.5 (34.64)	5.5/94.5 (62.93)
Apical	57.79/42.21 (11.02)	40.54/59.46 (13.38)	27.42/72.58 (31.93)	32.74/67.26 (24.40)	71.35/28.65 (30.19)	11.16/88.84 (54.93)
Total	56.63/43.37 (9.38)	36.37/63.63 (19.28)	29.80/70.20 (28.56)	36.72/63.28 (18.22)	69.65/30.35 (27.78)	12.09/87.91 (53.62)

Data are expressed as the mean percentage (standard deviation).

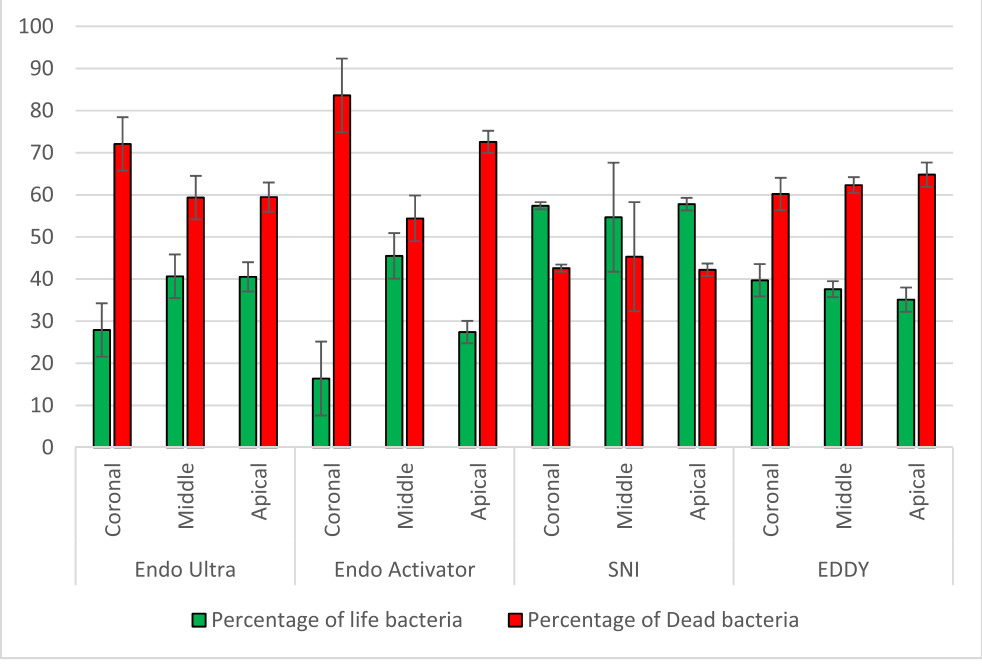

Fig. 1 Percentage of live and dead bacteria in each group.

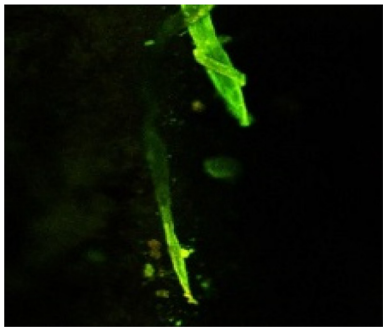

Fig. 2 Group A- standard needle irrigation.

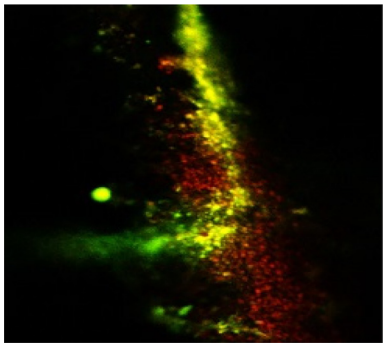

Fig. 3 Group B EndoUltra® ultrasonic activator.

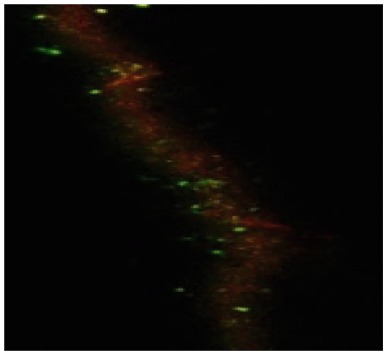

Fig. 4 Group C EndoActivator.

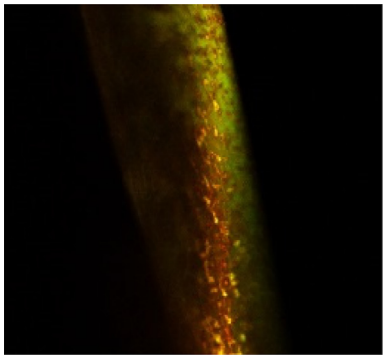

Fig. 5 Group D EDDY.

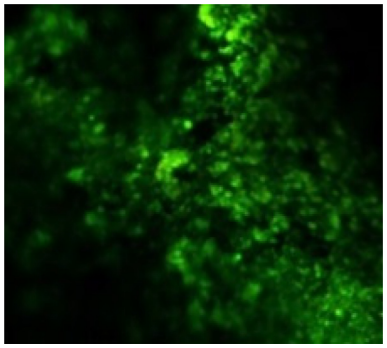

Fig. 6 Group E positive control.

dead (red) and live (green) bacteria for all groups are presented in Figs. 2–6. In these images, it is clear that the positive control group (Fig. 6) had all green stained live bacteria, while the EndoActivator group (Fig. 4) had mostly red stained dead bacteria, in contrast to single needle irrigation (Fig. 2) which had mostly live green stained bacteria. Both the EndoUltra® Ultrasonic Activator group (Fig. 3) and the EDDY group (Fig. 5) had a combination of dead and live bacteria.

4. Discussion

The goal of root canal therapy is the eradication of bacteria and prevention of re-infection. This can be achieved via effective mechanical instrumentation and irrigation. To improve bacterial eradication, irrigants must be in close approximation to the root canal. The standard needle irrigation technique pushes the irrigant for a maximum of 1.1 mm beyond the needle tip (Khalap et al., 2016). Although sodium hypochlorite can infiltrate dentinal tubules, the extent of its penetration is affected by time, temperature, and concentration (Zou et al., 2010). In addition, the antimicrobial efficacy of NaOCl was also found to be concentration-dependent (Wong and Cheung, 2014). The present study revealed that the disruption of *E. faecalis* bacteria with 2.5% NaOCl can be improved using ultrasonic and sonic techniques. The available research regarding these methods compared their ability to eradicate dentine debris (Ordinola-Zapata et al., 2014). The information regarding the ability of ultrasonic and sonic techniques to enhance elimination of biofilm-infected dentine is limited (Ordinola-Zapata et al., 2014). Shear forces in a sufficient amount to dislodge debris in instrumented canals have been demonstrated to develop from acoustic streaming (Ahmad et al., 1987). Files passively stimulated with ultrasonic energy produced acoustic streaming that was adequate to yield significantly more cleansed canals than hand filing alone (Plotino et al., 2007). The selection of the EndoUltra® Ultrasonic Activator, EndoActivator, and EDDY was based on novel ultrasonic and sonic devices from different markets. Large differences in the overall bacteria reduction rate were observed between the groups with activated irrigants and the standard needle irrigation group, despite utilizing the same irrigant application time. The EndoActivator system has been stated to deliver greater dissemination of the irrigant to every area within the root canal, and efficiently dislodge clumps of simulated biofilm and clean debris from lateral canals. It is possible that the higher frequency of activation by the sonic waves

results in higher flow velocity, which aids efficient debris dislodgement (Kumar et al., 2015). The EndoActivator device is a form of active irrigation; its primary function is to produce dynamic intracanal fluid agitation using acoustic streaming and cavitation. Acoustic waves form cavitation bubbles that ultimately disintegrate and release energy that is transmitted to the root canal, detaching any debris within the canal. This assisted in the reduction of bacteria within the apical and coronal segments of the root canals in the present study (Huffaker et al., 2010). The EndoUltra® Ultrasonic Activator produces acoustic streaming and cavitation in small canal spaces, resulting in disruption of the biofilm, enhanced penetration of irrigants into dentinal tubules, improved debridement, and the removal of vapor lock. Studies have demonstrated that other techniques, such as diode laser activation of irrigation, have resulted in fewer viable bacteria and improved antibacterial efficacy compared to standard needle irrigation, photon-initiated photoacoustic streaming, and the EndoActivator in experimentally infected root canals (Mathew et al., 2014). However, with regard to the sonic, ultrasonic standard needle irrigation techniques, the present study has demonstrated that the EndoActivator was superior. However, inclusion of different irrigation and agitation systems, as well as other ultrasonic and sonic systems and inclusion of a larger sample size, may produce various results; hence, the outcome of this study should be interpreted with consideration of these factors.

Our results are also in agreement with those of former studies that reported that EDDY offered improved intratubular bactericidal efficiency than standard needle irrigation in the middle and coronal segments of the root portions within the canal (Zeng et al., 2018). However, in our study, EDDY was only slightly better than the other two techniques in the middle segment of the canal only.

It might be a clinically useful addition to utilize the beneficial actions of sonic or ultrasonic agitation of irrigation during instrumentation of root canals in order to reduce the bacterial load within the canal, and ultimately improve root canal outcomes.

5. Conclusion

It is apparent that root canal irrigant activation systems with NaOCl can reduce the intracanal bacteria load to a significantly greater degree than passive standard needle irrigation alone. In the present study, EndoActivator, a sonic activation system, outperformed the EndoUltra® Ultrasonic Activator system and EDDY sonic activation system. However, none of the techniques could fully eradicate *E. faecalis* from the dentinal tubules of the infected root canal.

Ethical statement

- Has not been published in whole or in part elsewhere;
- The manuscript is not currently being considered for publication in another journal;
- All authors have been personally and actively involved in substantive work leading to the manuscript and will hold themselves jointly and individually responsible for its content.

Declaration of Competing Interest

The authors deny any conflict of interest related to this study.

Acknowledgment

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References

Ahmad, M., Ford, T.R.P., Crum, L.A., 1987. Ultrasonic debridement of root canals: acoustic streaming and its possible role. J. Endod. 13 (10), 490–499.

Ballal, N., Rao, S., 2017. Evaluation of smear layer removal using maleic acid with different irrigation techniques. Int. J. Clin. Dent. 10 (1), 45–54.

Dominici, J., Eleazer, P., Clark, S., Staat, R., Scheetz, J., 2001. Disinfection/sterilization of extracted teeth for dental student use. J. Dent. Educ. 65 (11), 1278–1280.

Huffaker, S.K., Safavi, K., Spangberg, L.S., Kaufman, B., 2010. Influence of a passive sonic irrigation system on the elimination of bacteria from root canal systems: a clinical study. J. Endod. 36 (8), 1315–1318.

Khalap, N.D., Kokate, S., Hegde, V., 2016. Ultrasonic versus sonic activation of the final irrigant in root canals instrumented with rotary/reciprocating files: an in-vitro scanning electron microscopy analysis. J. Conserv. Dent. JCD 19 (4), 368.

Kumar, V.R., Bahuguna, N., Manan, R., 2015. Comparison of efficacy of various root canal irrigation systems in removal of smear layer generated at apical third: an SEM study. J. Conserv. Dent. JCD 18 (3), 252.

Mancini, M., Cerroni, L., Iorio, L., Armellin, E., Conte, G., Cianconi, L., 2013. Smear layer removal and canal cleanliness using different irrigation systems (EndoActivator, EndoVac, and passive ultrasonic irrigation): field emission scanning electron microscopic evaluation in an in vitro study. J. Endod. 39 (11), 1456–1460.

Mathew, J., Emil, J., Paulaian, B., John, B., Raja, J., Mathew, J., 2014. Viability and antibacterial efficacy of four root canal disinfection techniques evaluated using confocal laser scanning microscopy. J. Conserv. Dent. JCD 17 (5), 444.

Mohmmed, S.A., Vianna, M.E., Penny, M.R., Hilton, S.T., Mordan, N., Knowles, J.C., 2017. Confocal laser scanning, scanning electron, and transmission electron microscopy investigation of *Enterococcus faecalis* biofilm degradation using passive and active sodium hypochlorite irrigation within a simulated root canal model. Microbiologyopen 6 (4), e00455.

Narayanan, L.L., Vaishnavi, C., 2010. Endodontic microbiology. J. Conserv. Dent. JCD 13 (4), 233.

Neelakantan, P., Romero, M., Vera, J., Daood, U., Khan, A., Yan, A., Cheung, G., 2017. Biofilms in endodontics—current status and future directions. Int. J. Mol. Sci. 18 (8), 1748.

Ordinola-Zapata, R., Bramante, C.M., Aprecio, R., Handysides, R., Jaramillo, D., 2014. Biofilm removal by 6% sodium hypochlorite activated by different irrigation techniques. Int. Endod. J. 47 (7), 659–666.

Plotino, G., Pameijer, C.H., Grande, N.M., Somma, F., 2007. Ultrasonics in endodontics: a review of the literature. J. Endod. 33 (2), 81–95.

Urban, K., Donnermeyer, D., Schäfer, E., Bürklein, S., 2017. Canal cleanliness using different irrigation activation systems: a SEM evaluation. Clin. Oral Invest. 21 (9), 2681–2687.

Vivacqua-Gomes, N., Gurgel-Filho, E., Gomes, B., Ferraz, C., Zaia, A., Souza-Filho, F., 2005. Recovery of Enterococcus faecalis after single-or multiple-visit root canal treatments carried out in infected teeth ex vivo. Int. Endod. J. 38 (10), 697–704.

Wong, D.T., Cheung, G.S., 2014. Extension of bactericidal effect of sodium hypochlorite into dentinal tubules. J. Endod. 40 (6), 825–829.

Zandi, H., Kristoffersen, A.K., Ørstavik, D., Rôças, I.N., Siqueira Jr., J.F., Enersen, M., 2018. Microbial analysis of endodontic infections in root-filled teeth with apical periodontitis before and after irrigation using pyrosequencing. J. Endod. 44 (3), 372–378.

Zehnder, M., 2006. Root canal irrigants. J. Endod. 32 (5), 389–398.

Zeng, C., Willison, J., Meghil, M.M., Bergeron, B.E., Cutler, C.W., Tay, F.R., Ma, J., 2018. Antibacterial efficacy of an endodontic sonic-powered irrigation system: An in vitro study. J. Dent. 75, 105–112.

Zou, L., Shen, Y., Li, W., Haapasalo, M., 2010. Penetration of sodium hypochlorite into dentin. J. Endod. 36 (5), 793–796.