

Evaluating the antibacterial effect of sonic activation of irrigants on *Enterococcus faecalis* infected root canals

Muthanna S. Ahmed¹, Raghad A. Rasheed², Salwa H. Mohammed³

¹BDS, HDD, MSc., Senior, Specialized dental center, Ministry of Health, Mosul, Iraq

²BDS, MSc., Ass. Prof., Department of Conservative Dentistry, College of Dentistry, Mosul University, Iraq

³BSC, MSc, Ph.D.Oral Microbiology, Lecturer, Department of Basic Dental Science, College of Dentistry, Mosul University, Iraq

Abstract: *Enterococcus faecalis* is frequently isolated from the failed root canals undergoing retreatment. It is well adapted for survival and persistence in a variety of adverse environments. The aim of this ex-vivo study was to evaluate the intracanal bacterial reduction using Vibringe[®] sonic activation of three irrigants. 42 extracted human mandibular first premolar teeth were inoculated with *Enterococcus faecalis*, incubated for 48 hours then randomly distributed into two groups of 21 root each: group A (Control): conventional irrigation (CI) with syringe using Navi Tip gauge 31 with no activation. Group B (sonic activated): the irrigants were delivered and activated using Vibringe[®] sonic activator for 60 second. Each group was subdivided into three subgroups according to the irrigant used (group I=0.9% normal saline), (group II=1.5 %NaOCl) and (group III=3 %NaOCl). After treatment, a sample of dentin was taken from each root for colony-forming unit counting (CFU_s). The results showed that sonic activated group showed a significant reduction in (CFU/ml) comparing to the CI protocol. The sonic activation of 0.9%NaCl showed (31.07%) reduction, 1.5%NaOCl showed (82.91%), while 3%NaOCl showed (98.43%). As a conclusion, sonic activation of irrigants gives better antibacterial results for root canal disinfection in comparison to CI protocol.

Key words: Vibringe[®], sonic activation, *Enterococcus faecalis*. Vapor lock.

Introduction

The main principle of endodontic therapy is to eliminate the causative agents, which providing an environment conducive to healing⁽¹⁾. These requires a combination of physical and chemical agents to eradicate soft-tissue debris, smear layer, and microorganisms⁽²⁾. Data from microbiological studies have revealed that *Enterococcus faecalis* is occasionally isolated from primary endodontic infections but frequently recovered from treatment failures. The rapid emergence of antimicrobial resistance among enterococci makes it difficult to treat the chronic infection⁽³⁾. Presence of *Enterococcus faecalis* in cases of root treatment failures are of particular importance. Prevalence of this microorganism is 22 to 77% of these cases⁽⁴⁾. Irrigation is complementary method to instrumentation in facilitating removal of bacteria, debris and necrotic tissue⁽⁵⁾, especially from areas of the root canal that have been left unprepared by mechanical instruments. The effectiveness of irrigation relies on mechanical flushing and the ability of irrigants to eliminate bacteria⁽⁶⁾ and dissolve tissue⁽⁵⁾. Irrigants have been traditionally delivered using a syringe and needle⁽⁷⁾. The problem with this irrigation technique is the resultant trapped air in the apical third of the root canal that develops vapor lock effect, which may also hinder the exchange of irrigants and decrease the efficacy of debridement⁽⁸⁾.

Paque et al⁽⁹⁾ reported that after NaOCl syringe/needle irrigation and instrumentation, 40–60% of the canals still contained cultivable bacteria. Over the last few decades, several mechanical devices have been developed to improve the dispersal, penetration and effectiveness of irrigation and to activate it in peripheral areas of the root canal space, including Sonic and ultra-sonic techniques⁽¹⁰⁾. The efficiency of sonic and ultrasonic devices is based on the creation of hydrodynamic phenomenon in well-shaped canals filled with an irrigant⁽¹¹⁾. Sonic activation devices operate at frequencies of 1–6 kHz⁽¹²⁾. One of the newest sonic devices is Vibringe[®] endodontic sonic activator that allows the delivery and sonic activation of the irrigating solution in one step. It employs a two-piece syringe with a rechargeable battery. The present study compared the ex-vivo intracanal reduction of *Enterococcus faecalis* populations promoted by three irrigants (0.9% NaCl, 1.5% NaOCl and 3% NaOCl) using two irrigation protocols: CI protocol and sonic activation protocol using Vibringe[®] sonic activator.

Materials and Method

Samples selection and preparation:

An 42 newly extracted, mature, single rooted human mandibular 1st premolar teeth were collected. Radiograph was used with two projections (bucco-lingual and mesio-distal) to examine the canal anatomy. Teeth with additional canal, complex anatomy or accessory canals were eliminated from the study. Decoronation done by removal of the crown at the cemento-enamel junction (CEJ) using a two-sided diamond coated sectioning disc under copious coolant. The length of each root was adjusted to 15 mm from the cervical border of the root to the apical foramen. While the working length kept at 1 mm less than the root canal length (14 mm). The canals were prepared using ProTaper Universal rotary nickel titanium files (ProTaper[®], DENTSPLY Maillefer, Ballaigues, Switzerland) according to the manufacturer's instructions to the size F4. A resin based composite restorative material (Spectrum Universal composite, DENTSPLY, Germany) shade A2 was used to seal the apical foramen (the apical 3 mm) of each root. While the external root surface with the apical 3 mm was painted with two layers of nail varnish.

Samples Mounting:

A silicone based poly vinyl siloxane impression material was mixed and adapted in a stainless steel metal container on which the sampled roots were mounted vertically about 3mm from the cervical border. Then sterilization was done by autoclave at (121 C°/15 pounds/inch² for 15 minutes).

Bacterial preparation:

The Enterococcus faecalis were isolated and identified from teeth canals of failed endodontic treatment with persisting periapical lesions as described by Pinheiro et al.,⁽¹³⁾.

Samples inoculation:

A sterile 1-ml insulin syringe was used to fill the sterile roots with 0.1 ml of the brain heart infusion broth (BHI) that contains 8×10^{-7} of the isolated Enterococcus faecalis without overflowing. The sampled roots were incubated at 37°C for 48 hours.

Samples grouping:

After the 48 hours, the total samples (n=42) were divided into 2 groups according to the management methods and irrigation protocols into:

1-) group A: (control group/n=21)

Irrigated by luer-lock hypodermic syringe with Navi tip (Navi Tip; Ultradent products, Salt Lake City, UT, USA) gauge 31 of two sides venting and closed apex at a rate of 5 ml/75 seconds (1ml/15 second) the needle tip was placed 1 mm from the working length. No activation method was used for this group which is divided into three sub-groups:

Group A-I: (negative control): (n=7) irrigated with 0.9% sterile saline.

Group A-II: (positive control): (n=7) irrigated with 1.5% NaOCl (ParcanTM; Septodont, Saint-Maur-des-Fossés, France).

Group A-III: (positive control): (n=7) irrigated with 3% NaOCl (ParcanTM; Septodont, Saint-Maur-des-Fossés, France).

At the end of treatment period, group A-II and A-III were neutralized with 1.2ml and 2.4ml of 5% sodium thiosulphate for 60 second.

2-) Group B: (sonic activated group/n=21)

Irrigated by Vibringe[®] sonic activator (Vibringe[®] B.V. Corp, Amsterdam). The syringe attached to Navi tips (Navi Tip; Ultradent products, Salt Lake City, UT, USA) gauge 31 of two sides venting and closed apex at a rate of 5ml/75 second (1ml/15 second) in an activation mode. The needle tip was placed 3mm from the working length according to the manufacturer instructions and moved 2mm up and down during activation period. Divided into three sub-groups:

Group B-I: (n=7) irrigated with 0.9% sterile saline.

Group B-II: (n=7) irrigated with 1.5% NaOCl (ParcanTM; Septodont, Saint-Maur-des-Fossés, France)

Group B-III: (n=7) irrigated with 3% NaOCl (ParcanTM; Septodont, Saint-Maur-des-Fossés, France).

At the end of treatment period, group B-II and B-III were neutralized with 1.2ml and 2.4ml of 5% sodium thiosulphate for 60 second. All the irrigants used were at room temperature during irrigation.

Determination of the Remaining Viable Bacteria (RVB):

After 1 minute of the treatments, 10 µL of a sterile ringer solution was placed in all root canals according to Pedullà et al.,⁽¹⁴⁾. A sample of dentin shaves was taken from each treated root by inserting a sterile size F5 file into the root canal. The file was rotated 360° clockwise direction within the canal, reaching the full working length to engage and remove dentin from the coronal, middle and apical third of the root.

The file with the dentin stuck on it was dispensed within a sterile screw-capped vial of 4.5 ml BHI, vortexed for 30 second then 10 fold serial dilution was carried till 10⁻⁷ for each root. 100µl of the last two dilutions was cultured on Enterococcus selective agar plates, incubated at 37°C for 48 hours to determine the remaining cultivable bacteria.

Statistical analysis

The statistical analysis was performed using (release 20.0; SPSS Inc. Chicago, IL, USA) for windows 7. Two way analysis of variance (ANOVA) and post hoc analysis with Duncan was used at a significance level of 0.05.

Results

The findings of this study showed that the root canal treated by sonic activation exhibits the least viable count bacteria within 1 minute after management. Sonic activation group showed a significant reduction in (RVB/ml) comparing with the CI group. The sonic activation of 0.9% NaCl showed (31.07%) RVB reduction, 1.5 % NaOCl sonic activation showed (82.91%), while sonic activation of 3% NaOCl showed (98.43%) compared to (14.11%) reduction when using 0.9% NaCl with CI, (68.11%) for 1.5% NaOCl with CI and (93.1%) eradication for 3% NaOCl (figure 1).

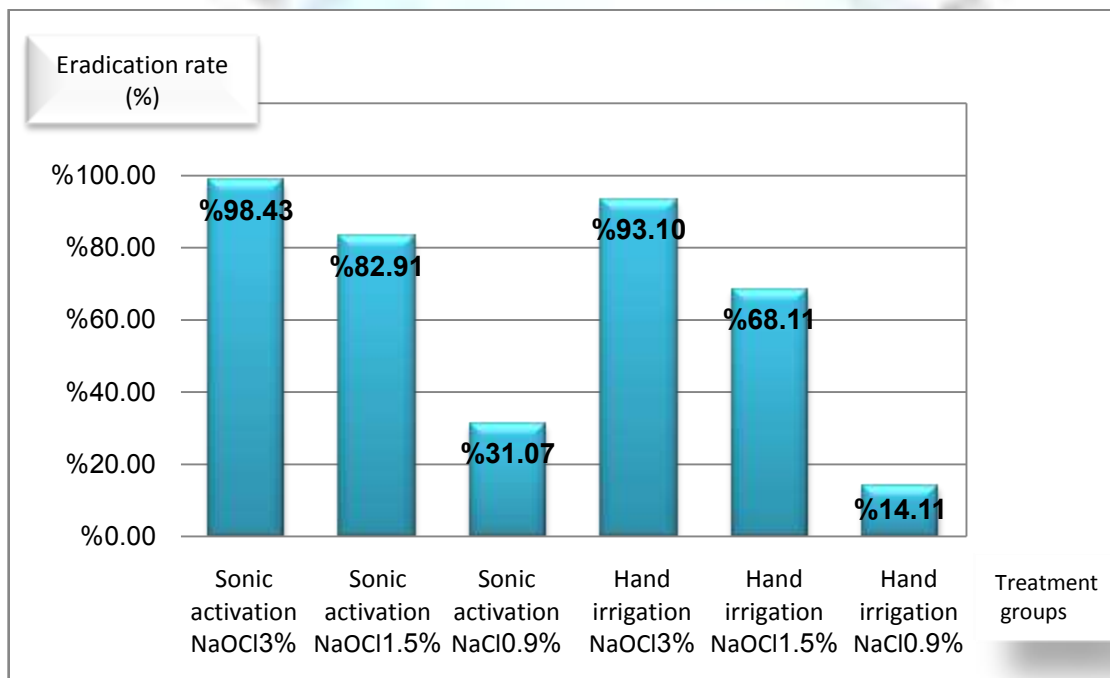


Figure 1: The eradication rate percentage for CI and Vibringe® sonic activation.

Discussion

The efficacy of sonic activated irrigation using Vibringe® system on the bactericidal property of irrigants has not been fully evaluated. The present ex-vivo study was conducted to compare the antibacterial efficacy of two activation protocols; CI and sonic activation protocol using Vibringe® sonic activator) for reducing intracanal Enterococcus faecalis populations. Sonic activated protocols showed a significant reduction in the bacterial population in intragroup analysis and were significantly more effective than the control group. Tay et al⁽⁸⁾ stated that CI protocol generates a “dead water zone” at the apical 0.5-1.0 mm. causing “apical vapor lock” inhibiting accurate bacterial eradication that needs a forceful current which can be created by sonic or ultrasonic devices. Many authors stated that entrapped air bubbles cannot be removed with CI^(8, 15, 16.). These results are in agree with our results where by the eradication rate of

1.5% and 3% NaOCl using CI were only 68.11% and 93.1% respectively. This study evaluated the antimicrobial property of Vibringe[®] that combines both manual delivery and sonic activation (150 Hz) of the irrigants.

Vibringe[®] contains a microprocessor that converts the piston of the device into a sonic waves generator making the irrigant delivered from the needle tip auto-activated sonically. In addition to the vibration of the needle tip with less concern about the size of canal preparation, that gives Vibringe[®] an advantage over the other file or polymer tip depending devices which can be entrapped in curved or narrow prepared canals, as higher degree of curvature restricts the movement and vibratory motion of the tips leading to less efficient cleaning⁽¹⁷⁾.

Pitt WG⁽¹⁸⁾ stated that using Sonic activation to generate acoustic streaming is an effective method for disinfecting root canals. Acoustic streaming is the creation of intense circular fluid movement or flow pattern around files known as eddies. The directional flow from apical to coronal and eddies produced through the acoustic streaming which created by the vibrating instrument is more intense in velocity and magnitude around its tip. The induced acoustic streaming leads to jets of irrigant that are directed towards the root canal wall⁽¹⁹⁾ increasing penetration depth of the irrigant into dentinal tubules.

Because the apical vapor lock cannot be displaced within a clinically relevant time frame through simple mechanical actions, it prevents further irrigants from flowing into the apical region. More importantly, acoustic micro streaming and cavitation can only occur in a liquid phase. Therefore, once a sonic or ultrasonically activated tip leaves the irrigant and enters the apical vapor lock, acoustic micro streaming and/or cavitation become physically impossible.

NaCl activation with Vibringe[®] sonic activator gave a 31.07% reduction in CFU compared to 14.11% reduction when NaCl was used in the CI method. This reflects the fact that sonic activation can disturb and dislodge the biofilm formed by the bacteria and dislodge the bacteria from root canal system.

The interaction between the sonic activator and the NaOCl is a sono-mechanical and sono-chemical where the irrigant is flushed with turbulent waves that increase the debris removal efficiency as reported by Rödigo et al.,⁽²⁰⁾. Vibringe[®] system allows for continuous refreshment of the irrigant with laterally directed shear force that pushes the irrigant towards the dentinal tubules, this may play a role in increasing the penetration depth and disrupting the bacterial cell wall hindering behind the negative effect of vapor lock by delivering the irrigant to all the canal anatomy spaces in a 3D delivery manner. According to the results of our ex-vivo experiments, sonic activation of NaOCl showed superior antibacterial results when compared to CI method of NaOCl, This statistical difference is in agreement with Seet et al.,⁽²¹⁾ and Pedullà et al.,⁽¹⁴⁾.

Conclusions

Under the conditions of the current study, sonic activation protocol with Vibringe[®] endodontic activator was superior in *Enterococcus faecalis* eradication to hand irrigation with CI protocol syringe.

References

- [1]. Sjögren U, Figdor D, Persson S and Sundqvist G. Influence of infection at the time of root filing on the outcome of endodontic treatment of teeth with apical periodontitis. *Int Endod J* (1997); 30(3):297-306.
- [2]. Kimura Y, Wilder-Smith P and Matsumoto K. Laser in endodontics: a review. *Int Endod J* (2000); 33:173-85.
- [3]. Isabela N R, Jose FS and Katia RNS. Association of *Enterococcus faecalis* with different forms of periradicular diseases. *J of Endod*(2004); 30(50):315-320.
- [4]. Fouad AF, Zerella J, Barry J and Spangberg LS. Molecular detection of *Enterococcus* species in root canals of therapy-resistant endodontic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* (2005); 99:112-8.
- [5]. Lee SJ, Wu MK and Wesselink PR. The effectiveness of syringe irrigation and ultrasonics to remove debris from simulated irregularities within prepared root canal walls. *Int Endod J*(2004);37:607-12.
- [6]. Gulabevala K, Patel B, Evans G and Ng YL. Effects of mechanical and chemical procedures on root canal surfaces. *Endodontic Topics*(2005); 10:7-102.
- [7]. Haapasalo M, Shen Y, Qian W and Gao Y. Irrigation in endodontics. *Dent Clin of North Am* (2010); 54:291-312.
- [8]. Tay FR, Gu L and Schoffel GJ. Effect of vapor lock on root canal debridement by using a side-vented needle for positive – pressure irrigant delivery. *J of Endod.* (2010); 36 (4):745-750.
- [9]. Paque F, Laib A and Gautschi H. Hard-tissue debris accumulation analysis by high-resolution computed tomography scan. *J of Endod* (2009); 35:1044-7.
- [10]. Bago I, Plečo V, Gabrić D, Schauerplv Z, Baraba A and Anić I. Antimicrobial efficacy of a high-power diode laser, photo-activated disinfection, conventional and sonic activated irrigation during root canal treatment. *Int Endod J* (2013); 46(4):339-347.
- [11]. Ruddle CJ. Endodontic disinfection: tsunami irrigation. *Endodontic topics*(2008); 11:7-15.
- [12]. Ahmad M, Pitt ford TR and Crum LA. Ultrasonic debridement of root canals: An insight into the mechanisms involved. *J of Endod*(1987); 13:93-101.

- [13]. Pinheiro ET, Gomes BPFA, Drucker DB, Zaia AA, Ferraz CCR and Souza-Filho FJ. Antimicrobial susceptibility of *Enterococcus faecalis* isolated from canals of root filled teeth with periapical lesions. *Int Endod J* (2004); 37 (4):756-763.
- [14]. Pedullà E, Genovese C, Campagna E, Tempera G and Rapisarda E. Decontamination efficacy of photon-initiated photoacoustic streaming (PIPS) of irrigants using low-energy laser setting: an ex vivo study. *Int Endod J* (2012); 45(9):865-870.
- [15]. De Gregorio C, Estevez R, Cisneros R, Heilborn C and Cohenca N. Effect of EDTA, sonic, and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals: An in-vitro study. *J of Endod* (2009); 35:891-5
- [16]. Gu LS, Kim JR, Choi KK, Pashley DH and Tay FR. Review of contemporary irrigant agitation techniques and devices. *J of Endod* (2009); 35:791-804.
- [17]. Walmsley AD, Lumley PJ and Laird WR. Oscillatory pattern of sonically powered endodontic files. *Int Endod J* (1989); 22:125-32.
- [18]. Pitt WG. Removal of oral biofilm by sonic phenomena. *Am J Dent* (2005); 18:345-52.
- [19]. Jiang LM, Verhaagen B and Versluis M. The influence of the ultrasonic intensity file on the cleaning efficacy of passive ultrasonic irrigation. *J of Endod* (2011); 37; 688-92.
- [20]. Rödigo T, Bozkurt M, Konietschke F and Hülsmann F. Comparison of the Vibrate® system with syringe and passive ultrasonic irrigation in removing debris from simulated root canal irregularities. *J of Endod* (2010); 36(8):1410-1413.
- [21]. Seet AN, Zilm PS, Gully NJ and Cathro PR. Qualitative comparison of sonic or laser energization of 4% sodium hypochlorite on an *Enterococcus faecalis* biofilm grown in vitro. *Aust Endod J* (2012); 38(3):100-6.

