ORIGINAL ARTICLE

Efficacy of passive ultrasonic irrigation in removing residual root filling material during retreatment: *ex vivo* analyses by micro-CT and CLSM

ABSTRACT

Aim: *To evaluate the volume of residual filling material after desobturation and passive ultrasonic irrigation (PUI) with irrigants and an organic solvent using micro-computed tomography (micro-CT), and assess the penetrability of the obturation of retreatment using confocal laser scanning microscope (CLSM).*
Methodology: *Thirty curved mesial roots of mandibular molars were submitted for endodontic treatment. After, the filling material was removed and the root canals were reinstrumented with ProTaper instruments (Stage 1). The specimens were randomly assigned according to the protocol of supplementary cleaning (Stage 2). In the Manual group, orange oil solvent, 2.5% NaOCl, and 17% EDTA were applied in the root canal with syringe and needle. In the PUI group, the same irrigants were submitted to ultrasonic agitation. The volume of residual filling material was measured by micro-CT. After, the specimens were filled and submitted to CLSM. The micro-CT data were analyzed by Mann-Whitney and Friedman tests at 5%. The Wilcoxon and Mann-Whitney tests were used for CLSM data (P<.05).*
Results: *Both supplementary cleaning protocols decreased the amount of residual filling material, when compared with stage 1 (P<.05). The PUI group showed significantly less percentages of residual filling material than did the Manual group, for all of the thirds of the root canal (P<.05). There were no statistically significant difference in the penetrability of the obturation of retreatment in the recessions areas between the Manual and PUI groups (P>.05).*
Conclusions: *The use of the PUI as a supplementary technique in removing residual filling material after desobturation was more effective than manual agitation.*

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Introduction

Root canal retreatment procedures recommend the complete removal of filling material, because it can cover necrotic material, debris and infected dentine (1, 2). The presence of contaminated root filling material can contribute to the persistence of infection, and consequently, can compromise the final retreatment outcome (3, 4). Furthermore, the removal of the filling material of primary treatment it is important in order to allow for the penetration and the adaptation of the new obturation in the retreatment. Different methods have been proposed to remove filling material, nevertheless, substantial amounts of filling material commonly remain in the canal after retreatment procedures, especially in areas of difficult access (5-8).

The Passive ultrasonic irrigation (PUI) has been used as an effective method in the cleaning and the disinfection of root canals, principally in areas that are inaccessible to instrumentation such as the isthmus, the oval extensions and the irregularities of the root canal walls (9). The PUI potentiates the action of the irrigants, providing an effective removal of the remnants of pulp tissue, dentine debris, intracanal medication (10), microorganisms (planktonic or biofilm) and their products (11, 12) and improves the penetration depth of the endodontic sealers into the dentinal tubules (13).

The PUI also can be used during endodontic retreatment as an auxiliary for removing root filling materials using different irrigants (14-18), including endodontic solvents (19-21). It has been reported that the application of PUI with essential oil can be an effective method in dissolving zinc oxide-eugenol (ZOE) based sealers (21). However, the results of research evaluating PUI in retreatment are inconclusive. Barreto et al. (20) evaluated, by using micro-computed tomography (micro-CT), the effects of PUI with sodium hypochlorite (NaOCl) and orange oil solvent in the endodontic retreatment and they observed that PUI did not improve the filling material removal. The authors also observed a larger volume of filling material in the specimens that contained isthmus.

To the best of our knowledge, no study evaluated the effectiveness of the PUI in the removal of filling material and the adaptation of the obturation during endodontic retreatment in same study. Therefore, the aims of this study were: 1) to evaluate the effect of the PUI with endodontic irrigants and orange oil as a supplementary cleaning technique in removing residual filling material, using micro-CT; 2) to assess the effects of the PUI in the penetrability of the obturation of retreatment in isthmus and irregularities areas of the root canal, using confocal laser scanning microscope (CLSM).

Materials and Methods

This manuscript was written according to the Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines (22). The PRILE 2021 flowchart, figure 3, summarizes the key steps in reporting the present study.

Approval for this study was granted by the research ethics committee of the Federal University of Santa Maria (0698 8212.2.0000.5346).

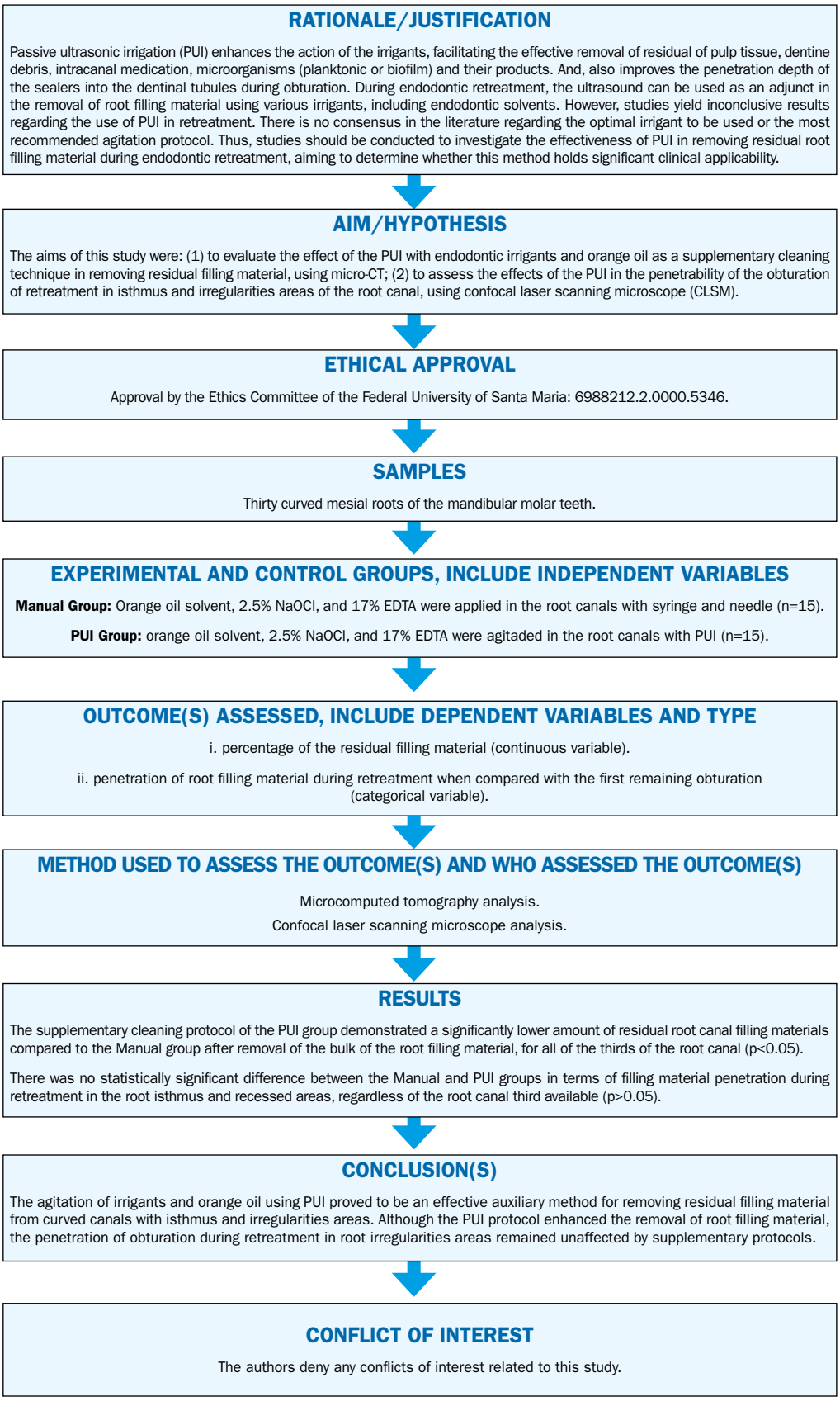
Sample Size

Previous studies (23, 24) have reported differences of nearly 10% for amount of residue when removing the filling material using different instruments and techniques. Assuming a difference of 10% in mean percentage of the volume of residual filling material in the root canal walls, with a standard deviation of 9, the sample size was calculated as 13 specimens per group. To compensate potential losses associated with experimental procedures, 2 samples were included, resulting in a total of 15 specimens per group. The study power was 80% and a significance level was 5%.

Teeth Selection

Thirty mandibular molar teeth with

Figure 3
PRILE 2021 Flowchart



curved mesial roots were collected. Each tooth's mesial and distal roots were sectioned and the distal root was discarded. The selection criteria for the mesial root included a curvature angle of 10°-20º and a curvature radius <12 mm in a bucco-lingual direction, determined through radiographic analysis according with the methodologies described by Schneider (25) and Schäfer et al. (26), respectively. Statistical analysis revealed no significant difference between PUI and Manual groups concerning the curvature angles (P=0.934) and the radii (P=0.575).
Afterward, all specimens were submitted to micro-CT scanning using the SkyScan 1174 scanner (Bruker-Micro CT, Kontich, Belgium) with settings at 50 kV, 800 µA, rotational angle of 180°, and an isotropic resolution of 14.1 µm to confirm the presence of isthmus. Specimens classified as types 2, 3, 5, 6 and 7 according to Vertucci's classification (27) were included in the study.

Cleaning and Shaping
The cavity access of the remaining crown was achieved by using 1014 diamond burs (KG Sorensen, Cotia, São Paulo, Brazil) under water-cooling. The crown of the mesial portion was intentionally maintained to allow a larger irrigant contact with the root canal. The patency of the canals was established using a size-10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) until its tip was visible at the apical foramen. The working length (WL) was determined by subtracting 1 mm from this measurement.
The root canal preparation was performed with ProTaper Universal files (Dentsply Maillefer) using a torque-control motor (X-Smart, Dentsply Maillefer) set at 300 rpm and 2.5 N/cm. S1, SX, S1, S2, F1 and F2 instruments were used in this sequence and between each instrument change, the canals were irrigated with 3 mL of 2.5% NaOCl(Biodinâmica, Ibiporã, Paraná, Brazil) using a syringe with NaviTip irrigation needle (Ultradent, Munich, Bavaria, Germany).
After the instrumentation, all of the root canals were submitted to PUI with 2.5% NaOCl and 17% ethylenediaminetetraacetic acid (EDTA) (Biodinâmica, Ibiporã, Paraná, Brazil). The PUI was performed using a piezoelectric ultrasonic unit (Gnatus, Ribeirão Preto, São Paulo, Brazil) at a high power with a smooth stainless steel wire of size 15 taper .02 (Dentsply Maillefer), 1 mm short of the WL, and oscillating in the toward the isthmus area.

Root Canal Filling
The root canal filling was performed with tapered gutta-percha cones (Dentsply Maillefer) and ZOE based sealer (Endofill, Dentsply Maillefer) using Tagger's hybrid technique (28). The sealer was mixed with 0.1% Rhodamine B dye (CI.45170) (Red) (Synth, São Paulo, Brazil) to allow fluorescence analysis by the CLSM (29). The access cavity was filled with a temporary restorative material (Cavit, 3M ESPE, St Paul, Minnesota, USA). The specimens were stored for 30 days at 37 °C with 100% humidity in order to allow the setting of sealer. Subsequently, all roots were scanned using micro-CT to determine the volume of the root filling.

Retreatment Techniques
The temporary restorations were removed, and a single drop of orange oil (Maquira, Maringá, Paraná, Brazil) was applied for 2 min to soften the gutta-percha at the root canal orifice. The bulk of the root filling material was removed using the ProTaper retreatment system (Dentsply Maillefer) according to the manufacturer's instructions (500 rpm and 3 N/cm).
The root canals were reprepared with F1-F4 ProTper rotatory instruments with 300 rpm and 2.5 N/cm using a torque-control motor (X-Smart, Dentsply Maillefer). After each instrument, the irrigation was performed with 3 mL of 2.5% NaOCl using a syringe with a NaviTip needle. Next, the teeth were scanned with Micro-CT, and the volume of the remaining radiopaque residue was determined (Stage 1). The specimens were randomly assigned (Random Allocation software, Microsoft, Redmond, Washington, USA) in a stratified manner when considering the Vertucci Classification (27) into two groups accord-

ing to the protocol of supplementary cleaning (Stage 2), as detailed below.

Manual group (n=15). First, orange oil-based solvent was used. The root canal was filled with the solvent and the solution was agitated for 3 min using 40 K-type instrument since the last instrument used for root canal preparation was F4 ProTaper instrument (Size 40/0.06 taper). The solvent was replaced at every minute. After, each root canal was irrigated with 2.5% NaOCl for 1 min using a syringe with a NaviTip needle positioned 1 mm short of the WL. Lastly, the root canal was filled with 17% EDTA, and the solution was agitated for 1 min using a 40 K-type instrument.

PUI group (n=15). First, each root canal was filled with orange oil and it was activated using PUI for 3 min. The solvent was replaced at every minute (21). Subsequently, the root canal was filled with 2.5% NaOCl and it was activated using PUI for 1 min. The solution was renovated every 20s. Finally, the root canal was filled with 17% EDTA, and the PUI was performed continuously for 1 min. The PUI was performed as previously described in the topic "Cleaning and Shaping".

After, for both of the groups, each root canal was irrigated with 2 mL of saline solution, dried with sterile absorbent paper points, and then stored at 37 °C with 100% humidity until a new 3D scan was conducted.

Preoperative Micro-CT Scanning and Evaluation Procedures

The specimens were positioned in condensation silicone on the scanning unit to avoid any interference during the process. The scanning procedures were performed by using a desktop X-Ray Microfocus CT Scanner (SkyScan 1174, Bruker-Micro CT, Kontich, Belgium) with a 50 kV, 800 µA, rotational angle of 180°, and an isotropic resolution of 14.1 µm, resulting in 700 to 900 slices per root. The images obtained were reconstructed in order to show 2-D slices of the root structures using NRecon 1.6.4.8 software (Bruker-Micro CT, Kontich, Belgium). CTAn 1.11.10 software (Bruker-Micro CT, Kontich, Belgium) was used to measure the volume of filling material (mm^3) in the apical, middle, and coronal levels (3 mm), from the root apex (30). The percentage of the residual filling material for each third of root canal was calculated for all of the specimens at each stage of the retreatment. A single calibrated and blinded observer performed the micro-CT analyses. Intra observer reproducibility was determined using intraclass correlation coefficient (ICC=0.90).

Obturation during Retreatment

The root canals were filled with tapered gutta-percha cones and AH Plus sealer (Dentsply DeTrey, GmbH, Konstanz, Germany) using Tagger's hybrid technique (28). The 0.1% fluorescein dye (CI.45350) (Green) (Synth) was mixed with the sealer to allow for the analysis by the CLSM.

CLSM Analysis

The specimens were transversally sectioned at 3, 6 and 9 mm from the apex by using a low-speed cutting machine (Isomet, Buehler, Illinois, USA) at 200 rpm and with continuous water cooling. Three root slices with each 2 mm thick were obtained, resulting in 30 slices per group. The slices were polished with a sanding granulation of 300, 600, 900 and 1.200. The coronal surfaces of the slices were evaluated using Olympus FluoView Confocal Laser 1000 Microscope (Olympus Corporation, Tokyo, Japan). The wave length for the absorption and the transmission of Rhodamine B and Fluorescein were 540/590 nm and 494/518 nm, respectively.

The penetration of root filling material during retreatment, compared to the initial remaining obturation was categorized into scores as follows: (0)-0%; (1)-0%-25%; (2)-25%-50%; (3)-50%-75%; and (4)-75%-100%. Two calibrated and blinded evaluators at the protocol of supplementary cleaning performed the analysis by using the LSM Image Browser Program (Zeiss Carl, Gottingen, Switzerland). Each evaluator performed two analyses at different moments. In cases of disagreement, a collective reanalysis was conducted until a consensus was reached. The Kappa co-

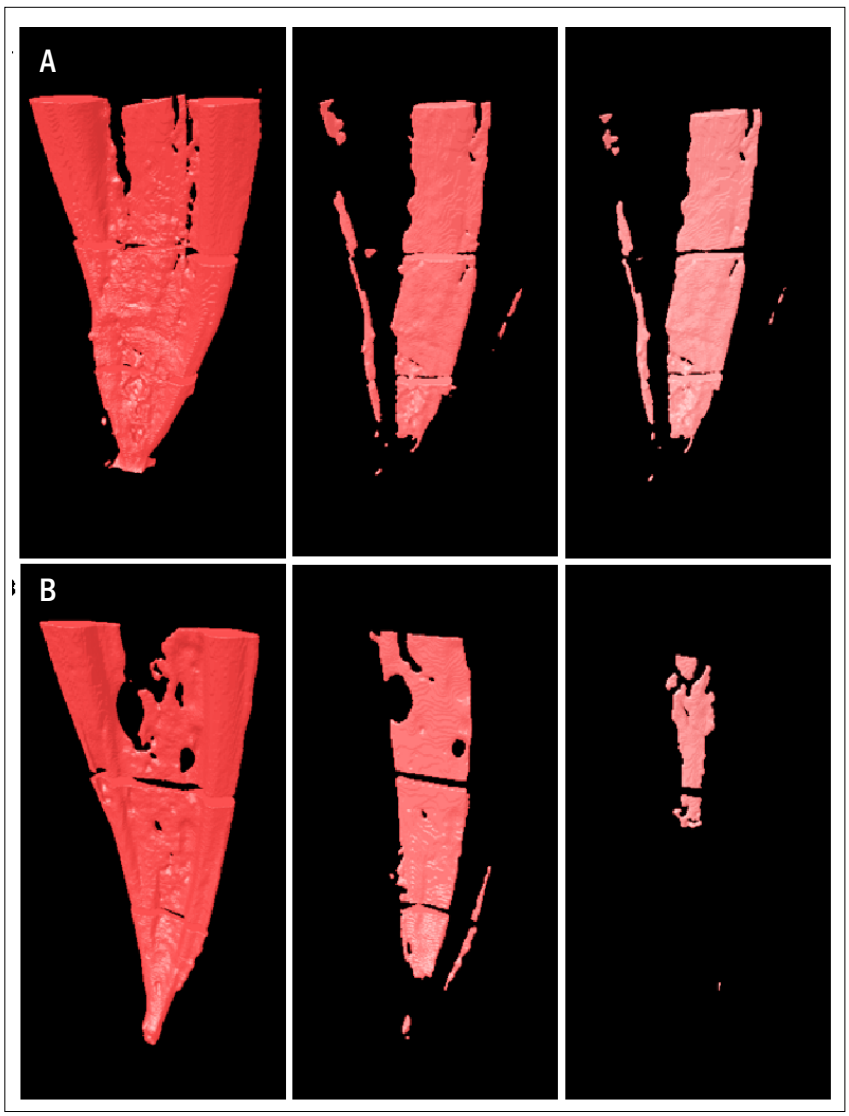
efficient was used to assess interexaminer and intraexaminer agreements. The analyses showed a Kappa coefficients of 0.91 and 0.87 for interexaminer and intraexaminer agreements, respectively.

Statistical Analysis

Data analysis was conducted using Prisma 5.0 software (GraphPad Software Inc., La Jolla, California, USA). The significance level was set at α=0.05. The Shapiro-Wilk normality test was used to assess the data distribution from the micro-CT and CLSM. The data showed non-normal distribution and nonparametric tests were applied.

Micro-CT: The Mann-Whitney test was used to compare the percentages of the residual root canal filling materials between groups at each retreatment step. The Friedman test was used to verify the differences of the residual root canal filling materials in the successive retreatment stages for each canal segment in the same group. The differences amongst the coronal, middle, and apical thirds, in each group and at each stage were also analyzed by using the Friedman test. The Dunn test was performed as the post-hoc multiple comparison method.

CLSM: The prevalence of the root filling material during retreatment was compared with the first one between the experimental groups using the Mann Whitney test. Comparison among the thirds in the same group was verified using the Wilcoxon test.



Results

Micro-CT

The mean percentages of the residual root canal filling materials during retreatment for each group after Stage 1 and Stage 2 are shown in Tables 1 and 2, respectively. The use of the Manual and PUI protocols for supplementary cleaning during retreatment (Stage 2) showed less residual root canal filling material than did Stage 1 (P<0.05). After stage 2, the PUI group showed a significantly less amount of residual root canal filling material compared to the Manual group, for all thirds of the root canal (P<0.05) (Figure 1) (Table 2). It was observed similar amount of residual root canal filling materials among the apical, middle, and cervical thirds in the PUI group (Stage 2) (P>0.05) (Table 2). None

Figure 1
Representative 3D reconstructions of root filling material in the mesial canals of mandibular molars. **(A)** Root filling material in the Manual group. *Left*, root filling material after obturation; *center*, residual root filling material after retreatment with ProTaper; *right,* residual root filling material after suplementary cleaning with Manual protocol. **(B)** Root filling material in the PUI group. *Left,* root filling material after obturation; *center,* residual root filling material after retreatment with ProTaper; *right,* residual root filling material after suplementary cleaning with PUI protocol.

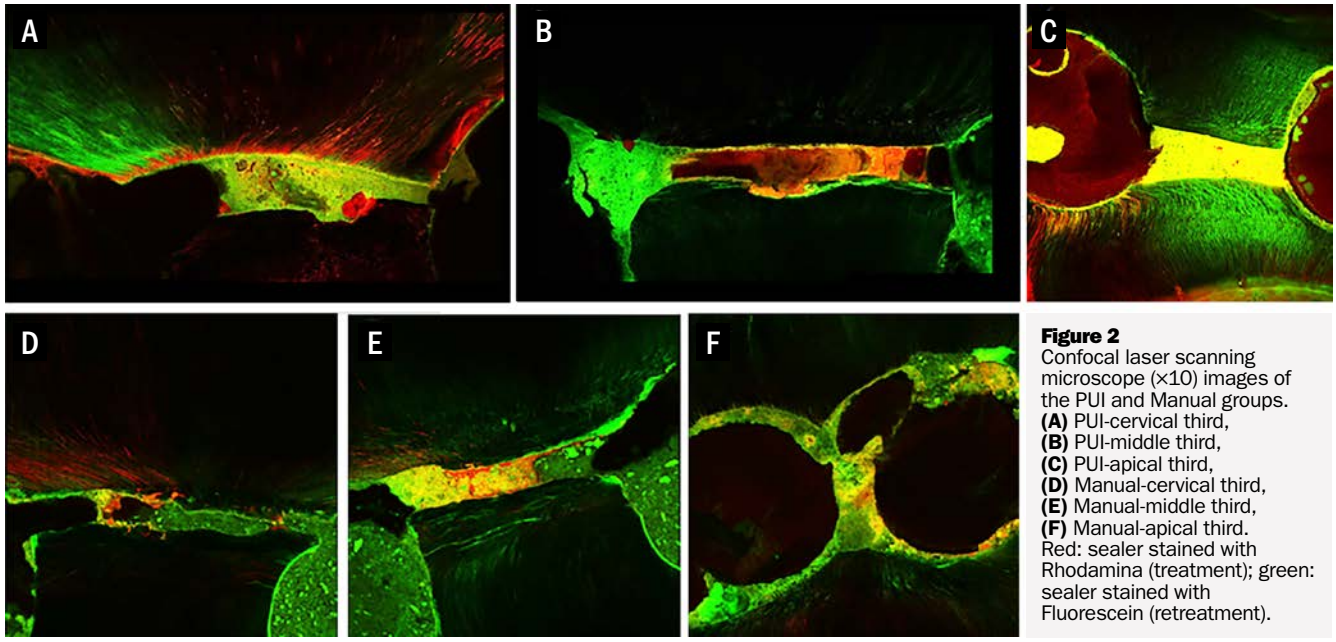


Figure 2
Confocal laser scanning microscope (×10) images of the PUI and Manual groups. **(A)** PUI-cervical third, **(B)** PUI-middle third, **(C)** PUI-apical third, **(D)** Manual-cervical third, **(E)** Manual-middle third, **(F)** Manual-apical third. Red: sealer stained with Rhodamina (treatment); green: sealer stained with Fluorescein (retreatment).

Table 1

Mean and Standard Deviation (SD) of residual filling material (in %) after removing bulk of the root filling material with ProTaper instruments (before randomization and common to both groups)

Canal Segment	ProTaper Instruments (Stage 1)		
	Manual Group (mean ± SD)	PUI Group (mean ± SD)	*P* value*
Apical	35.4±17.3^{A}	30.6±15.7^{A}	.5069
Middle	25.9±12.2^{A}	26.03±7.42^{A}	.7089
Coronal	10.8±6.97^{B}	9.93±4.69^{B}	.9339

Capital Letters: comparison among the thirds in the same group.
Different letters indicate statistically significant differences (P<0.05).
**Statistically significant differences between the groups if P<0.05.*

irrigation protocol completely removed the residual filling materials of root canal in all of the specimens.

CLSM

The distribution of absolute and relative frequency values of the scores of prevalence of the root filling material during retreatment for each experimental group is described in Table 3. Figure 2 showed confocal images for Manual and PUI groups. The penetrability of obturation during retreatment in the root isthmus and recesses was similar among thirds of the root canal when it was available in the same group (P>0.05). No significant statistical difference was observed between the Manual and PUI groups, independently of the third of the root canal (P>0.05).

Discussion

During endodontic retreatment, effective removal of the root filling is essential to facilitate disinfection and adaptation of new filling material (1-4). In this context, the authors aimed to assess the efficacy of the PUI using endodontic irrigants

Table 2

Mean and Standard Deviation (SD) of residual filling material (in %) after supplementary cleaning techniques (Stage 2)

Canal Segment	Manual Group (mean±SD)	PUI Group (mean±SD)	*P* value*
Apical	28.6±15.9^{A}	9.06±9.46^{A}	.0007
Middle	20.5±10.2^{AB}	8.14±8.81^{A}	.0019
Coronal	9.33±6.34^{B}	3.65±3.83^{A}	.0079

Capital Letters: comparison among the thirds in the same group.
Different letters indicate statistically significant differences (P<0.05).
**Statistically significant differences between the groups if P<0.05.*

Table 3

Distribution of absolute and relative frequency values of the scores of prevalence of the root filling material during retreatment for each third of the root canal within each experimental group

Score	Manual Group (n=15)			PUI Group (n=15)		
	Apical	Middle	Cervical	Apical	Middle	Cervical
0	4 (26.7%)	4 (26.7%)	1 (06.7%)	2 (13.3%)	2 (13.3%)	2 (13.3%)
1	3 (20.0%)	5 (33.4%)	8 (53.3%)	2 (13.3%)	5 (33.3%)	4 (26.7%)
2	2 (13.3%)	2 (13.3%)	2 (13.3%)	2 (13.3%)	1 (06.7%)	2 (13.3%)
3	2 (13.3%)	2 (13.3%)	3 (20.0%)	2 (13.3%)	3 (20.0%)	3 (20.0%)
4	4 (26.7%)	2 (13.3%)	1 (06.7%)	7 (46.7%)	4 (26.7%)	4 (26.7%)

and orange oil solvent as supplementary cleaning method to remove residual filling materials. The results of this study indicated that it was not possible to completely remove the existing root filling materials, principally in root isthmuses, recesses and oval extensions, which is in agreement with previous studies (8, 16, 24, 30, 31). However, the micro-CT analysis demonstrated that the use of PUI protocol significantly improved the removal of the residual filling material compared to the Manual group. The authors attribute the superior outcomes in the PUI group to the ultrasonic activation of the solvent. Ultrasound activation of the orange oil led to a temperature increase in the solvent by 2 °C (21), thereby enhancing its ability to dissolve the sealer (32). Furthermore, the replacement of the orange oil solvent prevented its saturation and facilitated extensive dissolution of the ZOE-based sealer, in according with a previous study (21).

During PUI, it is essential that the file moves freely within the root canal in order to allow for the solution to penetrate more easily into the root canal system (33). In this study, measures were taken to ensure free oscillation of the file within the root canal. The enlargement of the canal (Size 40/0.06 taper) during the retreatment allowed that the oscillation amplitude of the file to be minimally affected by contact with the root canal walls.

According to the endodontic literature, PUI has shown excellent results in disinfection and removal of dentinal debris

during endodontic treatment (9, 12). However, the protocol for using PUI during endodontic retreatment is not well established. The literature presents studies exhibiting considerable methodological variation, wherein factors like solution type, agitation duration, and endodontic sealer type can significantly influence outcomes (16-20). Grischke et al. (31) showed that PUI with NaOCl during retreatment was superior to all of the other irrigation techniques (syringe irrigation, CanalBrush, EndoActivator and RinsEndo) concerning the removal of the sealer from the root canal walls. Despite this, the results achieved with PUI were not entirely satisfactory, because although the root canal walls were free of sealer, the artificial grooves were still filled with sealer remnants. Some studies (15, 16) similarly concluded that PUI with NaOCl supplementary in the final irrigation during endodontic retreatment did not improve the removal of filling material within teeth with complex anatomical structures. In addition, Cavenago et al. (14) evaluated, using micro-CT, the percentage of the remaining filling material in the mesial root canals of mandibular molars after retreatment through three sequential procedures (mechanical cleaning, xylene, PUI with NaOCl). Their findings revealed no significant disparities between xylene and PUI with NaOCl. Despite methodological differences, there is a consensus that PUI has shown superior results compared to other irrigation methods such as syringe and needle, corroborating with the results of the present study.

When considering the 3D analyses, several cases in the PUI group could reasonably be called "extremely cleaned" (i.e., having less than 0.5% residue). The PUI group presented the best statistical results and some of the root canals extremely clean. However, the average and the standard deviation of the amount of the remaining filling material were notably higher in comparison to other studies (24, 30, 34). One possible explanation for this difference is that most of these studies utilized single straight root canals, aiming to simplify specimen standardization. In contrast, our study selected curved mesial roots of mandibular molars with several anatomical variations were selected, which were classified according to Vertucci Classification (27). The authors used teeth with more complex anatomy because they represent a common clinical scenario in endodontic practice (35) and investigations of nonsurgical retreatment techniques in these cases are not common.

The average percentages of remaining filling material in the root canals after Stage 1 were consistent with Abramovitz et al.'s findings (36). When these authors only used ProTaper retreatment files in the curved canals of the mesial roots of mandibular molars, they observed substantial residual root canal filling material in the apical third of the canals. In our study, the application of PUI after the use of rotary instruments during endodontic retreatment notably enhanced apical cleaning. PUI, recognized as the 'gold standard' in agitation studies, as acoustic streaming created around the instrument magnifies the performance of the solution agitation and enhances the removal of debris, smear layer, and root canal filling materials (9, 17).

Based on the CLSM results, the present study observed that the obturation during endodontic retreatment penetrated into the isthmuses areas similarly in both groups. The main objective of combining solvents with PUI was to clean those areas of difficult access to endodontic instruments. The action of the PUI with different irrigants proportioned a higher dissolution of the filling materials (micro-CT results), although this remotion was not fulfilled by a filling during retreatment in isthmus and irregularities areas (CLSM results). A possible explanation for this difference in results between the two assessments was the use of the Tagger hybrid technique for root canal filling, since warm compaction tecnhiques would be better for filling canals with isthmus and irregularities areas. Thus, if the obturation performed during root canal retreatment is capable of providing entrapment of the microorganisms that caused the failure of primary treatment, the absence of nutrients would prevent their survival. The viabili-

ty of these microorganisms could potentially contribute to the maintenance and progression of periapical infection (37) leading to subsequent failure of the endodontic retreatment.

The use of solvents during endodontic retreatment softens gutta-percha, facilitating instrument action, and thereby reducing the risk of deviation, perforations, and instrument fracture during material filling removal (38). Additionally, solvents are used to remove waste filling materials in the root canal (39). In this study, orange oil solvent was used as the irrigant for ultrasonic activation due to its capacity to dissolve sealers similarly to other solvents, yet without causing any deleterious effects on periapical tissues (40, 41). However, the association of ultrasound with a solvent may raise concerns regarding potential solvent extrusion of the solvent and subsequent adverse effects on the periapical tissues. Nevertheless, it was demonstrated that PUI does not increase apical extrusion of the irrigant (42).

It is important to emphasize that, in this study, the authors took care in standardizing the experimental stages to avoid potential biases. The angles and radii of curvature were assessed, and no significant differences were observed between the experimental groups. The root canal anatomy was assessed using Micro-CT, categorized following Vertucci's classification (27), and a stratified randomization was performed, considering this classification. Moreover, no statistical differences were observed between the groups after the use of rotary instruments. This suggests that the specimens exhibited similar characteristics concerning root canal anatomy and presence of filling material before allocation for the protocol of supplementary cleaning. Furthermore, one of the strengths of this study was the utilization of micro-CT, which enabled a three-dimensional evaluation of root filling material.

One potential limitation of this study was the use of different sealers in both endodontic treatment and retreatment, as the sealers employed possess distinct fluidity and flow capacity properties. However, our research could serve as a basis for future studies investigating agitation techniques using differents solutions for removing residual filling material. Furthermore, additional investigations should explore the efficacy of PUI with solvents in dissolving resin-based and bioceramic sealers.

Conclusions

None of the supplementary cleaning protocols succeeded in completely removing the root canal filling material from the root canal system. However, the agitation of irrigants and orange oil using PUI proved to be an effective auxiliary method for removing residual filling material from curved canals with isthmus and irregularities areas. Although the PUI protocol enhanced the removal of root filling material, the penetration of obturation during retreatment in root irregularities areas remained unaffected by supplementary protocols.

Clinical Relevance

This study aims to help clinicians choosing the best supplementary cleaning protocol for removing residual filling material during endodontic retreatment.

Conflict of Interest

The authors deny any conflict of interest related to this study.

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Nothing to declare.

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