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ORIGINAL ARTICLE

Bacterial Reduction in Oval-Shaped Root Canals After Different Irrigant Agitation Methods

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ABSTRACT

Objective: This study aimed to assess the antibacterial effect of Easy Clean®, passive ultrasonic irrigation and sonic irrigation against *Enterococcus faecalis* biofilm in oval-shaped canals.

Methods: Fifty-five extracted human teeth with similar dimensions were selected. Access cavities were prepared and the root canals were instrumented using Wave One® Primary files (25.08). The root canals were then contaminated with an *E. faecalis* suspension following incubation for thirty days. The contaminated roots were divided into three experimental groups (n=15) according to the irrigant agitation protocol (Easy Clean, passive ultrassonic irrigation and sonic irrigation), in addition to a positive control group (n=5) and a negative control group (n=5). Microbiological samples were taken from the root canals before instrumentation (S1), after instrumentation (S2) and after the final irrigation protocol (S3). The samples were assayed and incubated for 48 hours in order to obtain the residual titer of *E. faecalis* cells. Viable cells were quantified by colony-forming units (CFU) measurement. The collected data was submitted to statistical analysis by using Shapiro-Wilk`s test, Wilcoxon paired test, Kruskal–Wallis test and Dunn's post-hoc test. The level of significance was set at 5%.

Results: All experimental groups presented significant reduction on bacterial load after instrumentation ($P<0.05$) with similar reduction among the groups. After the agitation protocols, significant reduction in bacterial load was demonstrated for all groups ($P<0.05$). However, no differences were shown among Easy Clean®, PUI and SI ($P>0.05$).

Conclusion: The results showed that all tested groups exhibited similar disinfection effectiveness. However, none of them was able to render all root canals free from microorganisms.

Keywords: Endodontics, root canal irrigants, sonic, ultrasonic, vibration

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HIGHLIGHTS

- The present study compared the effect of Easy Clean®, passive ultrasonic irrigation and sonic irrigation against 30-day *Enterococcus faecalis* biofilm growth model.
- This study highlights the importance of irrigant agitation protocols in order to improve root canal disinfection in oval-shaped canals.
- This study demonstrates that irrigant agitation protocols are effective on reducing the bacterial content of root canals.

INTRODUCTION

The development of pulp and periradicular diseases usually comes from a microbial infection (1). During endodontic treatment, the root canal is enlarged in order to render mechanical removal of infected dentine and facilitate the penetration of irrigants through the canal, improving its disinfection (2). However, about 60% of the root canal surface remains untouched after root canal instrumentation, regardless of the instruments designs or technique approaches (3). In addition, there are unreached areas, such as isthmuses, ramifications and dentinal tubules where microorganisms may remain protected from root canal disinfecting protocols (4).

Under these circumstances, irrigation has a key role on disinfection of such areas along with root canal preparation (5). Regarding chemo-mechanical preparation of the Root Canal System (RCS),

conventional irrigation technique using syringe and needle is usually considered as an inefficient irrigation technique (6). It has been demonstrated that the binomial syringe/needle is not able to reach the apical third of the RCS due to the vapor lock effect (7). For this reason, supplementary irrigations' protocols have been developed to drive root canal irrigants into non-touched areas of RCS (8).

Other strategies such irrigant agitation protocols have been proposed in order to enhance the arrival of irrigants into non-touched areas of RCS and improve endodontic outcomes (4-9). From the currently available techniques, Passive Ultrasonic Irrigation (PUI) is the most widely described technique (5, 8-10). This technique is based on the transmission of ultrasonic waves from a file or an ultrasound insert to an irrigating solution, producing acoustic streaming capable of increasing sodium hypochlorite (NaOCl) agitation in root canals (11). Sonic irrigation (SI) is believed to generate sonic vibration that promotes smear layer and debris disintegration favouring their removal by the irrigation/aspiration process (12). Previous studies demonstrated that both techniques are able to reduce bacterial load, however, none of them seems to provide root canals free from microorganisms (5, 8-10).

Recently, Easy Clean® (BassiTM, http://bassiendo.com) has been introduced to activate irrigating solutions. This device consists of an instrument size 25/0.04, made with acrylonitrile butadiene styrene plastic, and used in reciprocating or rotary motion. EasyClean® has an "aircraft wing"-shaped cross section and presents great flexibility, enabling safe dispersion of irrigating solution. It seems to be a helpful method for both smear layer and hard tissue debris removal, mainly in the apical third of RCS (9). Easy Clean® may also be mounted on a dental handpiece. As the potential of Easy Clean® in reducing the bacterial load of endodontic infections has not yet been elucidated, the aim of this study was to assess, *ex vivo*, the antimicrobial effect of Easy Clean®, PUI and SI against a 30-day old Enterococcus faecalis biofilm in oval-shaped root canals. The null hypothesis of this paper was that there is no difference among the irrigant agitation protocols in the *E. faecalis* load reduction.

MATERIALS AND METHODS

Specimen preparation

This study was approved by Institutional Ethical Review Board (CAAE 48121115.2.0000.5259). Fifty-five extracted teeth were selected according to the following inclusion criteria: single-rooted mandibular human incisors, complete root formation, no evidence of root resorptions, no fracture or cracks, 20- to 22-mm in length, oval-shaped root canals (long/short cross section diameter ratio of ≥2.5 at 5mm from the apex) based in preoperative radiographs that were taken in the buccolingual and mesiodistal directions. The specimens were stored in 0.9% NaCl solution prior to their use (13).

Traditional endodontic accesses were executed by means of round and Endo-Z burs (KG Sorensen, São Paulo, Brazil). Dental crowns were maintained so a reservoir for *E. faecalis* suspension could be obtained. A size10 K-file was chosen to determine apical patency observing its tip in the apical foramen and the working length (WL) was delimited 1 mm shorter. The apical constriction was instrumented up to a size 20 K-file (Dentsply-Sirona, Ballaigues, Switzerland) to standardize the apical constriction size.

The smear layer was removed by irrigating with 3 mL of 17% EDTA for 3 minutes, in combination with 5 mL of 2.5% NaOCl. The bactericidal effect of NaOCl was inactivated by rinsing 5 mL 5% sodium thiosulfate.

The teeth received two layers of clear nail polish (Impala, Guarulhos, SP, Brazil) on the entire external surface, including the apical foramen. This procedure was performed to prevent microleakage of bacteria through lateral canals. Next, a sterile size 15 K-file was placed 0.5 mm beyond the apical foramen to remove any blockage from the nail polish layers (14).

Test apparatus

Eppendorf tubes had their caps replaced by rubber dam (Ind. Artefatos de borracha Inovatex Ltda - Madeitex, São José dos Campos, SP, Brazil), fixed with elastics. Each tooth was inserted vertically in a hole made in the center of the rubber-dam, up to the cervical region. The interface between the tooth crown and the rubber cap received a layer of cyanoacrylate adhesive (Henkel, São Paulo, Brazil). The entire model (Fig. 1) was sterilized in an autoclave (Prismatec, Itu, SP, Brazil) for 20 minutes at 127°C before infection procedures. Afterwards, 2 mL of sterile saline was added into the Eppendorf tubes to eliminate the entrapped air and to guarantee the vapor lock effect.

Specimen infection with *E. faecalis*

The infection of the study samples and sampling procedures were done according to de Brito et al. (15) with modifications.

A pure culture of *E. faecalis* (ATCC 29212) (American Type Culture Colection, Virginia, EUA) was incubated overnight in brain-heart infusion broth (Difco Laboratories, Maryland, EUA) and used to prepare an inoculum suspension. The bacterial suspension was adjusted spectrophotometrically to match the turbidity of 1.5x108 colony-forming units (CFU mL) equivalent

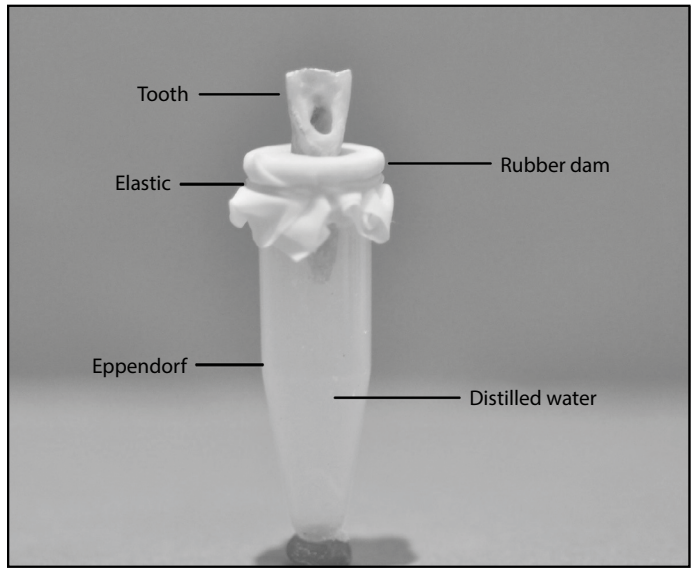

Figure 1. The experimental model system

to ±0.5 McFarland standard. Sterile pipettes were used, under Class I laminar flow to inoculate each specimen with 2 µL of the bacterial suspension. Fifty specimens were contaminated. A sterile size 10 K-file (Dentsply-Sirona) was used to spread the bacterial suspension along the entire root canal length. Subsequently, the infected specimens were incubated at 37°C for 30 days andevery 2 days received new sterile BHI medium to maintain the biofilm growth.

Subsequently, the root canals were irrigated with 1 ml of sterile NaCl and the first bacterial sample (S1) was taken by means of three sterile paper points size 15 (Meta® Biomed, Cheongjusi, Korea) introduced sequentially into the canals for 1 min. The paper points were then transferred to a sterile Eppendorf tube containing 1 ml of sterile saline solution. The CFU counts were performed as described in the quantification of the bacterial load.

Root canal preparation

One expert operator, using aseptic techniques, carried out canal preparation and sampling procedures on each specimen under a class I laminar flow hood, to prevent airborne contamination.

Wave One® Primary files (25.08) (Dentsply-Sirona) were used according to the manufacturer's instructions. Instruments were activated with a 6:1 contra-angle handpiece (Sirona, Bensheim, Germany) powered by a torque-limited electric motor (VDW Silver Reciproc; VDW, Münich, Germany) using the "WAVEONE-ALL" set-up. The instrument was introduced into the root canal with a slow in-and-out pecking motion without pulling it completely out of the canal. Forty-five contaminated specimens and five not contaminated were instrumented. Each third of the root length was prepared with 3 in-and-out pecking movements. The amplitude of the in-and-out movements did not exceed 3–4 mm. After the in-and-out movements, or when more pressure was needed to make the instrument advance further in the canal, or when resistance was encountered, the instrument was removed from the canal and were inserted into a clean endo stand (Dentsply-Sirona) for cleaning purposes. Canal patency was checked with a size 10 K-file.

A total volume of 8 mL of 2.5% NaOCl was used during the treatment. The irrigation was performed by means of a disposable 3 ml plastic syringe with a 31-gauge stainless steel NaviTip (Ultradent, South Jordan, UT, USA) placed passively down the canal, up to 3 mm from the WL without binding (14). The aspiration was done with Endo Tips 0.6 and 0.14 (Angelus, Londrina, PR, Brazil) connected to the suction pump. Due to possible differences in the total instrumentation time, the time that irrigating solution kept inside the root canal was standardized in 10 minutes. Subsequently, teeth were irrigated with 2 mL of 17% EDTA (Asfer, São Caetano do Sul, SP, Brazil) followed by additional 4 mL 2.5% NaOCl (15).

After the root canal preparation, the bactericidal effect of NaOCl was inactivated by rinsing the canals with 5 mL of 5% sodium thiosulfate. Then, the canals were filled with 2 mL of sterile saline and the second bacterial sample (S2) was collected. In order to collect dentine shavings for the S2 samples, a size 15 Hedströem file (Dentsply-Sirona) was used to file the dentine walls (16). Moreover, three paper points size 15 were placed at the root canal at the WL for 1 min, to absorb the liquid contents. Then, the file and the paper points were transferred together into the same Eppendorf tubes containing 1 mL saline solution. The CFU counts were performed as described in the quantification of the bacterial load.

Final irrigating protocol

The forty-five contaminated and instrumented specimens were randomly divided into three groups (n=15) according to the irrigant agitation protocol.

All specimens from all groups were irrigated with 2 mL of 2.5% NaOCl (16) and the different final irrigant agitation protocols were performed, as follows:

Easy Clean group – Easy Clean® instrument was activated 2 mm short of the WL in a continuous rotation motion (20.000 rpm) during 1 min.

PUI group – PUI was performed using an ultrasonic tip (20/0.01) (Irrisonic; Helse Dental Technology, São Paulo, Brazil) mounted in a piezoelectric ultrasound device (CV Dentus, São Paulo, Brazil), set at low power setting (10%). The ultrasonic tip was inserted 2 mm from the WL and activated promoting constant vibratory movement for 1 min (11).

SI group – EndoActivator medium tip (25/0.04) was inserted 2 mm short from the WL. The apparatus was then activated promoting vibratory and oscillatory movements at a speed of 10.000 cycles per minute (cpm) for 1 min. During this period, gentle in-and-out movements with amplitude of 2 to 3 mm were executed according to the manufacturer's instructions.

Positive control group – In this group, five root canals were contaminated but not instrumented. The bacterial biofilm formation status was confirmed in these specimens by using a 25kv Scanning Electron Microscopy (SEM) (Jeol, model JSM 5310, Itaberaba, São Paulo, SP, Brazil) at 500, 2000 and 7500 magnifications.

Negative control group – To assure the absence of cross contamination during the experiment, five samples that were sterilized but not contaminated were used as a negative control group (NCG). The NCG was subjected to instrumentation according to the protocol previously described and three specimens had the irrigating solution (NaOCl 2.5%) activated by different techniques: Easy Clean®, SI, and PUI. Two of them had no activation of the irrigating solution.

After the final irrigating protocol, the canals were rinsed with 5 mL of 5% sodium thiosulfate and then filled with 2 ml of sterile saline again and the third bacterial sample (S3) was taken as previously described for S2.

The methodology and experimental groups are summarized in Figure 2.

Quantification of bacterial load

The Eppendorf tubes containing the collected samples (S1, S2 and S3) were agitated in vortex for 1 min and serial dilutions with sterile saline up to 10-3 were prepared. 100 µL aliquots of each dilution were spread in *Mitis salivarius* agar plates (Dif-

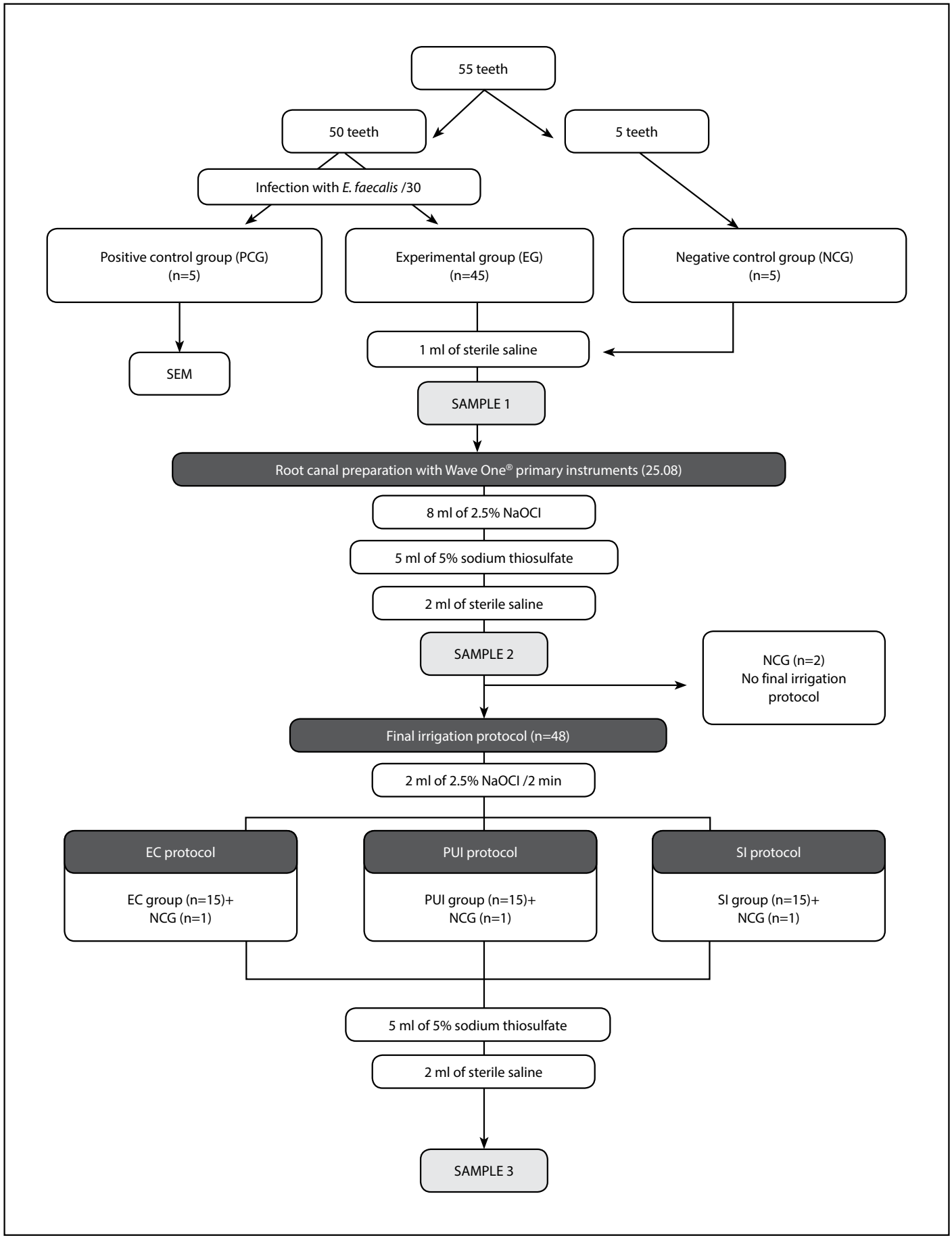

Figure 2. Flowchart of experimental procedures

TABLE 1. Mean values (log 10) of the bacterial content found before (S1) and after the root canal instrumentation (S2) as well as after the final irrigation protocols (S3), with their respective percentage of bacterial reduction

Groups	Before instrumentation (S1)	After instrumentation (S2)	Reduction percentage (S1-S2)	After agitation (S3)	Reduction percentage (S1-S3)
EA	4.65±0.48^{A}	2.47±1.50^{A*}	96.39%	1.17±0.99^{A**}	99.59%
EC	4.66±0.55^{A}	2.14±1.44^{A*}	97.23%	0.95±1.00^{A**}	99.83%
PUI	4.59±0.69^{A}	1.53±1.83^{A*}	96.06%	0.42±1.00^{A**}	99.80%

The values are mean±SD. *Significant statistical difference in bacterial content from S1 to S2. **Significant statistical differences in bacterial content from S2 to S3. The superscript letters represent a statistically significant difference at the same time (S1, S2 or S3, P<0.05)

co, Maryland, USA) and plates were incubated for 48h at 37°C. Each colony was counted as one Colony-Forming Unit (CFU).

Statistical analysis

The collected data (CFU counts) were statistically analyzed by SPSS 17.0 software for Windows (SPSS, Inc., Chicago, IL, USA). The Shapiro-Wilk`s test showed that the distribution of the variables studied deviated from normality. Therefore, the data were transformed to Log10. The intragroup quantitative analysis compared the bacterial reduction in three moments: from S1 to S2; from S1 to S3 and from S2 to S3, using the Wilcoxon paired test. The data for the quantitative comparisons among groups were obtained both by means of the absolute count in S1, S2 and S3, as well as by the analysis of percentage reduction of the bacterial load from S1 to S2 and S2 to S3, using the Kruskal-Wallis test with Dunn's post-hoc test. The level of significance was set at 0.05 (P<0.05).

RESULTS

Sterility of the specimens was confirmed by the negative control group showing no microbial growth. The maintenance of bacterial viability throughout the experiment was demonstrated by the positive bacterial growth in all samples of the positive control group, result that was also shown by SEM analysis. The culture technique showed the presence of bacteria in 100% of the specimens of the experimental groups (S1). The degree of homogeneity (baseline) of groups were confirmed in relation to the bacterial load in S1 (P>0.05) (Table 1). A significant reduction on bacterial load after root canal instrumentation (P<0.05) with similar reduction among all the experimental groups was verified (Table 1). After the final irrigation protocols (S3), a significant reduction in bacterial load when compared to S2 was observed in all groups (P<0.05) (Table 1). However, no differences were demonstrated among the use of Easy Clean®, PUI and SI (P>0.05).

DISCUSSION

In order to reach areas not touched by the instruments or intracanal dressings, where are usually located bacteria, some techniques (1-5), such as agitation of irrigating solution, have been proposed to enhance disinfection effectiveness (8-13, 16-18).

This study was conducted to access the effect of different agitation protocols: Easy Clean®, PUI and SI over *E. faecalis* biofilm. A significant bacterial reduction was observed in all groups after instrumentation (P<0.05), which is in consonance with previous studies about disinfection after the use of different instrumentation techniques (15, 19, 20). However, remaining bacteria inside the specimens was detected in all groups, confirming the need of supplementary disinfection protocols.

Our main result demonstrated that all final agitation protocols were effective in reducing the bacterial content of root canals (P<0.05). On the other hand, no differences were observed when Easy Clean®, PUI and SI were compared (P>0.05). Hence, the null hypothesis tested was accepted. This result corroborates with some previous studies that showed the improvement of bacterial load reduction after sonic and ultrasonic irrigation (15, 18). To the best of the author's knowledge, this is the first study to evaluate the efficacy of Easy Clean® on bacterial reduction in oval-shaped root canals as a final irrigant agitation method. This device has been previously tested regarding the smear layer and hard-tissue debris removal (9, 12). Kato et al. (9) compared Easy Clean® with PUI and demonstrated a better performance of the former in removing debris from the apical regions of the root canals. Furthermore, Silva et al. (12) demonstrated that PUI, EndoVac, SAF, and Easy Clean® promoted a similar removal of accumulated hard-tissue debris when they were used as final irrigation protocols. According to Duque et al. (20) activation methods enhance debris removal effectiveness of the canal and isthmus especially with the use of Easy Clean® in continuous rotation. These results can be understood as an improved cleanliness ability of the RCS. The continuous rotary movement at 20,000 rpm may create an intense flow of irrigating solution inside the root canal. Apart from it, the instrument might also touch on the canal walls, disorganizing biofilms. These actions together may help bacteria elimination when using Easy Clean®.

In order to prevent bias, specimens were carefully selected based on their anatomy. The use of single-rooted mandibular incisors aimed to produce a reliable and comparable anatomical baseline. Teeth with oval cross-section anatomy may harbour residual bacteria in flattened areas (21), even after root canal cleaning and shaping (17). Thus, the choice of oval-shaped canals for this study may be considered quite pertinent. Also, the agitation protocols have been conducted similarly, by standardizing the amount and irrigant exposure time, as well as the distance of the needle from the WL. All these variables are critical issues which should be controlled in studies like this (5, 9, 12, 16).

By the fact that not all manufactures recommend the same activation protocol, it was established a 1-minute uninterrupted activation of the irrigating solution in each specimen, avoiding the creation of bias in this research.

Due to its high frequency in persistent periapical infections (22) and numerous quotes with the same purpose, *E. faecalis* was selected (4, 16, 21, 22). This bacteria is able to penetrate deeply into the dentine tubules and form biofilm (23). To create a more complex and difficult to eradicate structure, a 30-day biofilm growth model was chosen rather than a 24-h colonization model (4).

It may arise arguments that a control group using syringe irrigation should have been performed. Nevertheless, the aim of this study was to evaluate different active irrigation protocols (Sonic activation, Easy Clean® and PUI) on root canal disinfection. This partly resulted from previous literature showing that the use of supplementary irrigation approaches are able to promote better disinfection when compared to the conventional syringe irrigation (6, 24). In future studies, a comparison among these agitation apparatus and a conventional syringe irrigation control group can be performed.

The methodology used to collect microbial samples from the root canal is well stated in the literature (8, 15-17), and was accurate for the objective of this study, however, some limitation do exist. Paper points and Hedström files can only collect bacteria from a superficial area of the dentine walls. Bacteria inside dentinal tubules and ramifications are likely not collected by this method. Besides that, it is not possible to compare the microbiota between the coronal and apical part of the RCS. For this propose, cryogenically ground samples may be used (25).

Even getting an improvement in root canal disinfection by the irrigating techniques, none of them was able to achieve bacteria-free root canals. This finding is in accordance with previous studies (16, 17, 24), and underscores the need of new techniques or solutions to enhance root canal disinfection. However, some caution must be taken when correlate clinical significance to results obtained by an *ex vivo* experiment. Thereby, clinical randomized trials investigating the effects of these protocols regarding root canal disinfection and/or other endodontic outcomes are required to increase reliability of a scientific-based final activation protocol.

CONCLUSION

The tested final agitation protocols of the irrigant solution showed similar disinfection effectiveness. The newer tested agitation device (Easy Clean®) was as well effective as the traditional PUI and SI. However, none of the protocols provided root canals free from microorganisms.

Disclosures

Conflict of interest: The authors declare that they have no conflicts of interest.

Ethics Committee Approval: The ethical clearance for this study was taken from the Institutional Ethical Review Board (CAAE 48121115.2.0000.5259).

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REFERENCES

1. Sundqvist G. Bacteriological studies of necrotic dental pulps. University of Umea, 1976.
2. Schilder H. Cleaning and shaping the root canal. Dent Clin North Am. 1974; 18(2):269–96.
3. Versiani MA, Leoni GB, Steier L, De-Deus G, Tassani S, Pécora JD, de Sousa-Neto MD. Micro–computed Tomography Study of Oval-shaped Canals Prepared with the Self-adjusting File, Reciproc, WaveOne, and ProTaper Universal Systems. J Endod 2013;39(8):1060–6. [CrossRef]
4. Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M. Effect of Long-term Exposure to Endodontic Disinfecting Solutions on Young and Old Enterococcus faecalis Biofilms in Dentin Canals. J Endod 2014; 40(4):509–14. [CrossRef]
5. Haapasalo M, Shen Y, Wang Z, Gao Y. Irrigation in endodontics. Br Dent J. 2014;216(6):299–303. [CrossRef]
6. Haapasalo M, Shen Y, Wang Z, Park E, Curtis A, Patel P, Vandrangi P Apical pressure created during irrigation with the GentleWaveTM system compared to conventional syringe irrigation. Clin Oral Investig 2016;20(7):1525–34. [CrossRef]
7. Pesse AV, Warrier GR, Dhir VK. An experimental study of the gas entrapment process in closed-end microchannels. Int J Heat Mass Transf 2005;48(25–26):5150–65. [CrossRef]
8. Neuhaus KW, Liebi M, Stauffacher S, Eick S, Lussi A. Antibacterial Efficacy of a New Sonic Irrigation Device for Root Canal Disinfection. J Endod 2016; 42(12):1799–803. [CrossRef]
9. Kato AS, Cunha RS, Da Silveira Bueno CE, Pelegrine RA, Fontana CE, De Martin AS. Investigation of the efficacy of passive ultrasonic irrigation versus irrigation with reciprocating activation: An environmental scanning electron microscopic study. J Endod 2016;42(4):659–63. [CrossRef]
10. Plotino G, Cortese T, Grande NM, Plotino, Cortese T, Grande NM, Leonardi D P, Di Giorgio G, Testarelli L, Gambarini G. New Technologies to Improve Root canal Disinfection. Braz Dent J 2016;27(1):3–8. [CrossRef]
11. Martin H, Cunningham W. Endosonic endodontics: the ultrasonic synergistic system. Int Dent J 1984;34(3):198–203.
12. Silva EJNL, Carvalho CR, Belladonna FG, Prado MC, Lopes RT, De-Deus G, Moreira EJL. Micro-CT evaluation of different final irrigation protocols on the removal of hard-tissue debris from isthmus-containing mesial root of mandibular molars. Clin Oral Investig 2018; 23(2):1–7. [CrossRef]
13. De-deus G, Reis C, Beznos D. Limited Ability of Three Commonly Used Thermoplasticized Gutta-Percha Techniques in Filling. J Endod 2008;34(11):1401–5. [CrossRef]
14. Kustarci A, Akdemir N, Siso SH, Altunbas D. Apical Extrusion of Intracanal Debris Using Two Engine Driven and Step-Back Instrumentation Techniques: An In-Vitro Study. Eur J Dent 2008;2(4):233–9. [CrossRef]
15. de Brito PR, Lima PM, Nogueira Leal Silva EJ, Fidel SR, Fidel RAS, Sassone LM. Effectiveness of ProTaper Next, ProTaper Universal and WaveOne systems in reducing intracanal bacterial load. Endo-Endodontic Pract Today 2016;10(3):167–73.
16. Brito PRR, Souza LC, Machado de Oliveira JC, Alves FR, De-Deus G, Lopes HP, Siqueira JF Jr. Comparison of the Effectiveness of Three Irrigation Techniques in Reducing Intracanal Enterococcus faecalis Populations: An In Vitro Study. J Endod 2009; 35(10):1422–7. [CrossRef]
17. Alves FRF, Almeida BM, Neves MAS, Moreno JO, Rôças IN, Siqueira JF. Disinfecting oval-shaped root canals: Effectiveness of different supplementary approaches. J Endod 2011;37(4):496–501. [CrossRef]
18. Nair PRN, Sjogren U, Kahnberg KE, Krey G SG. Intraradicular bacteria and fungi in root-filled, asymptomatic human teeth with therapy-resistant periapical lesions: a long-term light and electron microscopic follow-up study. J Endod 1990;16(12):580–8. [CrossRef]
19. Neves MAS, Provenzano JC, Rôças IN, Siqueira JF. Clinical Antibacterial Effectiveness of Root Canal Preparation with Reciprocating Single-instrument or Continuously Rotating Multi-instrument Systems. J Endod 2016; 42(1):25–9. [CrossRef]
20. Duque JA, Duarte MAH, Canali LCF, Zancan RF, Vivan RR, Bernardes RA, Bramante CM. Comparative Effectiveness of New Mechanical Irrigant Agitating Devices for Debris Removal from the Canal and Isthmus of Mesial Roots of Mandibular Molars. J Endod 2017;43(2):326–31. [CrossRef]

21. Grande NM, Plotino G, Pecci R, Bedini R, Pameijer CH, Somma F. Micro-computerized tomographic analysis of radicular and canal morphology of premolars with long oval canals. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endod 2008;106(3):70–6. [CrossRef]
22. Zhang C, Du J, Peng Z. Correlation between Enterococcus faecalis and Persistent Intraradicular Infection Compared with Primary Intraradicular Infection: A Systematic Review. J Endod 2015;41(8):1207–13. [CrossRef]
23. Haapasalo M, Ørstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66(8):1375–9. [CrossRef]
24. Mohmmed S alarab, Vianna ME, Penny MR, Hilton ST, Mordan N, Knowles JC. A novel experimental approach to investigate the effect of different agitation methods using sodium hypochlorite as an irrigant on the rate of bacterial biofilm removal from the wall of a simulated root canal model. Dent Mater 2016;32(10):1289–300. [CrossRef]
25. Alves FRF, Siqueira JF, Carmo FL, Santos AL, Peixoto RS, Rôças IN, et al. Bacterial community profiling of cryogenically ground samples from the apical and coronal root segments of teeth with apical periodontitis. J Endod 2009; 35(4):486-92. [CrossRef]