The Effectiveness of Three Irrigation Systems in the Enterococcus faecalis Reduction after Instrumentation with a Reciprocating Instrument

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Abstract

Objective This study aimed to analyze the effectiveness of three irrigation systems: EndoActivator, passive ultrasonic irrigation (PUI), and Easy Clean in the reduction of Enterococcus faecalis, after instrumentation with the reciprocating system, through microbiological collection and culture method.

Materials and Methods A total of 60 extracted human lower premolars were used and standardized at 16 mm in length. The teeth were accessed, contaminated with E. faecalis, and incubated for 21 days at 37°C. Initial collections (S1) were made with an absorbent paper cone to confirm the contamination; subsequently, instrumentation was performed with WaveOne Primary. The teeth were divided into four groups according to the final irrigation protocol (n = 15): group 1, EndoActivator; group 2, PUI; group 3, Easy Clean; and group 4, control group irrigated with saline solution sterile and without agitation. In the final irrigation, the agitation of the 17% ethylene amine tetra-acetic acid (EDTA) solutions was used, then 2.5% sodium hypochlorite (NaOCl); in both for this, three cycles of 20 seconds each. After the chemical–mechanical preparation and agitation of the irrigating solutions, the final collections (S2) for counting the colony-forming units (CFU/mL) occurred.

Results The Kruskal–Wallis test revealed that all the agitation systems reduced by 100% and the control group by 65.7%.

Conclusion The control group presented a significantly higher amount of CFU/mL after the chemical–mechanical preparation than the other groups, which were similar to each other (p > 0.05).

Keywords ► endodontics ► EndoActivator ► Easy Clean ► Enterococcus faecalis ► PUI

Introduction

The success of endodontic treatment depends on the eradication of microorganisms present in the root canal system and the prevention of reinfection. Therefore, it needs to be modeled with manual or automated instruments under constant irrigation, to remove inflamed or necrotic tissue, microorganisms, and other debris. Endodontic treatment aims to promote proper cleaning and disinfection of the root canal.
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The inclusion criteria were teeth with complete root development, without curvature, that did not undergo endodontic treatment, without calcification and apical diameter compatible with a K #15 file (Dentsply/Maillefer, Ballagigues, Switzerland). The teeth were standardized at 16 mm by cutting the coronary portion with a carbide disc, and the working length (WL) was determined at 15 mm. Canal exploration and apical patency occurred with K #10 file, and canals were instrumented by manual technique progressively up to K #20 file. At each instrument change, the canal was irrigated with 1 mL of 2.5% NaOCl.

The apical foramina of each root canal were sealed with photopolymerizable composite resin. The external water-proofing of the roots, except for the cervical region at the entrance of the root canal, was performed with two layers of Araldite epoxy adhesive. After the waterproofing, the specimens were wrapped with Optosil/Xantopren condensing silicone and then autoclaved at 121°C for 15 minutes. The canals were contaminated with pure cultures of the standard E. faecalis strain obtained from the American Type Culture Collection (ATCC 29212). For the reactivation of the strains, cultures were transferred using a bacteriological loop, to brain and heart infusion (BHI) broth and incubated in an incubator at 37°C for 24 hours, with 5% carbon dioxide (CO2). After microbial growth, tube suspension was prepared to contain 10 mL of sterile physiological solution, compatible with the McFarland scale 10.0 turbidity standard. In a sterile test tube, 5 mL of the suspension prepared was mixed with 5 mL of BHI broth to obtain the final concentration suspension.

The final concentration with 20 µL of E. faecalis suspension was placed into the root canals of the specimens with the aid of an insulin syringe and after each root canal entry, a sterile cotton ball embedded in the suspension. In four wells of each cell culture dish, sterile cotton, moistened with sterile distilled water, was introduced to ensure the humidity of the environment. The lid of the plate was closed and sealed with adhesive tape and the assembly incubated in a CO2 oven at 37°C for 21 days. BHI broth of 20 µL was added to the root canals every 2 days with the aid of a 0.3 cc insulin syringe and the cotton soaked in distilled water was exchanged in the wells of the plates.

Confirmation of the viability and purity of the microorganisms within the canal system occurred weekly using random collection in two teeth with the aid of a sterilized no. 20 absorbent paper cone held inside the canal for 1 minute, seeded in BHI broth and incubated in an oven at 37°C with 5% CO2 for 24 hours. After 21 days of contamination, a cone of sterilized no. 20 absorbent paper was inserted into the root canal of each specimen for one minute and then transferred with the aid of a sterilized forceps to a polypropylene flask with 1 mL of physiological sterilized solution (0.9% NaCl). It was stirred for 30 seconds on a tube shaker. From this suspension, serial dilutions up to 10 were handled. Aliquots of 0.1 mL of the suspension and each dilution were seeded into Petri dishes containing BHI agar. These were incubated in an oven at 37°C for 24 hours. Subsequently, the number of colony-forming units (CFUs) per plate was counted, and the number of CFU/mL was calculated.

**Materials and Methods**

The research was approved by the Research Ethics Committee of the São Leopoldo Mandic Dental Research Center of Campinas/SP, under the number 1,563.716 and performed at the laboratory of microbiology of said institution. Sixty unirradicular permanent human premolars were used, extracted for orthodontic or periodontal reasons. The sample number for each experimental group (n = 15) was determined from the analysis of publications with similar methodologies.

The inclusion criteria were teeth with complete root development, without curvature, that did not undergo endodontic treatment, without calcification and apical diameter compatible with a K #15 file (Dentsply/Maillefer, Ballagigues, Switzerland). The teeth were standardized at 16 mm by cutting the coronary portion with a carbide disc, and the working length (WL) was determined at 15 mm. Canal...
The tip E1 Irrisonic 20/0.01, fitted to an ultrasound, 1 mm from the WL.

Group 3: EC n = 15

EC (25.04) was introduced into the WL in the reciprocating motion. For this, the X-Smart Plus electric motor was used in Wavene mode.

Group 4: Control (C) n = 15

The Navitip irrigation needle was coupled to a plastic syringe with 2.5 mL of sterile saline solution and introduced into the canal without agitation of the irrigation solution.

All Groups

The teeth were instrumented with the WaveOne Primary system (Dentsply; Maillefer, Balaiques, Switzerland) with the X-Smart Plus electric motor. The irrigation of the canals occurred with the aid of a plastic syringe and Navitip needle, using a total of 20 mL NaOCl 2.5%. After the instrumentation of the canals, the agitation protocol was three cycles of 20 second of sodium hypochlorite, three cycles of EDTA, and three cycles of hypochlorite according to each system. After completion of instrumentation and final irrigation, 10% sodium thiosulfate was used to neutralize NaOCl before microbiological collection. Sample collection of the root canal contents was done with a sterile no. 25 absorbent paper cone, diluted and seeded in Petri dish containing BHI agar, to verify bacterial growth. After 1 minute in the canal, the cone was transferred to a polypropylene flap containing 1 mL of sterile physiological solution (NaCl 0.9%), shaken for 30 seconds on a tube shaker. From this suspension, dilutions up to 10 were prepared. Aliquots of 0.1 mL of the suspension and each dilution were seeded into Petri dishes containing BHI agar. These were incubated in a greenhouse with 5% CO₂ at 37°C for 24 hours. Subsequently, the CFU number was counted per plate, using the colony counter, calculating the number of CFU/mL.

Results

The Kruskal–Wallis test, applied to both dilutions, revealed significant differences between groups (p < 0.01). Dunn’s multiple comparison tests showed that the control group had a significantly higher amount of CFU/mL after the chemical–mechanical preparation than the other groups, similar to each other (p > 0.05 (– Tables 1 and 2). The results of the Wilcoxon’s test, applied to the intragroup data for both dilutions (reduction in the number of CFU/mL), are shown in (– Table 3. The results showed that all groups significantly reduced the number of bacteria in the root canals, except for the control group. The results showed that all groups significantly reduced the number of bacteria in the root canals, except for the control group (– Fig. 1).

### Table 3 Results of the Wilcoxon’s test for intragroup data

|                  | Dilution 1 × 10^{-3} | Dilution 1 × 10^{-2} |
|------------------|-----------------------|-----------------------|
| EndoActivator    | p = 0.0005            | p = 0.0020            |
| PUI              | p = 0.0010            | p = 0.0039            |
| EasyClean        | p = 0.0001            | p = 0.0010            |
| Control          | p = 0.3575            | p = 0.0857            |

Abbreviation: PUI, passive ultrasonic irrigation.

### Table 1 CFU/mL count of Enterococcus faecalis before and after chemical-mechanical preparation with three different irrigation systems, for 1 × 10 dilution

|                  | S1                  | S2                  | % of reduction S1 for S2 |
|------------------|---------------------|---------------------|-------------------------|
|                  | Average             | Median              | Amplitude               | Average | Median | Amplitude               | Average (Amplitude) |
| EndoActivator    | 4.0 × 10⁴           | 7.1 × 10⁴           | 0 a 1 × 10⁷             | 8.6 × 10⁴ | 0.0    | 0 a 1.2 × 10³            | 97.2 (66,7 a 100,0) |
| PUI              | 3.4 × 10⁴           | 1.8 × 10⁴           | 0 a 1 × 10⁷             | 2.7 × 10⁴ | 0.0    | 0 a 4.0 × 10³             | 100 (—)              |
| EasyClean        | 4.0 × 10⁴           | 2.0 × 10⁴           | 0 a 1 × 10⁷             | 0.0 (A)  | 0.0    | —                         | 100 (—)              |
| Control          | 4.0 × 10⁴           | 6.0 × 10⁴           | 0 a 1 × 10⁷             | 2.0 × 10⁷ (B) | 2.0 × 10⁴ | 0 a 1.0 × 10³             | 47.5 (0.0 a 100,0) |

Abbreviations: CFU, colony-forming unit; PUI, passive ultrasonic irrigation. Note: different letters indicate statistically significant differences between groups (α = 0.05).

### Table 2 CFU/mL count of Enterococcus faecalis before and after chemical-mechanical preparation with three different irrigation systems, for 1 × 10 dilution

|                  | S1                  | S2                  | % of reduction S1 for S2 |
|------------------|---------------------|---------------------|-------------------------|
|                  | Average             | Median              | Amplitude               | Average | Median | Amplitude               | Average (Amplitude) |
| EndoActivator    | 3.4 × 10⁴           | 4.8 × 10⁴           | 0 a 1 × 10⁷             | 0.0 (A)  | 0.0    | —                         | 100 (—)              |
| PUI              | 2.9 × 10⁴           | 3.6 × 10⁴           | 0 a 1 × 10⁷             | 0.0 (A)  | 0.0    | —                         | 100 (—)              |
| EasyClean        | 8.0 × 10⁴           | 1.3 × 10⁴           | 0 a 1 × 10⁷             | 0.0 (A)  | 0.0    | —                         | 100 (—)              |
| Control          | 2.7 × 10⁴           | 9.2 × 10⁴           | 0 a 1 × 10⁷             | 4.8 × 10⁴ (B) | 2.8 × 10⁴ | 0 a 1.9 × 10³             | 65.7 (0.0 a 100,0) |

Note: different letters indicate statistically significant differences between groups (α = 0.05).
Discussion

The purpose of endodontic treatment is to remove the cause of the inflammatory/infectious process of the root canal system to recover and maintain the health of the periapical tissues. Mechanical removal by instrumentation is particularly useful in disrupting bacterial biofilm. It reduces the presence of bacteria in the main root canal, but penetration of the irrigant into anatomically complex areas inaccessible to the instruments plays a decisive role in the control of microorganisms. The association of agitation systems with chemical irrigation solutions more effectively reduces the microbial load of the canal system.

*E. faecalis* was chosen as a bacteriological marker because it is one of the bacterial species most frequently found in persistent infections of the root canals, presenting a high capacity to penetrate the interior of the dentinal tubules. Besides, the pathogenicity of the bacteria is also influenced by environmental factors, such as high bacterial load, interactions between different species, causing collective pathogenicity, host resistance, changes in the virulence factors regulated by the environment, and resistance of the host when in contact with the periapical tissues and defense cells.

The teeth used in this study were previously autoclaved at 121°C for 20 minutes, according to recent research methodology. With autoclave sterilization, changes occur in dentin components, such as collagen, causing less bacterial adhesion when compared with fresh nonsterile or gamma-sterilized dentin. The microbiological collection of root canals does not allow to measure the depth of bacterial invasion in the dentinal tubules. Caution should be exercised when comparing the results of other studies or even among the specimens used in the same study due to the impossibility of standardization concerning dentin properties. The intratubular penetration of bacteria depends on the composition and architecture of the dentinal tubules and can be reduced in the apical third or elderly individuals with more regions of sclerotic dentine.

Preinstrumentation collection revealed quantitative differences in bacterial growth between the initial samples, although not statistically significant. One possible reason for this may be the impossibility of standardizing specimens to the age of donors. In older individuals, the formed biofilm may be less stable due to the higher deposition of mineralized dentinal tissue. The paper cone may not absorb bacteria present in apical branches or dentin tubules during collection and, therefore, this technique may fail to detect viable bacteria generating false results.

The PUI consists, after completing the root canal instrumentation, to position an instrument of small diameter at its center and closest to the apical region, so that the instrument acts passively, agitating the irrigator so that it quickly penetrates the apical third. The instrument should remain free within the root canal without touching its walls.

The EC system acts through a reciprocating or rotating mechanical movement, and as a plastic file can be introduced to the WL. In the present study, EC worked on WL, PUI at 1 mm below and EA at 2 mm from the WL. Duque et al. compared the efficacy of EA, EC, and PUI for the removal of isthmus debris from mesial roots of lower molars by scanning electron microscopy and concluded that rotational EC promoted better cleaning of the canal and isthmus. The positive results with the use of the EC device can be explained by the fact that it has been used in the rotational movement and its blades act on the walls of the root canals, releasing debris and microorganisms, as shown by the study by Simezo et al. Huffaker et al. compared the EA and the conventional syringe irrigation system to evaluate the microbial reduction in root canals associated with the use of intracanal medication with calcium hydroxide. There was no significant difference between the groups. In both the sonic and the ultrasonic systems, there is acoustic cavitation, which can be defined as the creation of bubbles. When associated with NaOCl, it results in a more significant number of small bubbles, and, activating three cycles of 20 seconds, promotes a
more efficient cleaning, removing debris from dentin, microorganisms, and organic matter.\textsuperscript{15} Andrade et al\textsuperscript{13} compared PUI, EC in the reciprocating motion, EC in rotational movement and control group without activation and the results revealed that EC in rotational movement and PUI, surpassed EC in the reciprocating movement. This result was different from that of Kato et al\textsuperscript{14} who demonstrated that EC in reciprocating movement presented better performance than PUI in the apical third. The sonic system presented similar performance to the ultrasonic and to the EC system, regarding the removal of microorganisms, this may have happened due to the use of teeth with straight roots.

**Conclusion**

It was concluded that the EC, PUI, and EA systems showed a microbial reduction in root canals contaminated with *E. faecalis*, different from the control group.

**Conflict of Interest**

None declared.

**References**

1. Chivatkarunakul P, Dashper SG, Messer HH. Dentinal tubule invasion and adherence by Enterococcus faecalis. Int Endod J 2008;41(10):873–882
2. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. Dent Clin North Am 2010;54(2):291–312
3. Machado ME, Sapia LA, Cai S, Martins GH, Nabeshima CK. Comparison of two rotary systems in root canal preparation regarding disinfection. J Endod 2010;36(7):1238–1240
4. Martinho FC, de Rabello DG, Ferreira LL, Nascimento GG. Comparison of PUI and 3% sodium hypochlorite as root canal irrigants. Eur J Dent 2015;9(4):529–534
5. Podar R, Kulkarni GD, Illuzzi G, Laneve E, Cocco A, Troiano G. Endodontic irrigants: Different methods to improve efficacy and related problems. Eur J Dent 2018;12(3):459–466
6. Polar R, Kulkarni GD, Iluuzzi G, Laneve E, Cocco A, Troiano G. Endodontic irrigants: Different methods to improve efficacy and related problems. Eur J Dent 2018;12(3):459–466
7. Gu LS, Kim JR, Ling J, Choi KK, Pashley DH, Tay FR. Review of contemporary irrigant agitation techniques and devices. J Endod 2009;35(6):791–804
8. Dioguardi M, Gioia GD, Iliuzzi G, Laneve E, Cocco A, Troiano G. Endodontic irrigants: Different methods to improve efficacy and related problems. Eur J Dent 2018;12(3):459–466
9. Podar R, Kulkarni GP, Dadu SS, Singh S, Singh SH. In vivo antimicrobial efficacy of 6% Morinda citrifolia, Azadirachta indica, and 3% sodium hypochlorite as root canal irrigants. Eur J Endod 2015;9(4):529–534
10. Pinheiro SL, Silva CC, Silva LAD, et al. Antimicrobial efficacy of 2.5% sodium hypochlorite, 2% chlorhexidine, and ozonated water as irrigants in mesiobuccal root canals with severe curvature of mandibular molars. Eur J Endod 2018;12(1):94–99
11. Roy RA, Ahmad M, Crum LA. Physical mechanisms governing the hydrodynamic response of an oscillating ultrasonic file. Int Endod J 1994;27(4):197–207
12. Al-Jadaa A, Paqué F, Attin T, Zehnder M. Acoustic hypochlorite activation in simulated curved canals. J Endod 2009;35(10):1408–1411
13. Ruddle C. Endodontic disinfection - tsunami irrigation. Saudi Endod J 2015;5:1–12
14. 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):659–663
15. Brito PRR, Souza LC, Machado de Oliveira JC, et al. Comparison of the effectiveness of three irrigation techniques in reducing intracanal Enterococcus faecalis populations. J Endod 2009;35(10):1422–1427
16. Siqueira JF Jr, Rochas IN, Santos SR, Lima KC, Magalhaes FA, de Uzeda M. Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals. J Endod 2002;28(3):181–184
17. Matos Neto M, Santos SS, Leao MV, Habitante SM, Rodrigues JR, Jorge AO. Effectiveness of three instrumentation systems to remove Enterococcus faecalis from root canals. Int Endod J 2012;45(5):435–438
18. Gordusys M, Nagas E, Torun OY, Gordusys O. A comparison of three rotary systems and hand instrumentation technique for the elimination of Enterococcus faecalis from the root canal. Aust Endod J 2011;37(3):128–133
19. Aydin C, Tunca YM, Senses Z, Baysalir M, Kayaoglu G, Orstavik D. Bacterial reduction by extensive versus conservative root canal instrumentation in vitro. Acta Odontol Scand 2007;65(3):167–170
20. Paragliola R, Franco V, Fabiani C, et al. Final rinse optimization: influence of different agitation protocols. J Endod 2010;36(2):282–285
21. Castelo-Baz P, Martin-Biedma B, Cantatore G, et al. In vitro comparison of passive and continuous ultrasonic irrigation in simulated lateral canals of extracted teeth. J Endod 2012;38(5):688–691
22. Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periododontis. Int Endod J 1998;31(1):1–7
23. Siqueira JF Jr, Rochas IN, Microbiological and treatment of acute apical abscesses. Clin Microbiol Rev 2013;26(2):255–273
24. White JM, Goodis HE, Marshall SJ, Marshall GW. Sterilization of teeth by gamma radiation. J Dent Res 1994;73(9):1560–1567
25. Love RM. Invasion of dentinal tubules by root canal bacteria. Endod Topics 2004;9:52–65
26. San S, Wang J, Jiang W, Zhu C, Liang J. Assessment of dentinal tubule invasion capacity of Enterococcus faecalis under stress conditions ex vivo. Int Endod J 2015;48(4):362–372
27. Tennert C, Fuhrmann M, Wittmer A, et al. New bacterial contamination in primary and persistent/secondary endodontic infections with respect to clinical and radiographic findings. J Endod 2014;40(5):670–677
28. Jensen SA, Walker TL, Hutter JW, Nicoll BK. Comparison of the cleaning efficacy of passive sonic activation and passive ultrasonic activation after hand instrumentation in molar root canals. J Endod 1999;25(11):735–738
29. Duque JA, Duarte MA, Canali LC, et al. 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):326–331
30. Simeo AF, da Silveira Bueno CE, Cunha RS, et al. Comparative analysis of dentinal erosion after passive ultrasonic irrigation versus irrigation with reciprocating activation: an environmental scanning electron study. J Endod 2017;43(1):141–146
31. Hufnaker SK, Safavi K, Spangberg LS, Kaufman B. Influence of a passive sonic irrigation system on the elimination of bacteria from root canal systems: a clinical study. J Endod 2010;36(8):1315–1318
32. van der Sluis LW, Vogels MP, Verhaagen B, Macedo R, Wesselink PR. Study on the influence of refreshment/activation cycles and irrigants on mechanical cleaning efficiency during ultrasonic activation of the irrigant. J Endod 2010;36(4):737–740
33. Andrade CV Jr, Batista MR, Alves MM, Alves F, Silva JN. Efficacy of a new activation device in irrigation penetration into simulated lateral canals. Euro Endod J 2016;3:1–5