

Effect of Passive Ultrasonic Irrigation on *Enterococcus faecalis* from Root Canals: An *Ex Vivo* Study

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Endodontic irrigation aims to clean and disinfect the root canal system. Passive ultrasonic irrigation (PUI) is based on the use of an ultrasound-activated instrument into the root canal filled with irrigant. The aim of this study was to evaluate, *ex vivo*, the effectiveness of PUI in eliminating *Enterococcus faecalis* from root canals. Seventy-five extracted human single-root teeth were used. After root canal preparation, specimens were inoculated with *E. faecalis* and incubated at 37 °C for 21 days. Specimens were distributed into five groups (n=15), according to the irrigation method: PUI + saline solution (PUI/SS); PUI + 1% NaOCl (PUI/NaOCl); conventional needle irrigation (CNI) + saline solution (CNI/SS); CNI + 1% NaOCl (CNI/NaOCl); No irrigation (control). Microbiological samples were collected at three time points: initial (21 days after inoculation), post-irrigation (immediately after irrigation), and final (7 days after irrigation). Data were obtained in CFU mL⁻¹ and subjected to analysis by ANOVA and Tukey's tests at 5% significance level. The post-irrigation samples did not demonstrate statistical difference between PUI/SS and CNI/SS nor between PUI/NaOCl and CNI/NaOCl (p>0.05), but PUI/NaOCl and CNI/NaOCl had lower CFU mL⁻¹ number than the other groups (p>0.05). Statistically significant difference was observed between the initial and post-irrigation samples and between the post-irrigation and final samples (p<0.05) in all groups, except in the control. The final samples of all groups presented bacterial counts similar to the initial samples. PUI or CNI with 1% NaOCl contribute to disinfection, but are unable to eradicate *E. faecalis* from the root canal system.

Key Words: endodontics, *Enterococcus faecalis*, passive ultrasonic irrigation, root canal disinfection.

Introduction

Microorganisms and their products play an essential role in the development of pulp and periapical diseases, and are responsible for endodontic treatment failure (1-3). Biomechanical preparation using antimicrobial irrigants aims to eliminate infection from the root canal system (RCS) (4,5). However, the root canal system anatomy favors the maintenance of infection in the isthmuses, lateral and apical ramifications and flattened areas (6).

The ideal irrigation solution needs to remove debris, lubricate the root canal walls, dissolve organic tissue and eliminate bacteria. Regardless of irrigation solution used, the endodontic microorganisms are reduced by the mechanical action of the irrigation. (7). Sodium hypochlorite (NaOCl) is the most widely used chemical irrigant in Endodontics, due to its antimicrobial properties (4,8,9) and its ability to dissolve organic tissues (5,10,11).

Enterococcus faecalis is the most frequently observed microorganism in persistent infections associated with endodontic failure (12), and has demonstrated the ability to invade the dentine tubules (13). Due to its great resistance, ability to organize into a biofilm and to form a monospecies infection, this microorganism has been extensively used in

studies evaluating the effectiveness of irrigating solutions and intracanal medicaments (1,5,8,12,14).

Several irrigation methods have been proposed to enhance the effectiveness of NaOCl in cleaning and disinfecting the RCS and then in eliminating the microorganisms (8,15,16). Ultrasound has been used in Endodontics to complement the cleaning ability of root canal irrigants. Passive ultrasonic irrigation (PUI) was first described by Weller et al. (17). PUI is based on the use of an ultrasound-activated instrument into the root canal filled with irrigant, inducing acoustic microstreaming in the solution around the tip (18,19).

Studies have demonstrated that NaOCl solution, in conjunction with PUI in the final step of irrigation, is able to remove more debris, bacteria and pulp tissue compared with conventional irrigation with a needle and syringe (10,15,16,20). However, *E. faecalis* is a very resistant bacteria and can remain viable within the root canal system. Therefore, it is necessary to evaluate the effect of PUI used immediately after root canal preparation, but also the effect on root canal system.

The aim of this study was to evaluate the effect of PUI compared with conventional needle irrigation (CNI) using

1% NaOCl or saline solution in eliminating *E. faecalis*. The null hypothesis is that the irrigation methods and irrigating solutions do not influence the elimination of *E. faecalis* from the RCS.

Material and Methods

Preparation of Root Canals and Tooth Surface

This study was approved by Ethics Committee of Araraquara Dental School, UNESP, Brazil. Seventy-five human single-rooted teeth with a single straight root canal were used. The crowns were removed and roots were standardized at 15 mm of length. A size #3 Gates Glidden drill (Dentsply-Maillefer, Ballaigues, Switzerland) was used on the coronal 3 mm of the root canal and foramen size was standardized with a size 20 K-file (Dentsply-Maillefer). The working length (WL) was established 1 mm short of the apical foramen and the root canal was instrumented up to a size 50 K-file (apical stop). After that, the root canals were instrumented by a step-back technique up to a size 70 K-file. Conventional needle irrigation (CNI) was used with 2 mL saline solution after each file. Subsequently, the root canals were filled with 17% trisodium EDTA (Biodinâmica, Ibiporã, PR, Brazil) for 3 min, followed by irrigation with 5 mL of saline solution. Following that, the root apices were sealed with light-cured composite resin (Z250 Universal Restorative; 3M ESPE, St. Paul, MN, USA) and all external root surfaces (except the root canal access) were made impermeable with two layers of epoxy adhesive (Araldite; Brascola Ltda., São Paulo, SP, Brazil), according to Aranda-Garcia et al. (21).

Specimens were randomly divided into five 24-well cell culture microplates (Corning Incorporated, Corning, NY, USA). Each microplate received 15 specimens, which were attached to the wells with self-curing acrylic resin (Clássico Artigos Odontológicos, São Paulo, SP, Brazil). The microplates containing the specimens were wrapped and sterilized by ethylene oxide (Acecil, Campinas, SP, Brazil).

Contamination of the Root Canals

These procedures were carried out in a laminar flow chamber (VecoFlow Ltda., Campinas, SP, Brazil). Standardized *E. faecalis* strains (ATCC 29212) were cultured in Tryptic Soy Broth – TSB (Difco, Detroit, MI, USA) for 24 h. Bacteria were seeded onto Tryptic Soy Agar – TSA medium (Difco) and incubated at 37 °C for 48 h. A bacterial suspension was prepared in sterile saline solution, at a concentration equivalent to 0.5 in the McFarland standard. The optical density of the suspension was adjusted using a spectrophotometer (Model 600 Plus; Femto, São Paulo, SP, Brazil) to a concentration equivalent to 1.6×10^7 CFU mL⁻¹.

Sterile TSB was mixed to the bacterial suspension at

a 1:1 ratio, and root canals were contaminated with 20 µL of this mixture. A sterile cotton pellet moistened with TSB was placed at each root canal entrance. Microplates were covered and kept in a microaerophilic environment at 37 °C. During the 21-day incubation period, sterile TSB was added to each root canal on alternate days by means of a 0.5 mL insulin syringe (Bectron Dickinson, Curitiba, PR, Brazil), as previously described by Aranda-Garcia et al. (21).

After that, initial samples were collected, in order to confirm contamination by *E. faecalis*. Two #50 sterile paper points (Tanari Industrial Ltda., São Paulo, SP, Brazil) were used in sequence in each root canal. The paper points remained in the root canal for 1 min and were subsequently transferred to tubes containing 1 mL sterile saline solution, which were shaken for 1 min (Vortex AP 56; Phoenix, Araraquara, SP, Brazil). Ten-fold serial dilutions were made and 20 µL aliquots were seeded in triplicate onto Petri dishes containing TSA. Following that, the plates were incubated in microaerophilic environment at 37 °C for 48 h. Bacterial growth was determined by the CFU mL⁻¹ counts of *E. faecalis*. The microplates containing the roots were randomly distributed in five groups (n=15) according to the irrigation method: PUI + saline solution (PUI/SS); PUI + 1% NaOCl (PUI/NaOCl); CNI + saline solution (CNI/SS); CNI + 1% NaOCl (CNI/NaOCl); No irrigation (control). (Table 1).

Irrigation Protocols

Root canals in the groups (except for the control) were irrigated by using a 27-G irrigation needle (Endo-Eze Irrigator; Ultradent Products, South Jordan, UT, USA) and a 5 mL syringe (Ultradent Products). A rubber stop was placed 14 mm from the needle tip. All specimens were irrigated for 2 min. For specimens in Groups PUI/saline and CNI/saline, the irrigant used was 5 mL of sterile saline solution (Fresenius Kabi Brazil Ltda., Aquiraz, CE, Brazil), while Groups PUI/NaOCl and CNI/NaOCl were irrigated with 5 mL of 1% NaOCl.

In Groups PUI/saline and PUI/NaOCl, a size 25 IRRI S file (VDW; Endo Ultrasonic Files, Endodontic Synergy, Munich, Germany) was inserted until 1 mm short of the WL. The file

Table 1. Experimental groups and control

Final irrigation protocol	Total irrigation time	Total volume	n
PUI + saline	2 min	5 mL	15
PUI + 1% NaOCl	2 min	5 mL	15
CNI + saline	2 min	5 mL	15
CNI + 1% NaOCl	2 min	5 mL	15
Control (no irrigation)	-	-	15

PUI: passive ultrasonic irrigation; CNI: conventional needle irrigation.

was attached to a piezoelectric ultrasonic device operating at 30 kHz (CVDent 1000; CVD Vale, São José dos Campos, SP, Brazil) set at power level 4. The irrigation protocol was conducted as follows: 2 mL of irrigant solution using CNI in the first 30 s, 20 s using PUI, 1 mL by CNI for 20 s, 20 s of PUI, followed by 2 mL of irrigant by using CNI for 30 s, totaling 2 min of irrigation, as previously described by Chávez-Andrade et al. (22).

Specimens in Groups CNI/saline and CNI/NaOCl were submitted to CNI, by applying finger pressure to the plunger of the previously described syringe/needle set. Half of the total irrigant volume was dispensed in the first minute. The solution was agitated in the root canal with a size 50 K-file for 20 s, followed by 40 s of final irrigation. Half of the total volume of irrigating solution (2.5 mL) was used in the first minute, followed by agitation in the root canal using a size 50 K-file for 20 sec. At the end, 2.5 mL of solution was used for final irrigation for 40 seconds, totalizing 2 min.

Microbiological Analysis

Immediately after irrigation, root canals in Groups PUI/NaOCl and CNI/NaOCl were filled with 1% sodium thiosulfate in order to neutralize the NaOCl (2,8). The other groups were filled with saline. Following that, a second microbiological sample was collected from each canal. Two #50 sterile paper points were used *per* specimen, as described for the initial sample. After that, root canals were filled with sterile saline solution and sterile cotton pellets were placed at each canal entrance. The microplates containing the specimens were covered and incubated in a microaerophilic environment at 37 °C. After 7 days, the final samples were collected according to the previously

described steps, in order to recover microorganisms that had remained in the dentine tubules and root canal system. Data obtained were submitted to base-10 logarithmic transformation and analyzed by the GraphPad Prism 3.0 software (San Diego, CA, USA). ANOVA and Tukey's tests were used for comparison among the groups. For comparison between samples within the same group, repeated measures ANOVA test was used. The significance level was set at $p < 0.05$.

Results

Contamination of the specimens with the standard *E. faecalis* strain was confirmed in the initial samples, collected after 21 days of incubation. All groups had similar CFU mL⁻¹ counts, as shown in Table 2. Viability of *E. faecalis* in the control group, which was not submitted to irrigation, was confirmed throughout the entire experimental period. Comparison between the post-irrigation samples from each group did not show statistically significant difference between PUI/saline and CNI/saline nor between PUI/NaOCl and CNI/NaOCl ($p > 0.05$). However, immediately after irrigation PUI NaOCl and CNI/NaOCl had lower CFU mL⁻¹ counts than the other two groups ($p > 0.05$). Significant difference was observed between the control group and all other groups, except for CNI/saline. In the final samples, all groups showed similar results with no statistical difference. As shown in Table 2, comparison between samples within each group demonstrated statistically significant difference between the initial and the post-irrigation samples, as well as between the post-irrigation and the final samples ($p < 0.05$) in all groups, except for the control.

Discussion

Several studies use *E. faecalis* to evaluate the antimicrobial effect of different medicaments, irrigants and treatment protocols (8,14,23,24). The methodology used in this study simulates the clinical infection in the RCS, using an *E. faecalis* 21-day incubation period, as described in previous study (21). The use of sterile paper points for sample collection allows recovery of bacteria present only in the root canal lumen. The final samples, collected 7 days after irrigation, showed permanence of viable bacteria within the RCS. *E. faecalis* has demonstrated resistance to several antibacterial agents (8,14). This microorganism invades the dentin tubules and is able to survive in the RCS, even after root canal treatment (12,13). Moreover, *E. faecalis* can be used in experiments after isolation and conventional culture methods (8,23).

The methodology used in this study allows standardization of samples. This was confirmed

Table 2. Comparison between the groups at the initial, post-irrigation, and final samples (mean and standard deviation of CFU mL⁻¹ log)

Final irrigation protocol	Initial	Post-irrigation	Final
PUI + saline	7.18 (± 0.34) ^{A,a}	5.02 (± 0.38) ^{C,b}	6.90 (± 0.46) ^{A,a}
PUI + 1% NaOCl	6.97 (± 0.29) ^{A,a}	1.41 (± 1.07) ^{D,b}	6.89 (± 0.46) ^{A,a}
CNI + saline	6.96 (± 0.64) ^{A,a}	5.18 (± 0.41) ^{CB,b}	6.89 (± 0.58) ^{A,a}
CNI + 1% NaOCl	7.15 (± 0.36) ^{A,a}	0.89 (± 1.14) ^{D,b}	7.16 (± 0.11) ^{A,a}
Control (no irrigation)	6.84 (± 0.41) ^{A,a}	6.64 (± 0.54) ^{AB,a}	6.82 (± 0.13) ^{A,a}

Different letters indicate statistically significant difference ($p < 0.05$). Uppercase letters for comparison between groups and lower-case letters for comparison between samples. PUI: passive ultrasonic irrigation; CNI: conventional needle irrigation.

by the homogeneity of the initial samples, 21 days after inoculation. Analysis of the different irrigation protocols revealed that 1% NaOCl was the most effective irrigant against *E. faecalis*, regardless of the irrigation method (CNI or PUI). However, seven days after irrigation, increase in the bacterial counts was observed in all groups, demonstrating permanence of *E. faecalis* in the RCS. These results are in agreement with those of previous studies (1,23,24) that did not observe significant difference between CNI and PUI when NaOCl was used as irrigant.

The 1% NaOCl was used in this study to evaluate the effect of PUI using a low sodium hypochlorite concentration. Previous studies (1,14) using this concentration observed antibacterial effect using the association of 1% NaOCl and PUI immediately after irrigation. However, Tardivo et al. (23) observed that 5.25% and PUI did not increase antibacterial effect against *E. faecalis*, in comparison with manual irrigation using this solution.

PUI has been suggested as an alternative to CNI to more effectively clean and disinfect the RCS (10,17,18). The present study evaluated the effect of PUI in an infected root canal system model. The results from the present study did not show significant difference after irrigation by using PUI. The irrigating solution was essential, with better results with the use of NaOCl. The final samples showed no differences between the groups, including specimens irrigated using NaOCl with or without ultrasonic activation. Gründling et al. (24) also evaluated PUI and CNI using 2% NaOCl in conjunction with 17% EDTA and distilled water, and did not find significant differences between these irrigation protocols.

Some studies report different outcomes regarding the antimicrobial efficacy of PUI. These differences may be related to the use of bacterial strains more susceptible to disinfection procedures (25) as well as to shorter incubation periods, or still to microbiological analysis being only performed immediately after treatment (1). Cohenca et al. (15) showed better root canal disinfection using PUI associated with 2.5% NaOCl than CNI in root canals of dog's teeth with apical periodontitis.

Other methodologies, such as the one described by Harrison et al. (14) involving scanning electron microscopy (SEM) specimen analysis, showed that irrigation with 1% NaOCl for 1 min after biomechanical preparation is effective to promote reduction of *E. faecalis* within the root canals. Bhuvu et al. (1) also performed SEM to evaluate the effectiveness of PUI with 1% NaOCl for removal of *E. faecalis* biofilm and concluded that this treatment was able to completely remove intracanal biofilm. However, these authors did not observe significant difference between PUI and CNI, which is in agreement with the results obtained in the present study.

In conclusion, both PUI and CNI with 1% NaOCl contribute for disinfection, but are unable to eliminate *E. faecalis* from the root canal system. The present results suggest that the use of an irrigating solution with antimicrobial activity plays an essential role in root canal disinfection.

Resumo

A irrigação endodôntica visa à limpeza e desinfecção do sistema de canais radiculares. A irrigação ultrassônica passiva (IUP) baseia-se na utilização de um instrumento ultrassônico ativado no interior do canal radicular preenchido com a solução irrigadora. O objetivo deste estudo foi avaliar, *ex vivo*, a eficácia da IUP na eliminação de *Enterococcus faecalis* dos canais radiculares. Foram usados 75 dentes humanos unirradiculares extraídos. Após o preparo biomecânico dos canais radiculares, foram inoculados com *E. faecalis* e incubados a 37 °C por 21 dias. Os espécimes foram distribuídos em cinco grupos (n=15), de acordo com o método de irrigação: IUP + solução salina (IUP/SS); IUP + NaOCl a 1% (IUP/NaOCl); irrigação manual convencional (IMC) + solução salina (IMC/sol); IMC + NaOCl a 1% (IMC/NaOCl); Sem irrigação (controle). Foram realizadas três coletas microbiológicas: inicial (21 dias após a inoculação), pós-irrigação (imediatamente após a irrigação), e final (7 dias após a irrigação). Os dados foram obtidos em UFC mL⁻¹ e submetidos aos testes de ANOVA e Tukey. A coleta pós-irrigação não demonstrou diferença estatisticamente significante entre IUP/SS e IMC/SS nem entre IUP/NaOCl e IMC/NaOCl (p>0,05), mas IUP/NaOCl e IMC/NaOCl apresentaram menor número de UFC mL⁻¹ comparado aos outros grupos (p>0,05). Diferença estatisticamente significante foi observada entre as coletas iniciais e pós-irrigação e entre a pós-irrigação e a coleta final (p<0,05) em todos os grupos, exceto no controle. Na coleta final, todos os grupos apresentaram contagem bacteriana similar à coleta inicial. IUP ou IMC com NaOCl a 1% contribuem para a desinfecção, mas não eliminam *E. faecalis* do sistema de canais radiculares.

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Received January 12, 2015

Accepted 17 April, 2015