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ORIGINAL ARTICLE

Antimicrobial Effectiveness of High-Power Sonic and Ultrasonic Devices Combined with Stepwise Intraoperative or Final Activation of Sodium Hypochlorite

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ABSTRACT

Objective: This study investigated the intratubular decontamination promoted by high-power sonic and ultrasonic devices using either a stepwise intraoperative activation (SIA) technique or a final conventional activation (CA) approach during root canal chemomechanical preparation.

Methods: Fifty human lower premolars were contaminated with *Enterococcus faecalis* and assigned into five groups (n=8): conventional syringe irrigation (CSI); final ultrasonic activation (FUA) using the ultrasonic insert 25/25 IRRI S; final sonic agitation (FSA) using the high-power sonic insert 20/28 Eddy system (both CA techniques); stepwise ultrasonic activation (SUA); and stepwise sonic agitation (SSA) using the same devices during and after canal preparation (SIA techniques). Remaining specimens served as controls. Root canal preparation was performed with the Reciproc system and 5.25% NaOCl, followed by final irrigation with 17% EDTA. Bacterial viability was assessed via confocal microscopy with Live/Dead technique. Statistical analysis was employed using non-parametric tests (α=0.05).

Results: SUA showed the lowest bacterial viability, followed by FSA, both statistically similar. SSA and FUA were similar but less effective than SUA and FSA ($p<0.05$). The CSI group had significantly higher bacterial viability compared to all other groups ($p<0.05$).

Conclusion: High-power sonic agitation and ultrasonic activation enhanced intratubular decontamination against *E. faecalis*. The SIA technique, using IRRI S or Eddy systems, effectively reduced bacterial viability and represents a promising approach for root canal disinfection.

Keywords: Endodontics, *Enterococcus faecalis*, root canal irrigants, root canal preparation, ultrasonics

HIGHLIGHTS

- The IRRI S ultrasonic and Eddy tip sonic devices reduce the intratubular bacterial viability.
- The irrigation techniques when used during and at the end of the biomechanical preparation can reduce infection levels.
- From a microbiological point of view, the SIA technique is favorable for root canal system disinfection.

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INTRODUCTION

Chemomechanical preparation is a crucial step in endodontic treatment, aimed at effectively cleaning, shaping, debriding, and disinfecting root canal systems (RCSs) (1–3). However, the action of instruments and irrigating solutions may not fully achieve these objectives, as unprepared root canal surfaces can harbor residual bacteria (1–3). Among the endodontic pathogens, *Enterococcus faecalis* (*E. faecalis*), a

facultative Gram-positive anaerobic bacterium, is often associated with persistent infections (4, 5). Studying it as a marker at the intratubular level is particularly important, given the narrow spaces within dentinal tubules compared to the larger branches and isthmuses of the RCS (6–9).

To ensure direct contact of irrigants with all components of the RCS and enhance irrigation efficacy, various agitation techniques have been proposed, including sonic and ultrasonic devices (10–12). Ultrasonic activation is the most used method, leveraging cavitation and acoustic streaming to improve cleaning (12–17). The 25/25 IRRI-S ultrasonic insert (VDW, Munich, Germany) has demonstrated high efficiency in removing dentine debris (10, 11). Similarly, high-power sonic devices like the Eddy tip (VDW GmbH, Munich, Germany) have proven to be an effective alternative. This polyamide instrument, size 25/04, is activated at a frequency of 6 kHz by an air-driven handpiece (10, 11, 18). It has shown excellent debris removal capability (10) and delivers results comparable to ultrasonic activation (19, 20).

An irrigant activation approach, called stepwise intraoperative activation (SIA), has been introduced. This technique involves activating the irrigant during instrumentation, meaning each time the file is withdrawn from the main root canal, rather than only at the end of the procedure, yielding favorable results (11). According to Plotino et al. (21), increasing the activation time through stepwise intraoperative activation (SIA) has been shown to enhance the debris removal efficiency of both Eddy and IRRI S from a simulated isthmus connecting two curved canals, reinforcing their effectiveness in optimizing endodontic cleaning protocols (21). This evidence supports the selection of these devices to investigate decontamination in smaller spaces, such as dentinal tubules. Therefore, this study aimed to evaluate the effectiveness of these devices compared to conventional syringe irrigation (CSI) and the SIA technique compared to final conventional activation (CA) in reducing *E. faecalis* viability, as evaluated by confocal laser scanning microscopy (CLSM). The null hypotheses tested were that there are no differences regarding antimicrobial efficacy between the two devices or between the two agitation techniques.

MATERIALS AND METHODS

Sample Preparation

This study was conducted in accordance with the Declaration of Helsinki and was approved by the University of São Paulo, Bauru School of Dentistry Research Ethics Committee (approval number: #2.618.257/2018, date: 24/04/2018). Sample size determination was based on a previous study of intratubular decontamination (22) and by calculation performed using G*Power 3.1 software for Macintosh (Heinrich Heine University of Düsseldorf, Germany). For 6 experimental groups, using an effect size of 0.54, with a significance level of alpha error $\alpha = 0.05$ and power 0.8, the resultant total sample size was 50, with 8 specimens for each experimental group.

To ensure sample standardization, only teeth with single, oval-shaped root canals, a root curvature of less than 10° (measured using a protractor and compass), and an initial apical diameter compatible with a size 15 K-file were selected. Teeth that showed root canal calcifications or resorptions were excluded from the experiments. Each tooth underwent microtomography using a micro-CT system (SkyScan 1174v2; Bruker-microCT, Kontich, Belgium), following previously established procedures (8). A total of fifty freshly extracted human lower premolars were chosen and immersed in a 0.1% thymol solution to eliminate organic debris from the surfaces. Conventional endodontic access was prepared using specialized burs (EndoAccess Bur; Dentsply Maillefer, Ballaigues, Switzerland).

Root canals were initially probed with 10 K-files (Dentsply-Maillefer, Ballaigues, Switzerland), advancing until the instrument tip was visible at the apical foramen. The working length (WL) was determined by subtracting 1 mm from this measurement. To standardize the specimens, the cusps were adjusted, ensuring a uniform root length of 20±1 mm. The canals were subsequently enlarged with size 15 K-files to create adequate space for bacterial inoculation. Irrigation was performed sequentially with 5 mL of each solution as follows: sterilized saline, 17% EDTA (CanalPro, Coltene, Altstätten, Switzerland) for 3 minutes, and a final rinse with sterilized saline, all delivered using Navitip 27G needles (Ultradent Products Inc., South Jordan, UT, USA). These steps aimed to remove the smear layer generated during enlargement with 15 K-files. Subsequently, the roots underwent three ultrasonic cleaning cycles, each lasting 10 minutes, using 1% NaOCl, 17% EDTA, and 1% sodium thiosulfate to eliminate residual irrigation substances and open the dentinal tubules (23).

To prevent bacterial infiltration into the intratubular region through the root canal, the external surfaces of the roots were coated with two layers of red nail polish (Colorama, Rio de Janeiro, RJ, Brazil). Each specimen was placed in individual microtubes containing 1.5 mL of distilled water and sterilized in an autoclave (Gnatus, Ribeirão Preto, SP, Brazil) at 121°C. Following sterilization, the teeth were placed in sterile Brain Heart Infusion (BHI) culture medium (Difco, Detroit, MI, USA) and underwent a final 10-minute ultrasonic bath to facilitate deep penetration of the culture medium into the dentinal tubules (7, 8, 9, 22, 24–26). Two additional specimens were used as negative controls (C-), in which the teeth were not infected, to confirm sterility. Sterility was assessed using CLSM and by collecting samples with paper points, which were then plated on agar plates.

Intratubular Contamination

The bacterial reference strain *E. faecalis* (ATCC 29212) was acquired, and its colony morphology and Gram staining results were assessed daily for five consecutive days during the contamination protocol to confirm purity. The microorganisms were cultivated in BHI broth (Difco, Detroit, USA) with successive subcultures. Dilutions were prepared based on the absorbance value obtained using an SF325NM spectrophotometer (Bel Photonics do Brazil Ltda, Osasco, Brazil) to achieve a concentration of 3×10^8 CFU/mL at 540 nm.

The specimens were contaminated over five days in BHI medium at 37°C, following the centrifugation sequence of Ma et al. (6) and the contamination protocol of Andrade et al. (24), which has been previously reproduced (7–9, 22, 25,

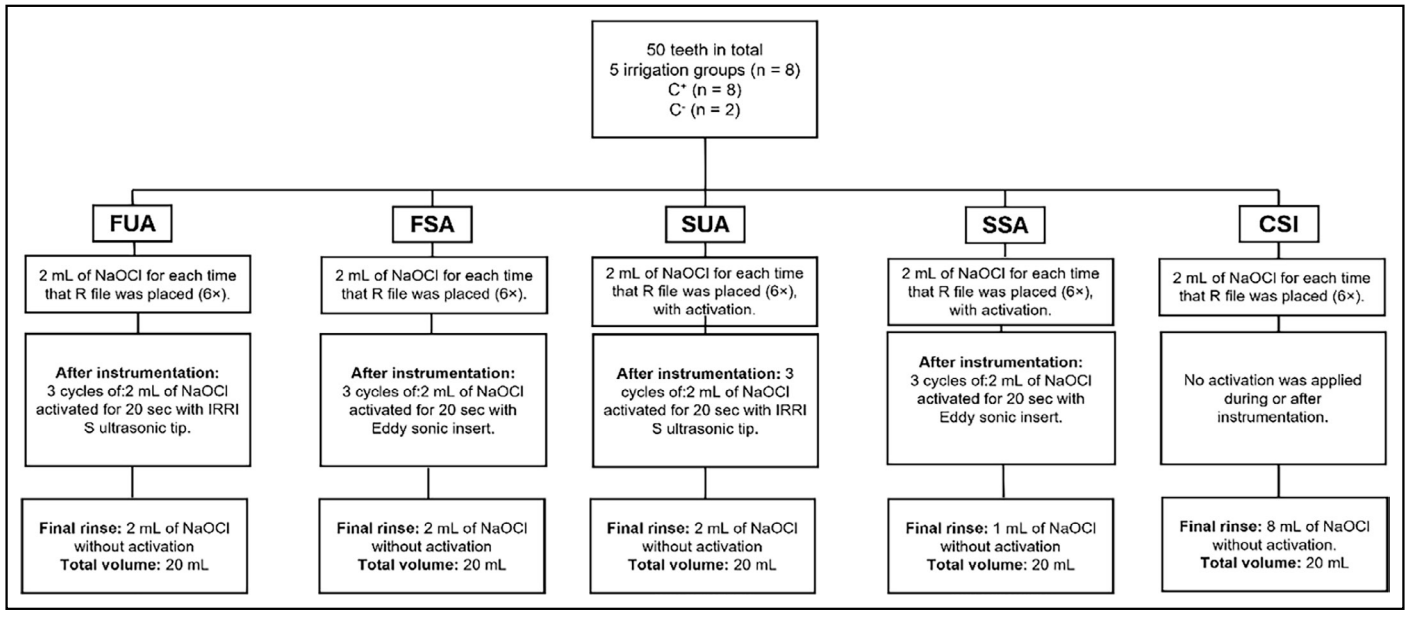

Figure 1. Flowchart of the different irrigation groups investigated

FUA: Final ultrasonic activation, FSA: Final sonic agitation, SUA: Stepwise ultrasonic activation, SSA: Stepwise sonic agitation, CSI: Conventional syringe irrigation

26). This five-day intratubular contamination period was sufficient to infect the entire root thickness, from the main canal to the cementum, forming biofilms with viable bacteria, as demonstrated in the cited studies.

Canal Preparation and Irrigation

The specimens were randomly assigned to five experimental groups based on the irrigation protocol (n = 8 per group): Conventional Syringe Irrigation (CSI) – Irrigation performed using a syringe without additional activation; Final Ultrasonic Activation (FUA) – Irrigation activated at the end of canal preparation using the ultrasonic insert 25/25 IRRI S (CA technique); Final Sonic Agitation (FSA) – Irrigation activated at the end of canal preparation using the high-power sonic insert 20/28 Eddy system (CA technique); Stepwise Ultrasonic Activation (SUA) – Irrigation activated both during and at the end of canal preparation using the ultrasonic insert 25/25 IRRI S (SIA technique); Stepwise Sonic Agitation (SSA) – Irrigation activated both during and at the end of canal preparation using the high-power sonic insert 20/28 Eddy system (SIA technique). Additionally, eight extra specimens were used as positive controls (C+) to confirm intratubular contamination on CLSM analysis.

A laminar flow hood was the experiment work field. Root canal preparation was carried out by a single operator using the Reciproc system with a VDW Silver Reciproc electric motor set to the 'RECIPROC ALL' mode (VDW, Munich, Germany). The R25 file was gently inserted into the canal until engagement, followed by three pecking movements (up and down) to advance the instrument. Irrigation was performed with 2 mL of 5.25% NaOCl (Niclor OGNA, Milan, Italy) for 20 seconds (flow rate: 0.1 mL/s) using a 27G endodontic Navi Tip needle (Ultradent, Salt Lake City, USA) positioned at the instrument's insertion point. This instrumentation and irrigation process was repeated twice more, until the working length (WL) was reached. The same steps were then performed with the Reciproc R40 file, totaling 12 mL of NaOCl used during the chemomechanical procedures.

In the final irrigation phase, the root canals were treated with 6 mL of 5.25% NaOCl for 80 seconds (flow rate: 0.075 mL/s), following the specific protocols for each group. For the FUA group, the final irrigation consisted of three consecutive cycles, each using 2 mL of 5.25% NaOCl (flow rate: 0.10 mL/s), activated for 20 seconds with the IRRI S 25/25 ultrasonic tip (VDW). The tip was passively positioned 1 mm short of the working length (WL) in the root canal and activated with the piezoelectric ultrasonic device P5 Newtron (Satelec Acteon, Merignac, France) at power level 30. After activation, the root canals were irrigated with 2 mL of 5.25% NaOCl for 20 seconds (flow rate: 0.10 mL/s) without activation. In the FSA group, the same final irrigation procedure was followed, using the Eddy sonic insert 20/28, powered by the Sonic Borden 2000N device (Kavo Keer, Detroit, MI, USA) at power level 1.

In the SUA and SSA groups, the final irrigation was performed as in the previous groups, with the addition of activating the irrigant for 20 seconds each time the canal was irrigated during instrumentation. The ultrasonic or sonic tip was positioned at the same level as the instrument and needle. After the final activation, the root canals were irrigated with 2 mL of 5.25% NaOCl for 20 seconds (flow rate: 0.10 mL/s) without additional activation.

In the CSI group, no activation was applied during or after the instrumentation procedures. For the final irrigation, the root canals were irrigated with 8 mL of 5.25% NaOCl for 80 seconds. Subsequently, all root canals from all groups were irrigated with 5 mL of 17% EDTA for 2 minutes, followed by 5 mL of 1% sodium thiosulfate for another 2 minutes to eliminate any remaining irrigants. Thus, across all irrigation groups, a total of 20 mL of NaOCl was used, considering chemomechanical preparation, activation techniques, and final irrigation. Figure 1 presents the flowchart of the different irrigation groups.

Evaluation Method

The specimens were longitudinally sectioned using a diamond disk (Erios, São Paulo, SP, Brazil) on a cutting machine

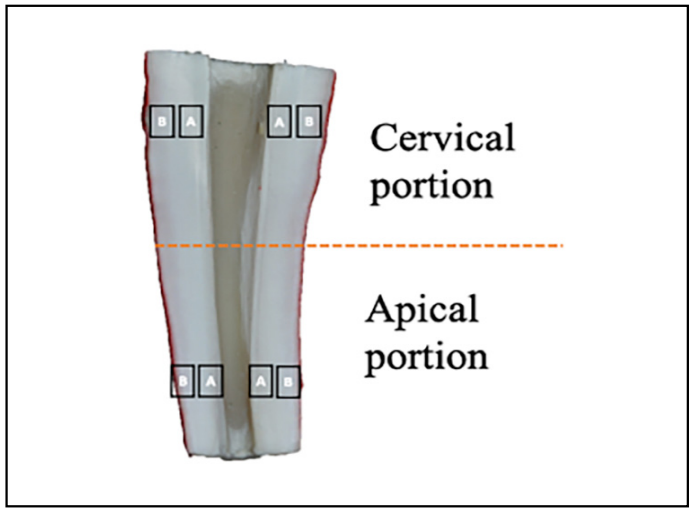

Figure 2. Representative image of the sectioned dentine cylinder and dentinal tubules zones analyzed by confocal laser scanning microscope in different root portions (cervical and apical): a: Superficial, b: Deep

under continuous irrigation with sterile saline. To eliminate the smear layer formed during cutting, specimens were immersed in 17% EDTA for 5 minutes and then rinsed with saline, following established protocols (7, 8, 9, 22, 24–26). Each root half was stained with 30 µL of LIVE/DEAD® BacLight dye (Invitrogen Molecular Probes, Eugene, OR, USA), which uses SYTO 9® which stains intact bacterial membranes (green) and propidium iodide which stains damaged bacterial membranes (red). After 20 minutes, samples were rinsed with phosphate-buffered saline (PBS) to remove excess dye.

Confocal imaging was performed with a Leica TCS-SPE microscope (Leica Microsystems GmbH, Mannheim, Germany) at 40× magnification with a 1 µm step depth and a resolution of 1024×1024 pixels by an expert in confocal laser scanning microscopic analysis blinded to the experiment. Data acquisition was performed for each specimen, capturing images from the cervical and apical portions (Fig. 2), encompassing both superficial and deeper dentinal regions, as previously described (7, 8, 9, 22, 24–26). Bacterial quantification was conducted using Leica LAS X Life Science software.

Statistical Analysis

Data are presented as medians with 95% confidence intervals (CI). The normality of the data was evaluated using the Shapiro-Wilk test. Bacterial viability, as determined from CLSM images, was analyzed with the Kruskal-Wallis test followed by Dunn's post hoc comparisons. The Mann-Whitney test was used to examine differences in bacterial viability across the root canal thirds within each group. All statistical analyses were performed using GraphPad Prism 9.5 software (GraphPad, San Diego, CA, USA) (α=0.05).

RESULTS

Table 1 shows the median values of intratubular bacterial viability (in percentage) for each group. The positive control group exhibited the highest median percentage of intratubular bacteria (90.36%), assuring the effectiveness of the contamination protocol across the entire root structure. The absence of bacteria in the negative control specimens confirmed the effectiveness of the sterilization procedure.

Figure 3 illustrates the percentage of bacterial viability in the cervical and apical portions of root canals following different irrigation protocols. Figure 4 illustrates the presence of bacteria within the dentinal tubules of contaminated samples across all groups. Treatments caused bacterial damage, resulting in lower contamination levels compared to the control ($p<0.05$). The SUA group exhibited the lowest bacterial viability (0.51%), followed by the SSA group (2.19%), with no significant difference between them; however, both were significantly different from the FUA and FSA groups ($p<0.05$). Additionally, the SIA groups (SUA and SSA) demonstrated better disinfection outcomes compared to the CSI group ($p<0.05$). No significant differences were observed between the FUA and FSA groups ($p>0.05$), nor among the analyzed root canal portion in any of the groups ($p>0.05$).

The analysis of results at the cervical portion revealed that CA and SIA were statistically similar to each other and significantly different from the CSI group (Table 1). In the apical portion, both CA and SIA showed less contamination than the CSI group; however, the SIA technique demonstrated significantly better performance than the CA technique ($p<0.05$).

DISCUSSION

Building on previous favorable findings regarding smear layer and debris removal by the SIA technique, the present study provided a preliminary antimicrobial investigation focused on intratubular disinfection, using the ultrasonic IRRI S and the high-power sonic Eddy inserts. The first null hypoth-

TABLE 1. Median (min-max) of the percentage (%) of intratubular bacterial viability, inside the coronal and apical portions and the total viability

Groups	Cervical	Apical	Total
Control	$91.40^{A,a}$ (47.95–97.85)	$90.08^{A,a}$ (66.00–97.72)	90.36^{A} (47.95–97.85)
FUA	$2.16^{C,a}$ (0.15–24.62)	$4.19^{C,D,a}$ (0.01–40.95)	2.61^{C} (0.01–40.95)
FSA	$0.86^{C,a}$ (0.03–40.97)	$4.91^{B,C,a}$ (0.05–70.33)	1.83^{C} (0.03–70.33)
SUA	$0.89^{C,a}$ (0.0–28.79)	$0.38^{D,a}$ (0.0–25.53)	0.51^{D} (0.0–28.79)
SSA	$0.81^{C,b}$ (0.07–10.39)	$3.64^{C,D,a}$ (0.10–73.36)	$2.19C,^{D}$ (0.07–73.36)
CSI	$7.99^{B,a}$ (1.13–89.4)	$18.80^{B,a}$ (1.45–87.35)	12.54^{B} (1.13–89.40)

Different lowercase superscript letters in the lines, represent significant differences between thirds in each group by the Mann-Whitney test ($p<0.05$). Different uppercase superscript letters in a column represent significant differences among the groups by the Kruskal-Wallis and Dunn's test ($p<0.05$). FUA: Final ultrasonic activation, FSA: Final sonic agitation, SUA: Stepwise ultrasonic activation, SSA: Stepwise sonic agitation, CSI: Conventional syringe irrigation

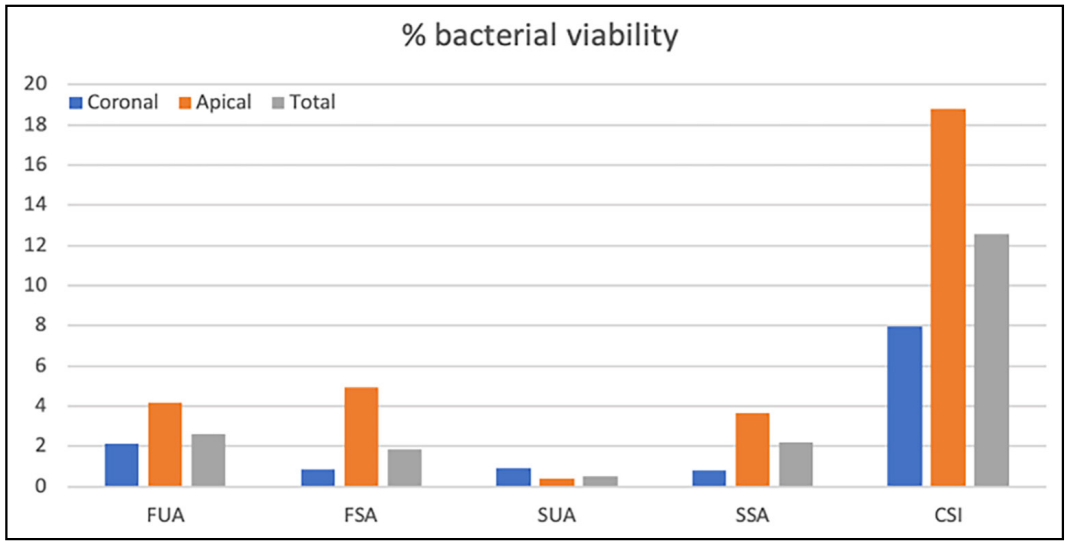

Figure 3. Percentage of bacterial viability in the cervical and apical portions of root canals following different irrigation protocols. Groups include Final Ultrasonic Activation (FUA), Final Sonic Agitation (FSA), Stepwise Ultrasonic Activation (SUA), Stepwise Sonic Agitation (SSA), and Conventional Syringe Irrigation (CSI). Bacterial viability was assessed using confocal laser scanning microscopy (CLSM). Cervical values are represented in blue, apical values in orange, and total bacterial viability in gray

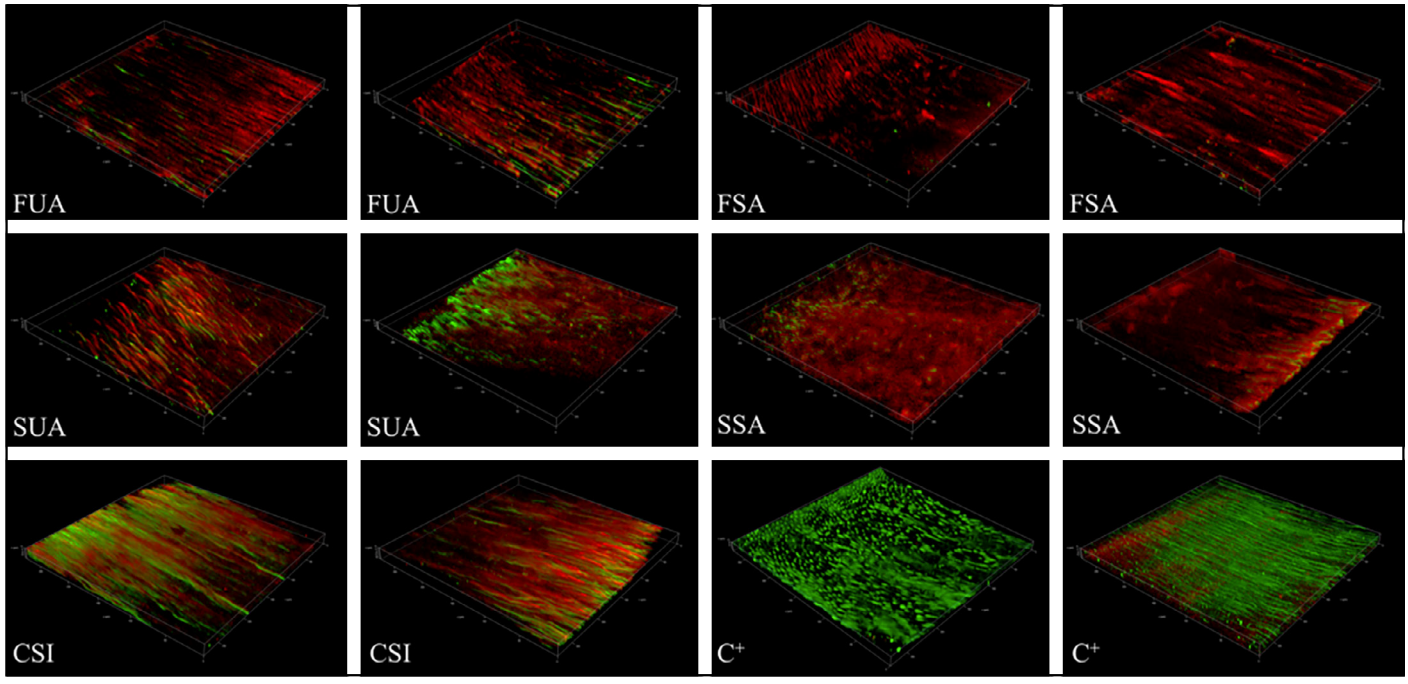

Figure 4. Representative laser scanning confocal microscopy images (magnification 40×) of the groups. Each group includes images of the cervical (left) and apical portion (right) of the root canals. Green indicates viable bacteria, while red staining represents nonviable bacteria

FUA: Final ultrasonic activation, FSA: Final sonic agitation, SUA: Stepwise ultrasonic activation, SSA: Stepwise sonic agitation, CSI: Conventional syringe irrigation, C+: Positive control

esis was not rejected, as no statistical difference was found between the two devices, with both demonstrating significantly better disinfection compared to CSI ($p<0.05$), consistent with other studies (10, 11, 19, 27, 28).

These results confirm that, without activation, the chemical effect of NaOCl solution encounters increased difficulty in reducing intratubular bacterial viability. NaOCl is known to have limited penetration due to its rapid consumption when reacting with the organic biofilm substrate, an effect amplified at lower NaOCl concentrations (9, 27, 29). This consumption is further accelerated by elevated temperatures during activation. While lower concentrations are less cytotoxic, a 5.25% concentration was selected due to the number of activation cycles and the rapid depletion of free chlorine in this context.

The Eddy sonic device, ultrasonic activation, and conventional irrigation have been previously evaluated using colony-form-

ing unit (CFU)/mL counts in both curved and straight root canals. Sonic irrigation at 6 kHz with the Eddy insert was found to be equal to or better than ultrasonic activation (30). However, this method cannot collect bacteria from irregular areas, such as dentinal tubules, where bacteria remain undetectable by conventional culture methods. This highlights the importance of evaluating bacterial viability within the dentinal tubules.

CLSM enables detailed visualization of bacteria within dentinal tubules (7, 9, 22, 26). It captures optical sections of dentine at varying depths, up to 23 µm, which are then stacked to generate a three-dimensional reconstructed image. This provides a thorough view of the dentine mass, particularly in single-rooted teeth (7, 9, 22, 26). *E. faecalis* was chosen for this study due to its ability to penetrate and colonize dentinal tubules, a key factor in bacterial resistance (7, 9, 22, 26). Therefore, a validated research model using an axenic biofilm consisting in traced bacteria provides a useful framework for the initial evaluation of disinfection methods and innovative techniques (30).

Although the present study focused on straight root canals, existing literature suggests that any activation is better than no activation (30, 31). Sonic activation has shown improved decontamination even in curved canals (30). However, further studies with more complex investigations are needed being important to recognize the limitations of experimental models.

In this study, the apical 3 millimeters were removed, leaving a dentine tube that was then divided into a more apical portion and a more cervical portion. This approach allowed for the separate evaluation of these two areas, in addition to the overall assessment of the specimens' dentine. CLSM was not used to assess the entire apical third of the teeth, as this region contains few dentinal tubules with reduced diameters and highly variable anatomy (7, 8, 9, 22, 24–26). These factors result in very narrow or sometimes absent dentinal tubules in the most apical area, limiting consistent bacterial contamination (32).

As an analogy, previous studies have reported decreased sealer penetration from the coronal to the apical third, attributed to the smaller tubular diameter and lower density in the apical region (33). Including the entire apical portion could have led to false-positive or false-negative results. Nevertheless, the successful contamination established in this study provided a reliable baseline. The findings demonstrated minimal differences between the cervical and apical portions, with consistent decontamination in the agitation device groups, potentially mitigating any anatomical variations in the apical third.

The Eddy sonic insert is highlighted for performing similarly to ultrasonic activation in terms of dentine debris and smear layer removal (10, 11). Since commercially available sonic devices have lower power, they typically exhibit lower efficacy when compared to ultrasonics (11). Sonic devices operate at frequencies of 1–8 kHz, whereas ultrasonic devices function at 25–40 kHz (34). The findings of this study verify that the oscillation frequency of the Eddy high-power sonic tip (6000 Hz) can achieve a similar level of root canal cleaning as ultrasonic devices (10, 19, 20, 28). Additionally, plastic sonic inserts provide benefits compared to metal ultrasonic tips. When a metal ultrasonic insert touches the dentine of root canal walls, it can reduce the energy of the oscillating instrument and limit its movement, particularly in curved canals where free oscillation is restricted. Moreover, although ultrasonic files feature a non-cutting tip, their metal composition is harder than dentine, which may lead to deformation of the root canal walls (10, 35). In contrast, plastic sonic tips are safely inserted into root canals with curvatures (10, 11, 19, 36).

Based on the present results, the second null hypothesis evaluated was rejected. The SIA technique, performed both during and after root canal instrumentation with ultrasonic activation and the high-power sonic Eddy inserts, resulted in statistically superior intratubular disinfection compared to the CA technique using the same devices ($p<0.05$). These results are attributed to the greater number of activation cycles performed in the SIA groups during this irrigation technique, representing a significant improvement in the physical-mechanical action against root canal infection, as longer activation times typically lead to enhanced cleaning and disinfection (10, 11, 28).

The apical preparation size of 40 may have contributed to improved intratubular disinfection by allowing the activated instruments to oscillate more freely within the root canal, thus promoting better exchange of the irrigating solution (36). However, about 35% of the wall areas in the premolars are not reached by the Reciproc R40 file (2). Unprepared areas of the canal walls may harbor remaining dental pulp tissue, microorganisms, and dentine debris sustaining bacterial viability in the dentinal tubules (2), that could be better removed by agitation. These factors highlight the importance of evaluating intratubular contamination in dentine, as done in the present study using CLSM.

CONCLUSION

In this *in vitro* study, both the IRRI S and Eddy devices demonstrated greater bacterial reduction compared to conventional syringe irrigation. The SIA technique resulted in improved intratubular bacterial disinfection, highlighting its potential as an effective strategy for optimizing irrigation protocols.

Disclosures

Ethics Committee Approval: The study was approved by the University of São Paulo, Bauru School of Dentistry Research Ethics Committee (no: #2.618.257/2018, date: 24/04/2018).

Informed Consent: Informed consent was obtained from all participants.

Conflict of Interest Statement: All authors declared no conflict of interest.

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