

Filling Material Removal with Reciprocating and Rotary Systems Associated with Passive Ultrasonic Irrigation

- D Natália Nascimento GOMES, D Guilherme Moreira de CARVALHO,
- 🗓 Emílio Carlos SPONCHIADO JÚNIOR, 🗓 Lucas da Fonseca Roberti GARCIA,
- D André Augusto Franco MARQUES, Fredson Marcio Acris de CARVALHO

ABSTRACT

Objective: The purpose of this ex vivo study was to evaluate the filling material removal ability, and the time required to perform this procedure, of reciprocating and conventional rotary systems when associated with passive ultrasonic irrigation.

Methods: The palatal roots of 40 maxillary molars were submitted to root canal preparation and filling. The desobturation of root canals was initially performed with Largo burs in the coronal portion (4 mm) to drill the gutta-percha and to facilitate the action of the instruments used then. Next, the palatal roots were randomly distributed (n=10) according to the systems and irrigation protocols used for filling material removal: ProTaper universal retreatment (PTR), PTR+passive ultrasonic irrigation (PUI) (PTR+PUI), Reciproc system (RS), and RS+PUI. Passive ultrasonic activation was performed in the root canals completely filled with 2.5% sodium hypochlorite solution using a smooth and straight ultrasonic tip, coupled to a low-power (20%) ultrasonic device for 1 min (3 cycles of 20 s). After retreatment, the roots were longitudinally sectioned to the remaining filling material quantification using an operating microscope. Environmental scanning electron microscopy (ESEM) micrographs at 97, 105, and 250 X magnifications were also taken to evaluate the quantity of filling material present at the apical portion of the palatal roots.

Results: The RS group presented greater quantity of filling material attached to the root canal walls than the other groups (P>0.05). PTR+PUI and RS+PUI groups were statistically similar (P>0.05). Reinstrumentation of root canals using RS was faster than PTR, irrespective of the irrigation protocol used (P>0.05).

Conclusion: The association between PUI and the different systems for reinstrumentation yielded greSater filling material removal. The reciprocating system was faster.

Keywords: Environmental scanning electron microscopy, irrigant agitation protocols, reciprocating motion, root canal retreatment

Please cite this article as: Gomes N.N., de Carvalho G.M., Sponchiado Júnior E.C., Garcia L.F.R., Marques A.A.F., de Carvalho F.M.A. Filling Material Removal with Reciprocating and Rotary Systems Associated with Passive Ultrasonic Irrigation. Eur Endod J (2017) 2:6.

Received 20 September 2016, revision requested 31 October 2016, last revision received 14 December 2016, accepted 23 December 2016.

Published online: 27 February 2017 DOI: 10.5152/eej.2017.16037

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HIGHLIGHTS

- No retreatment technique is able to completely clean the root canal walls.
- Association between passive ultrasonic irrigation with reciprocating and rotary systems provided greater filling material removal.
- Passive ultrasonic irrigation enhances filling material removal during root canal retreatment.

INTRODUCTION

Amongst the different stages of endodontic retreatment, filling material removal may be performed by using manual files and rotary instruments (1-4). Further, desobturation may be associated with ultrasonic activation and different types of solvents, although they are currently used with less frequency (5). However, no retreat-

ment technique is capable of a clean root canal system in its entire length after reinstrumentation (5, 6).

Recently, a novel NiTi rotary system-ProTaper Universal Retreatment (PTR)-was developed for filling material removal (7). This system is an improvement over the conventional ProTaper instruments, with significant changes in instruments design, favoring endodontic retreatment (7).

On the other hand, reciprocating instruments, such as Reciproc system (RS), have greater flexibility and cyclical fatigue resistance, being more efficient in comparison with conventional rotary NiTi instruments (8). Developed for root canal preparation, they also emerge as an alternative for filling

material removal during endodontic retreatment (5, 6). Several studies reported that reciprocating systems have great potential in root canal preparation; however, their effectiveness in root canal retreatment, especially when associated with passive ultrasonic irrigation (PUI), has not been sufficiently explored so far (2, 3).

Irrigating solutions associated with passive ultrasonic activation can remove microorganisms, remaining pulp tissue, and debris more efficiently than conventional irrigation protocols (9). The movement of irrigating solutions increased by ultrasonic activation ensures solution penetration within the surface of root canal walls, mainly in critical areas (e.g., the apical portion) where proper cleaning is difficult to achieve (9). Thus, it is also believed that passive ultrasonic irrigation (PUI) might potentiate filling material removal, improving the cleaning of root canal after retreatment (9).

This ex vivo study aimed at evaluating the filling material removal efficacy, and the time required to perform this procedure, of a reciprocating single-file system (Reciproc) and a conventional rotary system (PTR) associated with PUI. Root canal wall cleaning was assessed using an operating microscope and environmental scanning electron microscopy (ESEM).

MATERIALS AND METHODS

Sample selection

A total of 40 freshly extracted maxillary molars were included in this study, with previous authorization by the State University of Amazonas Research Ethics Committee, where the experiment was carried out (Protocol No: CAAE no 43028015.0.0000.5016). The teeth were donated by the Bank of Teeth of State University of Amazonas, with the complete understanding and consent of the patients, allowing the authors to use their extracted teeth, and reproduce radiographs or images of them. The sample size was calculated in order to determine the proper number of specimens necessary to determine a significant difference of 5% (α) among the experimental groups.

The selected teeth had a single and straight palatal root canal, no signs of vertical fracture or external root resorption, and fully formed apex. The crowns of the selected teeth were sectioned with the aid of a double-faced diamond disk (KG Sorensen, Barueri, SP, Brazil), mounted on a high-speed apparatus (Silent-MRS 400, Dabi Atlante, Ribeirão Preto, SP, Brazil), in order to standardize the palatal root length to 16 mm. A radiographic examination of the teeth was performed to assess the internal anatomy of the palatal canal. Root canals with calcifying degenerative processes and internal resorption were excluded from the final sample.

The selected roots were sterilized in an autoclave (12 LX, Dabi Atlante), and then stored in receptacles (Bioplast, Porto Alegre, RS, Brazil) containing distilled water at 5°C until use.

Root canal preparation and irrigation

Initially, the palatal root canals were negotiated with a size 10 K-file (Dentsply/Maillefer, Ballaigues, Switzerland) in the apical direction until the foramen was increased and was indented 1

mm to set the working length (15 mm). Then, a size 15 K-file was inserted at the working length for the standardization of the apical anatomic diameter of the root canals.

In order to maintain the roots in the same position during root canal preparation, an acrylic matrix measuring $2.0 \times 2.0 \times 2.0$ cm containing 18 compartments filled with condensation silicone (Clonage, Nova DFL, Jacarepaguá, RJ, Brazil) was used. The palatal roots were coupled to the acrylic matrix compartments, and they were prepared using the ProTaper Universal system (Dentsply/Maillefer) according to the manufacturer's recommendations. The SX (0.19/0.035) instrument was used at the root canal's coronal and middle portions to create a proper glide path for the following instruments. The S1 (0.17/0.02) and S2 (0.20/0.04) instruments were used for coronal and middle root portions' preparation, respectively, and apical finishing was performed with F1 (0.20/0.07), F2 (0.25/0.08), and F3 (0.30/0.09) instruments. The instruments were coupled to a 6:1 contra-angle device (VDW Silver Reciproc, Sirona Dental Systems GmbH, Bensheim, Germany) driven by an electric motor (VDW Silver Reciproc Motor, Sirona Dental Systems) at a constant speed of 250 rpm and 2 N cm torque. As an irrigating solution, 2 mL of 2.5% sodium hypochlorite (Fórmula e Ação, São Paulo, SP, Brazil) was used after each change of the instrument. The smear layer was removed from the root canals using 1 mL of 17% ethylenediamine tetraacetic acid (Biodinâmica, Ibiporã, PR, Brazil) for 3 min prior to final irrigation with 5 mL of 2.5% sodium hypochlorite.

Root canal filling

Root canal filling was performed using the Tagger method. The root canals were dried with sterile absorbent paper cones (Dentsply/Maillefer), and then, they were filled using a size F3 gutta-percha master cone (Dentsply/Maillefer) and FM cones (Dentsply/Maillefer) wrapped in a sealer (AH Plus, Dentsply/Maillefer). A McSpadden instrument (size: 50/25 mm) coupled to a hand-piece device (Dabi/Atlante), rotating clockwise, and oscillating at 8000 rpm was introduced within the root canal 4 mm from its working length for filling material plasticization.

The roots were submitted for radiographic examination for filling quality evaluation. The root canals were evaluated in the buccolingual and mesiodistal directions by a properly calibrated examiner, taking into consideration the presence of voids within the root canal (Figure 1). Palatal roots, which presented failures in root canal filling, were discarded from the final sample.

Afterwards, the selected palatal roots had their canal orifice sealed with a temporary filling material (Coltosol, Coltène/Whaledent Inc., Cuyahoga Falls, OH, USA), and they were stored in artificial saliva at 37°C for 30 days to allow complete filling material setting and to simulate oral conditions.

Root canal retreatment

The filling material removal was initially performed as follows: sizes 1 and 2 Largo burs (28 mm) (tips 0.70 and 0.90, respectively) were used in the coronal portion (4 mm) of the root canal length to drill the gutta-percha and to facilitate the action of the instruments used then. Next, the teeth were assigned in accordance with the system/irrigation protocol used for filling material removal (n=10):

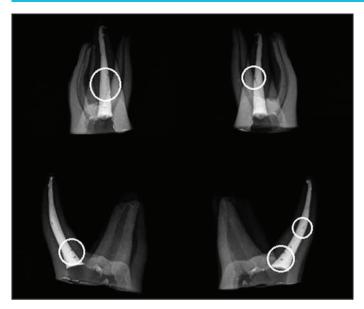


Figure 1. Radiographic examination for filling material quality evaluation. Palatal root discarded from the final sample. Note the presence of voids within the root canal (circle)

PTR group: PTR system (Dentsply/Maillefer)-D1 instrument (size: 0.30/.09 taper) in the coronal portion, D2 (0.25/0.08) in the middle portion (4 and 2 mm from the working length, respectively), and D3 (0.20/0.07) in the working length (0.5 mm from the apex). Finishing and reinstrumentation of the apical portion was performed with F4 (0.40/0.06) and F5 (0.50/0.05) instruments using the ProTaper Universal system (Dentsply/ Maillefer) in the working length. Each instrument was gradually inserted in the root canal 3 consecutive times, in the apical direction, with slow pecking movements of 3-mm amplitude limit. At each change of instruments, 2 mL of 2.5% sodium hypochlorite was used to irrigate the root canals. The instruments were coupled to a 6:1 contra-angle device (VDW Silver Reciproc) driven by an electric motor (VDW Silver Reciproc Motor), at 600 rpm for D1, 400 rpm for D2 and D3, and 300 rpm for F4 and F5, with a 2 N cm-1 torque, as recommended by the manufacturers. At the end of the reinstrumentation, 2 mL of 2.5% sodium hypochlorite, followed by 1 mL of 17% ethylenediamine tetraacetic acid (Biodinâmica) for 3 min, and a final irrigation with 5 mL of 2.5% sodium hypochlorite was performed to finish the retreatment procedure. Instruments from the ProTaper system were used to prepare only 3 root canals, and they were then discarded.

PTR+PUI group: Root canal instrumentation with PTR system, as performed in the PTR group. After root canal reinstrumentation, the pulp chamber was completely filled with 2 mL of 2.5% sodium hypochlorite, and PUI was performed using a smooth and straight ultrasonic tip (TRA-12, Trinks, São Paulo, SP, Brazil), coupled to a low-power (20%) ultrasonic device (NacPlus, Adiel, Ribeirão Preto, SP, Brazil) for 1 min (3 cycles of 20 s). Next, the irrigating solution was aspirated, and 1 mL of 17% ethylenediamine tetraacetic acid (Biodinâmica) was placed within the root canal for 3 min. A final irrigation with 5 mL of 2.5% sodium hypochlorite was performed to finish the retreatment procedure.

RS group: RS (VDW GmbH, Munich, Germany)-R50 instrument (0.50/0.05) was gradually inserted in the root canal 3 consecutive times, with slow pecking movements of 3-mm amplitude limit. As the R50 instrument advanced inside the root canal, it was removed for cleaning with sterile gauze, and the root canals were irrigated with 2 mL of 2.5% sodium hypochlorite. The R50 instrument was coupled to a 6:1 contra-angle device (VDW Silver Reciproc) driven by an electric motor (VDW Silver Reciproc Motor) in the mode "RECIPROC ALL," as recommended by the manufacturers. Final irrigation was performed as described in the PTR group. Each R50 instrument was used for reinstrumentation of only one root canal.

RS+PUI group: Root canal instrumentation with the RS, as performed in the RS group, followed by passive ultrasonic agitation as described in the PTR+PUI group.

A single operator, specialist in endodontics, performed the procedures listed above.

Filling material removal evaluation

After root canal reinstrumentation, longitudinal grooves were made on the buccal and palatal surfaces of the roots. The roots were longitudinally sectioned using a double-faced diamond disc (Microdont, São Paulo, SP, Brazil), and then cleaved into two halves with a N° 5 LeCron spatula (SSWhite/Duflex, Rio de Janeiro, RJ, Brazil). Both root hemi-sections were photographed under 16 X magnification by a camera (Sony Cybershot DSC-W350, Sony Brazil, São Paulo, SP, Brazil) coupled to an operating microscope (Alliance, São Carlos, SP, Brazil).

After image acquisition with the microscope, the outer contour of each root canal's hemi-section and the areas containing the remaining filling material were delineated (Figure 2a, b).



Figure 2. a, b. Remaining filling material evaluation using an operating microscope. (a) Calculation of the root canal area (outlined) and (b) remaining filling material attached to the root canal wall (outlined)

A properly calibrated examiner, using the ImageJ software (National Institute of Health, Bethesda, MD, USA), measured the root canal periphery and the areas containing the remaining filling material attached to the root canal walls. The area corresponding to the two hemi-sections of each root canal was considered as 100%, and the area containing the filling material was marked as "X."The quantity of filling material attached to the root canal walls and the total area were expressed in mm². Then, the data obtained in mm² were transformed into percentages for comparison among groups.

The time required for filling material removal was calculated from the introduction of the first instrument within the root canal until the establishment of the working length. A digital chronometer (Oregon Scientific-Sl928 m, Portland, OR, USA) was stopped every time each instrument was removed from the root canal, and restarted, as the root canal instrumentation continued using the subsequent instrument. Time measurements were expressed in seconds (s).

Environmental scanning electron microscopy (ESEM) analysis

After the quantification of the remaining filling material in root canal walls by means of the operating microscope, the apical portion of each root hemi-section was submitted to a qualitative analysis using ESEM (ESEM-Quanta 250, FEI Co., Hillsboro, OR, USA). The samples were fixed onto numbered metal stubs using a double-faced carbon tape, and then, they were taken to the SEM set operated at 20-25 kV. The root canal wall surface was analyzed at 97, 105, and 250X magnifications. A properly calibrated and blinded examiner evaluated the ESEM micrographs. For the qualitative evaluation of the filling material attached to the root canal walls, the following criteria were considered: root canal walls with small particles of remaining filling material and debris produced by reinstrumentation, filling material and debris attached to less than 50% of the root canal walls, and root canal walls completely covered by remaining filling material and debris (10).

Statistical analysis

The normality of the data distribution was tested using the Kolmogorov-Smirnov test, and the values obtained for the remaining filling material (Kruskal-Wallis, Dunn's multiple comparisons test, P<0.05) and time required for retreatment (1-way ANOVA, Tukey test, P<0.05) were statistically analyzed using the GraphPad InStat for Mac OS software (GraphPad Software, La Jolla, CA, USA).

RESULTS

Filling material removal effectiveness

The mean values (%) of the remaining filling material on the root canal walls are shown in Table 1.

The remaining filling material was observed to be attached to the root walls of all the samples, irrespective of the system/irrigation protocol used for retreatment. The RS group had the highest values, with significant difference when compared to the other groups (P>0.05). PTR+PUI and RS+PUI groups had the lowest values, which were statistically similar (P<0.05).

TABLE 1. Mean values (%) and standard deviation (±SD) of remaining filling material in root canal walls after retreatment

Groups	Mean value (±SD)
PTR*	6.67 (3.87) ^a **
PTR+PUI	2.56 (1.55) ^b
RS	9.64 (8.58) ^c
RS+PUI	2.85 (1.47) ^b

*PTR: ProTaper retreatment system; PTR+PUI: ProTaper retreatment system+passive ultrasonic irrigation; RS: reciproc system; RS+PUI: reciproc system+passive ultrasonic irrigation; **Different lowercase letters indicate a statistically significant difference (Kruskal–Wallis, Dunn's multiple comparisons test, P<0.05) n=10

TABLE 2. Mean values (s) and standard deviation (±SD) of time required to perform filling material removal

Groups	Mean value (±SD)
PTR*	219.73 (56.01) ^a **
PTR+PUI	208.13 (67.46) ^a
RS	127.29 (40.81) ^b
RS+PUI	138.42 (30.79) ^b

*PTR: ProTaper retreatment system; PTR+PUI: ProTaper retreatment system+passive ultrasonic irrigation; RS: reciproc system; RS+PUI: reciproc system+passive ultrasonic irrigation; **Different lowercase letters indicate a statistically significant difference (Kruskal–Wallis, Dunn's multiple comparisons test, P<0.05) n=10

Time required for retreatment

The reinstrumentation of the root canals was faster in groups where the reciprocating system was used (P>0.05) (Table 2).

ESEM analysis

As evident from Figure 3 and 4, the remaining filling material and debris attached to the root canal walls at the apical portion were observed in all the experimental groups, irrespective of the reinstrumentation system or irrigation protocol used. The palatal roots that were not submitted to PUI had greater amount of remaining filling material and debris in the apical portion in comparison with the groups where PUI was performed. The RS group exhibited a greater quantity of filling material, with the root canal walls almost completely covered by residues of gutta-percha and sealer (Figure 4a). In addition, it was observed that scattered amounts of debris produced by the reinstrumentation process covered the root canal walls, obliterating the entrance of the dentinal tubules (Figure 4b). Despite the better results obtained when the irrigation protocol was performed, all the samples still had debris and remaining filling material attached to the root canal walls at the apical portion, irrespective of the system used for reinstrumentation (reciprocating or rotary) (Figure 3c, 4c).

DISCUSSION

Previous studies have reported that no technique or instrumentation system can eliminate the filling material present in the root canal system after endodontic retreatment (5, 7). These results corroborate the findings of the present study, as all the samples had remaining filling material attached to the root canal walls.

NiTi instruments powered by electric motors are more efficient than manual instruments to perform filling material removal

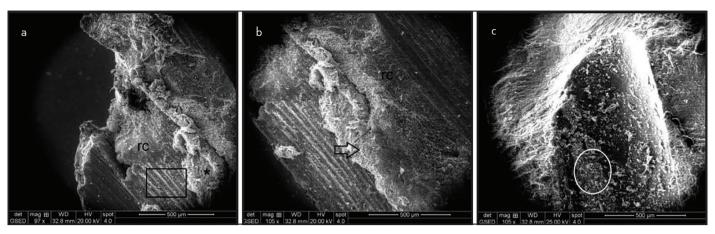


Figure 3. a-c. Representative ESEM micrographs of the root canal surfaces at the apical portion of the PTR group. (a) Most of the apical portion still had remaining filling material (*) attached to the root canal walls (rc). Note the presence of debris produced during reinstrumentation attached to the root canal surface (box). Original magnification: 97X. (b) The apical portion of the same sample in higher magnification. Note the remaining filling material (gutta-percha cone) (arrow) attached to the root canal wall (rc) despite reinstrumentation. Original magnification: 105X. (c) Representative ESEM micrograph of the root canal surfaces at the apical portion of the PTR+PUI group. Smaller amount of debris due to the association of PTR system to PUI protocol. Note the presence of debris attached to the root canal walls (circle). Original magnification: 105X

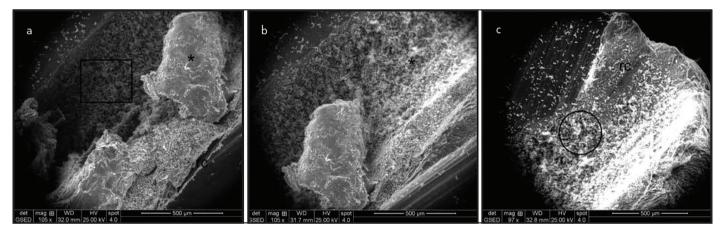


Figure 4. a-c. Representative ESEM micrographs of the root canal surfaces at the apical portion of the RS group. (a) Note the larger amount of remaining filling material (gutta-percha cone) (*) attached to the root canal walls (rc). It was also possible to detect a greater amount of debris produced by the reinstrumentation process covering the root canal walls, obliterating the entrance of the dentinal tubules (box). This feature could not be observed under an operating microscope (box). Original magnification: 105X. (b) In the same sample, another portion of the root canal can be observed to be covered by debris that was not removed during reinstrumentation (*). Original magnification: 105X. (c) Representative ESEM micrograph of the root canal surfaces at the apical portion of the RS+PUI group. As observed in the PTR+PUI group, a smaller amount of debris in comparison with the RS group, due to the association of RS with PUI protocol, was noted. However, a significant portion of the root canal walls (rc) remained covered by debris produced by reinstrumentation (circle). Original magnification: 97X

within the root canal (11, 12). The use of such instruments for root canal retreatment have seen significant increase in the last few years; therefore, the authors of this study decided to compare the PTR system, which was developed exactly for this purpose, and the RS, a system that has an innovative motion kinematics and has been developed for root canal preparation (5-8). Several studies have evaluated the effectiveness of reciprocating systems to perform gutta-percha and root canal sealer removal; however, the benefits of their association with PUI must be evaluated (6, 7).

The use of irrigating solutions during root canal preparation is crucial for proper cleaning, irrespective of the system or technique used for it (13-15). In this study, during each change of instrument, 2 mL of 2.5% sodium hypochlorite was used to ir-

rigate the root canals, irrespective of the systems or irrigating protocol used. However, when retreatment was performed with the PTR system, a larger number of instruments than that used in the RS were used; therefore, the total volume of irrigating solution used was also greater. The time to perform root canal preparation when using single-file systems, such as Reciproc, is significantly reduced. Therefore, the amount of irrigating solution used is also reduced, compromising chemical debridement (13-15). Such a fact might explain the worst performance of the RS when compared to the PTR system.

However, it is valid to emphasize that when both the systems were associated with PUI, the results were similar. Despite the lower content of irrigating solution used during root canal retreatment with the RS, the ultrasonic activation performed at

the end of the reinstrumentation optimized the remaining filling material removal, even in critical areas, such as the apical portion (16-18).

The use of ultrasound promotes numerous effects within the root canal, such as the cavitation phenomenon (19). Cavitation occurs when the osmotic pressure of a liquid is higher than the hydraulic pressure that the liquid exerts on its recipient walls, forming bubbles in its interior and subsequent implosion, creating temporary cavities. These, upon rupture, produce shock waves on the recipient surface where the liquid is located (19, 20).

In passive ultrasonic activation, cavitation produces irrigating solution displacement toward the root canal walls (19, 20). The impact caused by the phenomenon promotes smear layer removal, mainly in the areas where instruments cannot reach (19-21). Thus, it is valid to state that the same phenomenon may occur with the filling material (19, 20).

According to Zuolo et al. (5), the quantity of filling material attached to the root canal walls after retreatment may range from 4.5% to 12.0% depending on the instruments (manual, rotary, or reciprocating) used. These results support the findings of the present study, taking into consideration the root canal reinstrumentation with no association to PUI. When reinstrumentation was associated with PUI, both systems exhibited a significant decrease in the remaining filling material values, confirming the effectiveness of such association.

The apical portion of the palatal root canals was also analyzed by ESEM in the present study. The analysis of non-conductive and non-hydrated samples is one of the main advantages of ESEM equipment in comparison with conventional SEM (22, 23). This technique allows an accurate analysis without previous metallization of the samples and without the reduction of the natural contrast from the beam-sample interaction (22). The main element used for the samples metallization in conventional SEM is gold (Au), which has a high atomic number. For this reason, it produces high topographic contrast and images with high resolution. However, despite the high contrast produced, some details of the sample may not be detected due to the metallization process, compromising the analysis of important features of root canal morphology (22-25). Therefore, the authors decided to use the ESEM equipment for apical portion analysis. Despite the better results obtained when reinstrumentation was associated with PUI, a significant portion of root canal walls remained covered by remaining filling material and debris. It was not possible to detect these features by using an operating microscope, only when ESEM analysis was performed. The apical area is considered a critical zone; therefore, proper cleaning of this specific area is more difficult to achieve (4). Likewise, reinstrumentation and complete filling material removal in this area are challenging procedures, as our findings demonstrated (4).

With regard to the time required for reinstrumentation, the groups submitted to preparation with a reciprocating system were significantly faster than conventional rotary groups, corroborating the findings of Souza et al. (26). The number of instruments used by the reciprocating system, as well as

how easy the Reciproc R50 instrument can reach the working length in straight root canals might explain these results (5, 26, 27).

Despite having performed the reinstrumentation of root canals faster, it is worth emphasizing that the RS presented worse results when passive ultrasonic activation was not performed. PTR instruments had a design specifically developed for root canal retreatment with negative cutting-angle blades and lack of radial guide, which promoted larger amounts of filling material removal. These features might explain the better performance of PTR instruments in comparison with the RS.

In recent studies, Silva et al. (7) and de Souza et al. (26) reported no difference between PTR and RS, which is not in agreement with the results of the present study. The conflicting results could be explained by the use of chloroform to soften the gutta-percha before reinstrumentation in the study by de Souza et al. (26). In the present study, no solvent was used to promote gutta-percha softening. Gutta-percha softening helps to decrease the working time during root canal retreatment; however, softened gutta-percha could be compacted within the dentinal tubules and root canal surface irregularities, hindering filling material removal (28).

In their study, Silva et al. (7) used a radiographic method to evaluate filling material removal. In the present study, digital images of root hemi-sections were evaluated under an operating microscope, and the apical portion of the root canals was also analyzed by the ESEM equipment. Despite the fact that volumetric analysis by micro-computed tomography to measure residual filling material amount is considered superior than conventional methods, studies demonstrated that the analysis of root hemi-sections are the proper method for this purpose and more accurate than radiographic examination, ensuring reliable results (5, 6, 27, 28).

CONCLUSION

Filling material attached to root canal walls was observed in all the groups after retreatment, irrespective of the system or the irrigation protocol used. PUI provided greater filling material removal in both the systems. The reciprocating system was faster; however, this finding does not mean that such a system was more effective for filling material removal.

Disclosures

Ethical Approval: Ethics committee approval was received for this study from the ethics committee of State University of Amazonas (Protocol No: CAAE n° 43028015.0.0000.5016).

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept - A.A.F.M., F.M.A.C.; Design - N.N.G., G.M.C.; Supervision - A.A.F.M., F.M.A.C.; Resource - A.A.F.M., F.M.A.C.; Materials - A.A.F.M., F.M.A.C.; Data Collection and/or Processing - A.A.F.M., F.M.A.C., L.F.R.G., N.N.G.; Analysis and/or Interpretation - A.A.F.M., F.M.A.C., L.F.R.G., N.N.G.; Literature Review - N.N.G., L.F.R.G.; Writer - N.N.G., L.F.R.G., E.C.E.J.; Critical Review - A.A.F.M., F.M.A.C., E.C.E.J.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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