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Efficacy of Mechanical, Subsonic, Ultrasonic and Photon-Induced Photoacoustic Streaming techniques in reducing *Enterococcus faecalis* in severely curved root canals: An *ex vivo* study

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ABSTRACT

The aim of the study was to compare in vitro the efficacy of Photon-induced Photoacoustic Streaming (PIPS) with EndoActivator (EA), Ultrasound (PUI), XP-endo Finisher (XP) and conventional needle irrigation (CNI) in reducing Enterococcus faecalis from severely curved root canals. Fifty-four extracted human mandibular premolars with 35-45 degree canal curvature were prepared and irrigated with 5.25% sodium hypochlorite and 17% EDTA. After preparation the canals were autoclaved and then inoculated with E. faecalis. Fifty teeth were assigned to 5 experimental groups (n=10 each), while 4 teeth were used as positive and negative controls (n=2 each). In the experimental groups, the inoculated canals were irrigated with 5.25% sodium hypochlorite and the solution agitated with EA, XP, PUI or PIPS. In Group 5, the canals were irrigated without agitation using the CNI technique. Samples of canal contamination were obtained before and after irrigant agitation using sterile paper points. The infected material was vortexed in sterile saline. After serial dilutions, aliquots were plated on blood agar plates and incubated for 30 days. Microbial reduction/removal in the canals was determined by counting colony formed units (CFUs). Data was analyzed by the Kruskal-Wallis and Dunn´s multiple comparison test. Significance was established at $P<0.05$. The positive controls presented high concentration of CFUs. The negative controls showed no bacterial growth. There were no significant differences ($P>0.05$) between EA, XP, PUI and PIPS groups in their antimicrobial efficacy against *E. faecalis* but there was a significant difference ($P<0.05$) with the CNI Group. Although EA, XP, PUI and PIPS were comparable in their ability to reduce *E. faecalis* from severely curved root canals none of them including the CNI samples were capable of effectively eliminating the total microbial load.

KEYWORDS: EndoActivator, Ultrasonic agitation, Endodontics, Photon-induced Photoacoustic streaming, Root canal disinfection, XP-Endo Finisher

The Clinical Relevance

The use of EndoActivator, XP-Endo Finisher, Ultrasonic or Photon-induced Photoacoustic streaming for agitation of 5.25% NaOCl irrigating solution appears as effective adjuncts to reduce E. faecalis contamination in mandibular premolars with severely curved root canals. However, none removed the total load of the microorganisms.

INTRODUCTION

The main objective of the chemomechanical preparation of infected root canals is to eliminate or reduce the bacterial load to a level that allows for healing of the affected periradicular tissues [1]. The use of mechanical debridement along with irrigating solutions has been shown to be unable to clean and disinfect all canal irregularities, intracanal communications, isthmuses, and fins [2,3]. Several studies have demonstrated that after cleaning and shaping completely negative cultures are not obtained [3-5]. Gulavibala et al. [3], demonstrated that a high percentage of the canal wall surfaces showed bacterial remnants after chemomechanical instrumentation, especially if conventional needle irrigation (CNI) was used. Among a wide spectrum of anaerobic gram-positive bacteria *Enterococcus faecalis* is frequently found in infected root canals, demonstrating high resistance to antibiotics and intracanal disinfection procedures [6,7]. To maximize the efficacy of the irrigants different techniques have been proposed focusing on the agitation of the solutions including sonic, ultrasonic, mechanical and laser activation [8].

For root canal irrigation/agitation, the EndoActivator system (EA; Dentsply, York, USA) and the XP-endo Finisher instrument (XP; FKG, La Chaux-de-Fonds, Switzerland) have been widely used with different success rates [9-13]. EA is a sonic handpiece device that uses three different sized disposable polymeric plastic tips to agitate the irrigant solution at 2.000 - 10.000 cycles/min. The tips are unable to cut dentine and are used as final irrigation for approximately 30-60s [5,8,10].

Passive Ultrasonic Irrigation (PUI) employs the use of an ultrasonically activated non-cutting instrument in the root canal space for irrigation/agitation of the irrigating solution. The cleaning action of PUI is generated by the oscillation of the instrument, which produces an acoustic microstreaming [11].

XP is a non-tapered flexible rotary NiTi instrument that corresponds to a #25 file and is designed to agitate the irrigants in the final step of irrigation/disinfection. It has a linear shape at room temperature, but when used inside the root canal the body temperature broadens the instrument, however, without removing dentin [11-13].

More recently a Photon-Induced Photoacoustic Streaming technique (PIPS) was reported to be effective for bacterial disinfection during root canal therapy [13-15]. In this technique agitation of the irrigation solution is produced by generating a pulsed energy that forces the irrigant into dentin irregularities [16].

The antibacterial efficacy of the above described techniques has been analyzed in a series of in vitro studies showing controversial results [13,17,18]. These controversies may have been caused by differences in root canal size and configuration between experiments. Frequently, clinicians have to treat moderately or severely curved root canals (SCRC) [19] and this may have negative implications on the effectiveness of different irrigation/agitation techniques [20]. Studies are frequently performed in teeth with straight canals [12-15]. However, studies in teeth with severe root canal curvatures are scarce. This study aimed to compare the efficacy of EA, XP, PUI, PIPS and CNI to reduce/remove *E. faecalis* in vitro in SCRC. The null hypothesis tested was that all the irrigation/agitation techniques including the CNI technique have similar antibacterial effects against *E. faecalis.*

MATERIALS AND METHODS

This study was approved by the Institutional Research Ethics Committee of the Argentine Dental Association (#0123/2022-1/AOA). Single rooted human mandibular premolars, stored in 1% thymol solution, with curved root canals ranging from 35° to 45° were selected for the study. The sample size was calculated using the F test family, ANOVA, of G* Power 3.1 software [21]. A minimum of fifty-four (n=54) teeth was indicated as the appropriate sample size. The teeth had completely developed roots and were without root caries, fissures, root resorption or

previous endodontic treatment. The configuration and presence of a single canal was confirmed by buccolingual (BL) and mesio distal (MD) radiographs. The degree of curvature in the BL and MD directions was determined by using the Schneider method [22]. The macroscopic observation of the roots and the radiographs allowed for an assessment of gross similarities in anatomy, which formed the basis for a reasonable equitable distribution in five experimental groups of 10 teeth each (n=10). The remaining four teeth were used as positive (n=2) and negative (n=2) controls.

Specimen Preparation

The teeth were decoronated under water coolant using a high-speed diamond fissure bur to obtain a standardized root length of 18 mm. After the gross pulp tissue was removed the working length (WL) was determined by advancing a size #15 K-file (Maillefer, Ballaigues, Switzerland) into the canals until the tip of the file was visible at the apex with the aid of X10 magnifying loupe and then subtracting 1 mm. The canals were prepared to the WL in a reciprocating crown-down motion with WaveOne Gold NiTi files (WOG; Dentsply, Tulsa Dental Specialities, Tulsa, OK) to a Medium 35/.06 instrument while maintaining apical patency. The instruments were used in an electric X-Smart IQ motor with torque control (Dentsply Sirona) according to the manufacturer's instructions. Throughout instrumentation the canals were irrigated with 2 mL of 5.25% NaOCl using disposable syringes and #30-gauge needles (Ultradent Products Inc; South Jordan, UT). In each tooth a coronal reservoir resembling a pulp chamber was created to a depth of 5 mm using #2 to #4 Gates Glidden drills (Dentsply, Maillefer). After a repeat irrigation with 5.25% NaOCl the canals were flushed with 10% sterile thiosulfate solution to neutralize NaOCl remnants [14]. They were then irrigated with 2 mL of 17% EDTA for 1 min to remove the smear layer, followed by a final saline rinse. Each WOG instrument was used for the preparation of three canals and then discarded. After the canals were dried with paper points, a bonding system (Single Bond-U; 3M, St. Paul, MN) and a composite resin (Filtek Z-350 XT; 3M) were used to block the apical foramens. Care was taken to apply the resin over the root apex without invading the canal space. The remaining root surfaces were covered with the bonding agent to avoid lateral contamination. The prepared roots were then immersed in brain heart infusion broth (BHI; Difco Laboratory, Detroit, MI), ultrasonicated for 1 min and autoclaved at 121°C for 30 min. They were then stored under sterile conditions in 100% relative humidity at 37ºC.

Canal Inoculation

A suspension of 1.5x108 colony forming units (CFUs) per mL of Enterococcus faecalis (ATCC 29212) equivalent to 0.5 turbidity on the McFarland scale was prepared from a 24-hour culture of bacterial growth in BHI, which was used to contaminate the canals. The canals were filled to the access opening with the *E. faecalis* suspension using 1 mL disposable sterile insulin syringes with 30-gauge tip needles (Ultradent Products) and incubated aerobically at 37°C for 30 days in 100% humidity to allow bacterial colonization inside the canal space and on the canal walls. The culture media was replenished every 5 days.

Irrigation/Agitation Protocols

All procedures were performed under sterile conditions in a laminar air flow cabinet (Antech Scientific Co., Ltd., Qingdao, China) at 37°C. 5.25% NaOCl was used as the final irrigant solution in all experimental groups. The groups were subjected to the following irrigation/agitation protocols:

Group 1 (n=10): Sonically agitated irrigation using the EA system: After the canals were passively filled with 1 mL of 5.25% NaOCl, a size 25/.04 tip in the EA hand piece, operating at 10.000 cycles/min speed, was used for 1 minute in an up and down motion starting 2 mm short from the WL.

Group 2 (n=10): Mechanically agitated irrigation using the XP: The canals were passively filled with 1 mL of 5.25% NaOCl and the irrigant was agitated with the XP torque-controlled hand piece operating at 800 rpm for 1 min in a up and down motion starting 2 mm short of the WL.

G***roup 3 (n=10)***: PIPS-agitated irrigation using Er:YAG laser irradiation: After the canals were passively filled with 1 mL of 5.25% NaOCl, the samples were irradiated with 2940 nm Er:YAG laser (Light Walker AT, Fotona, Dallas, TX, USA). The pulse energy was 20 mJ, with a frequency of 20 Hz and 50 µs pulse duration. For this operation a 400 µm diameter quartz tip (Fotona) in a hand piece was placed for 1 min at 4 mm depth in the coronal reservoir and kept idle by means of the custom-made stand and without advancing into the root canals. During agitation the air and water spray were in the off setting.

Group 4 (n=10): Ultrasonically agitated irrigation using passive ultrasonic irrigation (PUI). In this Group, a non-cutting #20 Irrisafe Acteon file (Satelec, Merignac, France) driven by a Suprasson P5 Newtron (Satelec) setting

at 60% power was used. The canals were passively filled with 1 mL of 5,25% NaOCl and the instrument was placed centrally in the root canal 2 mm short of the WL. During the irrigation/agitation the hand piece remained immobile by means of a custom-made stand.

Group 5 (n=10): Conventional needle irrigation (CNI): The root canals were irrigated for 1 min with 1 mL of 5.25% NaOCl using an open-ended #30 gauge needle (Ultradent). Due to the severe canal curvature, the needle was placed 3 mm short of the WL and used with short-in-and-out vertical movements.

In groups 1 to 4 the agitation of the irrigant was repeated three times (1 min each) while the irrigant was constantly replenished using sterile disposable insulin syringes and #30-gauge needles.

Positive Controls

Two (n=2) teeth were prepared and treated as the experimental samples. After inoculation and incubation, the root canals were subjected to passive irrigation with disposable syringes and 2 mL sterile saline, however, without any type of agitation.

Negative Controls

Two (n=2) teeth were prepared and treated as the experimental ones but without inoculation. After incubation the root canals were passively irrigated with disposable syringes and 2 mL sterile saline.

Samples from the positive and negative teeth were obtained using sterile paper points and subjected to bacterial analysis like the experimental groups.

Bacterial Analysis

In all groups the root canals were sampled before (S1) and after (S2) irrigation/agitation according to the following methods. After 10 mL of sterile saline was injected into the root canals, samples (S1) of each contaminated canal were taken using three sequential sterile paper points inserted to the WL and left in place for 1 min. Upon retrieval, the paper points were transferred to sterile Eppendorf tubes (Eppendorf; Hamburg, Germany) containing 3 mL of sterile saline. The samples were vortexed for 1 min. followed by a 10-fold dilution in saline. The number of CFUs was determined by plating 10 µL aliquots on blood-agar plates (Becton Dickinson, NJ, USA). After aerobic incubation at 37°C in 100% humidity for 48h the viable CFUs were counted. Each dilution was plated in triplicate. Mean values for each treatment group were calculated based on the known dilution factor. After the S1 samples were collected, the root canals were treated by one of the above-mentioned irrigation/agitation methods. To obtain postoperative (S2) samples, three new sequential sterile paper points were used according to the procedures described above for S1 samples.

Evaluation

Viable CFUs of Enterococcus faecalis were identified on the blood agar plates using a laser mass spectrometer (MALDI-TOF MS; Bruker Daltonics GmbH, Leipzig, Germany) and their means were calculated for each tooth and the results of S1 and S2 compared. The results were expressed as the number of surviving CFU before (S1) and after the irrigation/agitation protocols (S2). Statistical analysis was performed using the SPSS statistics version 17.0 (IBM SPSS Inc, Chicago, IL). Data was analyzed by the Kruskal-Wallis test to determine if there were significant differences among groups. The Dunn´s multiple comparison test was used for intra-group comparisons. The significance level was set at P<0,05.

RESULTS

The positive controls had a similar concentration of CFUs as the initial inoculation. The negative controls showed no bacterial growth. The viable counts of *E. faecalis* before and after treatment protocols are shown in **Table 1**. The initial S1 levels of CFU in samples recovered from root canals were significantly higher in all groups when compared to S2 samples (P<0.05). The mean microbial reduction percentage ranged from 84.08 to 85.57. No significant differences (P>0.05) were found when the EA, XP, PUI and PIPS treatment groups were compared. However, there were significant differences (P<0.05) between the above mentioned groups and the CNI group. Therefore, the null hypothesis was partially accepted.

GROUP	n	S1 CFU SAMPLE	S2 CFU SAMPLE	MICROBIAL REDUCTION (%)
PC	2	107.3 ± 0.00		
NC	2	-		
EA	10	98.7 ± 0.65[a]	14.8 ± 0.67[b]	85.00
XP	10	105.4 ± 0.82[a]	15.2 ± 0.82[b]	85.57
PIPS	10	110.6 ± 0.60[a]	17.6 ± 0.70[b]	84.08
PUI	10	108.3 ± 0.30[a]	19.2 ± 0.60[b]	82.27
CNI	10	110.9 ± 0.55[a]	98.8 ± 0.60[c]	10.91

Table 1: Viable counts of *E. faecalis* before (S1) and after (S2) irrigation/agitation treatments.

Viable counts are expressed as means ± standard deviation. PC: Positive Control; NC: Negative control. Values with the same superscript letter within the same column are not significantly different (P>0.05). Values with different superscript letters within the same line are significantly different (P<0.05).

DISCUSSION

In a previous study, Swimberghe et al. [20] demonstrated that the degree of canal curvature had significant implications on the efficacy of irrigation/agitation techniques. In this in vitro study we compared the efficacy of EA, XP, PUI, PIPS and CNI plus irrigation with 5.25% NaOCl to remove/reduce *E. faecalis* from SCRC (>30°) [19,22]. E. faecalis was chosen because they are frequently implicated in the etiology of primary and secondary endodontic infections and because they are highly resistant to disinfection procedures [6,7]. Sodium hypochlorite was used as the irrigation solution because of its antimicrobial properties and its capacity to dissolve organic tissues [23]. In all teeth the apical foramen was sealed to avoid the extrusion of NaOCl through the apical foramen. The efficacy of EA, XP, PUI, PIPS and CNI to remove *E. faecalis* from root canals has been extensively investigated in the past but these investigations showed controversial results [13,17,18, 24-26]. For instance, the present study revealed that there were no significant differences between EA, XP, PUI and PIPS in their ability to remove *E. faecalis* from the main canal space. Our findings are in agreement with those of Balić et al. [17] who found that the behavior of EA was equal to PIPS but do not agree with Azim et al. [13], who reported that XP was more efficient than PIPS and EA. De Gregorio et al. [24] and Alves et al. [25] reported that the superior efficacy of EA and XP over other techniques is based on the amplitude of their movements making the instruments capable to expand its radius of action thus adapting better to the root canal anatomy. These results were further confirmed by Elnaghy et al. [26]. Ordinola Zapata et al. [18] reported a more effective microbial removal using PIPS in comparison to EA, but the differences may be due to Ordinola Zapata et al. [18] using a very different experimental model.

The superior efficacy, previously reported for EA [5,10,24] and XP [13,25] to remove *E. faecalis* from root canals, was not observed in this study. The efficacy of EA was probably reduced as the plastic tip cannot be pre bent and is therefore 2 mm short of the WL in SCRC. This may have led to contact with dentin walls causing tip deflection, a phenomenon which also may occur with XP, resulting in a limited effect on the antimicrobial load.

It has been reported that the Er:YAG laser possesses a high absorption level in water and its wavelength correlates with the absorption levels of hydroxyapatite [27]. As demonstrated by De Groot et al [16] and Meire et al. [27], high absorbed laser energy produces effective decontamination of the root canal space via disruption of the microbial membrane of oxygen reactive species such as *E. faecalis*, thus causing a rapid destruction of the microorganisms while agitating the irrigant solution. Moreover, Meire et al. [28] indicated that one of the most remarkable properties of PIPS has been attributed to its remote effectiveness in killing microorganisms, independently of the level of the laser tip placement. Taking into consideration these features the results of the current study were somewhat surprising. As per protocol, the laser tip was used at the level of 4 mm depth into the prepared coronal reservoir that mimicked a pulp chamber. We may thus speculate that the degree of curvature as well as the anatomical variables that are present in the most apical part of root canals may be a factor in reducing the remote effects of PIPS. The prepared canal size may also play a role in the effectiveness of PIPS, because it may reduce its efficacy due to the major dispersion of the pulsed energy absorbance [29]. Matsuoka et al. [30] indicated that placing the laser tip only at the level of the pulp chamber could be too remote to activate the fluid flow in the apical third in curved root canals and suggested that for better disinfection the laser tip should be placed approximately 4 mm short of the WL. However, this recommendation was not supported by George et al. [31] and Yao et al. [32], who pointed out that the placement of the laser tip near the WL allows for an increase in apical pressure, which may facilitate the extrusion of irrigants through the apical foramen.

In this study, PUI showed similar antimicrobial effects as the other irrigation/activation techniques. According to Nair et al. [33] the formation and implosion of bubbles produces the formation of shockwaves resulting in shear stress on the canal walls which collaborate in the biofilm removal from the root canal space. However, in the case of severely curved root canals such as those used in the current study, the efficacy of PUI may be reduced because of the contact of the ultrasonic tip with the dentin walls, causing a reducing of the amplitude and oscillation of the ultrasonic tip [34].

CNI has been used for many years with different success rates. Ram [35] demonstrated that when using the CNI technique the irrigating solutions do not reach a depth more than 1 mm from the needle tip. Thus, along with the generated apical pressure and apical stagnation an irrigation-free zone is formed. The lack of efficacy of the CNI compared to the other groups can be explained as follows. In our study no activation of the irrigant was performed and since the tip of the needle could not be placed closer than 3 mm from the WL, due to the curvature of the root canals, it is theorized that a greater number of microorganisms remained in the most apical part of the canals. Support for this explanation is based on the demonstration that the effectiveness of the CNI technique is generally limited to straight areas and is significantly reduced in curved root canals [3].

It should be kept in mind that the present study was performed under well-controlled conditions in a laboratory environment and used a single microbial species. Therefore, considering the high number of variables and the presence of other microbial species that exists in a clinical setting, one may expect different outcomes from clinical trials.

CONCLUSION

Within the limitations of this in vitro study, we conclude that EA, XP, PUI and PIPS when used with 5.25% NaOCl irrigation/agitation were significantly more effective than CNI, while they were equally capable to significantly reduce the initial volume of *E. faecalis* in SCRC. However, none of the used techniques were effective in removing the total load of the inoculated microorganisms.

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